62 The development of gene-based vectors for immunization

David B. Weiner
Gary J. Nabel

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 depends on helper T cells, which elaborate a variety of cytokines and stimulate B cells to produce antibodies and induce the formation of cytotoxic T lymphocytes (CTL). CTLs recognize processed antigen on major histocompatibility complex (MHC) molecules and lyse infected cells.

Both humoral and cellular immunity are elicited by vaccines, each with its own effector functions that can inactivate pathogens in different ways (Table 62-1). Although 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 patients with acquired immunodeficiency syndrome (AIDS), malaria, or 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. A variety of vectors, nonviral and viral, have been developed for these purposes (Figure 62-1), the most common of which are reviewed here.

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

Nonviral vectors and DNA vaccines

The possibility of vaccination with naked DNA generated excitement in the early 1990s when four independent groups simultaneously reported that plasmid gene delivery could result in in vivo antigen expression with resulting immune responses to the plasmid-encoded antigens. Stephan Johnson's laboratory used a device called the gene gun to "shoot" gold beads coated with a plasmid encoding human growth hormone as a gene therapy technique in mice.[3] Surprisingly, the animals seroconverted to this gene therapy. Other groups were attempting to harness the power of plasmids for vaccination purposes and presented their work in a session at the Cold Spring Harbor Vaccines meeting in the fall of 1992. The laboratories of Margaret Liu (Merck) and Harriet Robinson (University of Massachusetts) vaccinated mice intramuscularly with plasmids expressing influenza antigens and showed that antigen-specific immunity[4,5] could be induced. David Weiner (University of Pennsylvania) reported that plasmids containing HIV envelope or tumor antigens were capable of driving cellular and humoral immune responses in mice.[6] These novel results, combined with the simplicity and obvious manufacturing utility of DNA, spawned research into DNA vaccines for a plethora of viral, bacterial, parasitic, and cancer targets.[7–12]

The DNA vaccine platform was perceived by the scientific community to be important as, conceptually, DNA has multiple advantages over traditional live attenuated, killed, peptide-based, and viral vector vaccines.[13,14] For example, DNA is easy to manipulate, and it combines the simplicity of synthetic chemistry or bacterial production with the power of genomics, allowing the rapid design and construction of potential vaccines, removing entirely the requirement to develop vaccines using pathogen-derived materials. Second, DNA vectors are extremely stable, reducing the need for a cold chain and increasing product shelf life. Therefore, the ease, stability, and cost effectiveness of this platform make it ideal for manufacturing vaccines for the developing world. Furthermore, DNA vectors themselves are not immunogenic, which allows repeat homologous vaccination without immune interference. Importantly, DNA vaccines combine the immune power of live replicating vaccines for induction of broad cellular and humoral immune responses with the safety and ease of

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

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

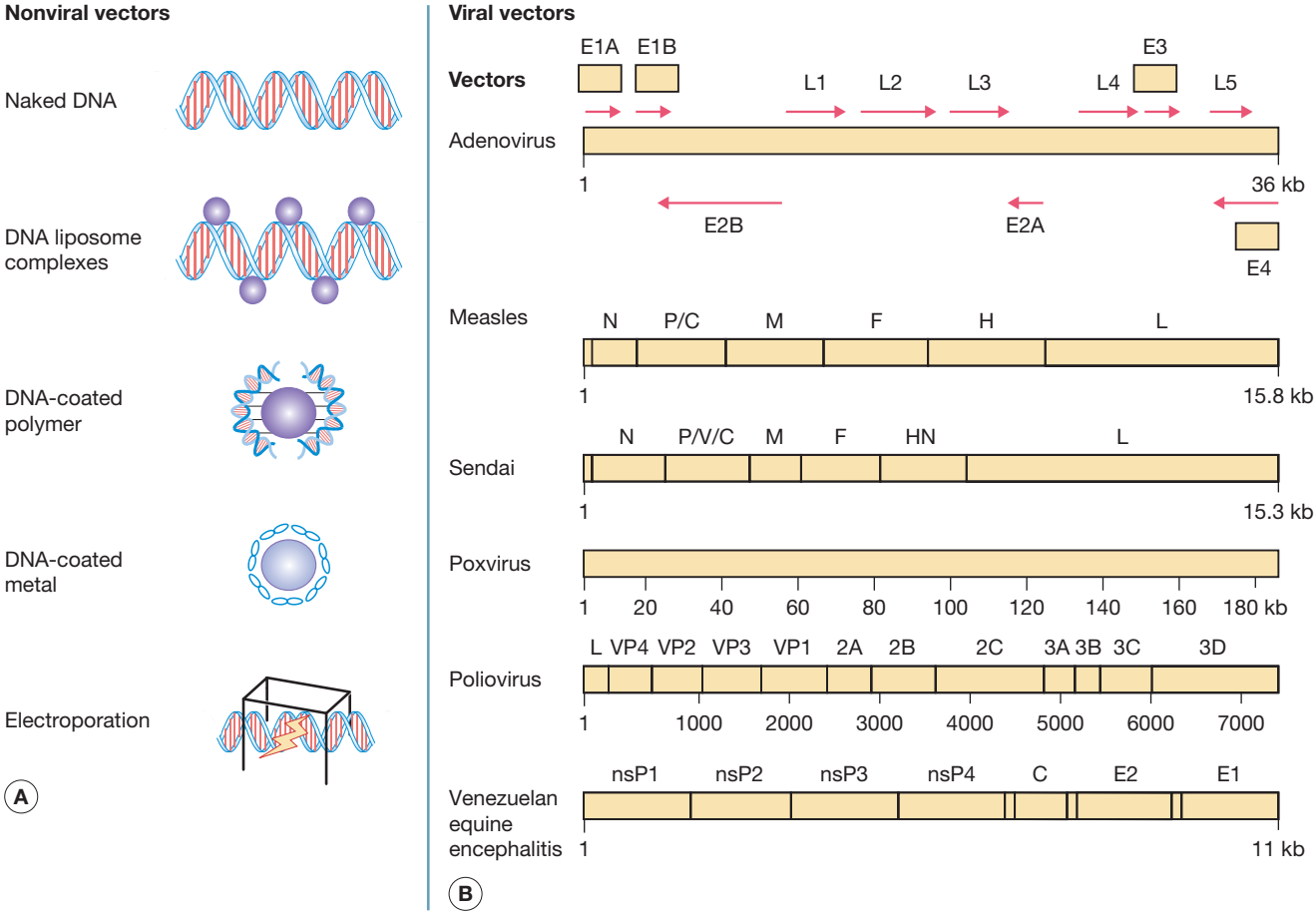

Figure 62-1 Representative vector platforms for gene-based vaccines that have advanced into clinical trials. Vaccination by gene delivery with nonviral (A) and replication-defective recombinant viral gene-based vectors are shown (B). In B, the genetic organization and virus structure of the natural replication-competent virus are shown.

manufacturing of a nonlive, nonspreading platform.[15,16] As DNA vaccines are nonreplicating, they eliminate the risk of attenuation reversions, spread in the subject, or unintended secondary infections in vaccinated populations. With some constraints, DNA vaccine products can be developed in repeat-use manufacturing facilities, thus providing enormous savings in product development and manufacturing. The implied safety advantage implicit in DNA vaccine technology has so far translated into the clinic, where DNA has provided an unparalleled safety profile over the past decade and a half of clinical study.[17] This safety profile allows expanded studies of this platform in development of vaccines for at-risk groups, including subjects with compromised immunity, the elderly, and persons on chemotherapy.

Mechanism of action

Understanding the mechanisms by which DNA vaccines induce antigen-specific immunity is of considerable importance. To put it simply, an antigen sequence of interest is

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

Advantages	Limitations
• Potent immunogenicity in animal and human infectious diseases	• High level of immunity to some vectors in humans
• Ability to induce cellular immunity with or without humoral immunity	• Need for qualified packaging cell lines
• Relative ease of production for many viral and nonviral vectors	• Induction of antivector immunity after initial injection of viral vaccines, limiting efficacy of homologous boost
• 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	

optimized and inserted into a mammalian expression plasmid vector.[18–25] For clinical use, such vectors tend to share specific features, including a high-copy-number origin of replication for production purposes, a human cytomegalovirus immediate-early (CMV-IE) promoter to drive expression in vivo, an RNA polyadenylation sequence (usually derived from bovine growth hormone or a similar sequence) to facilitate ribosome function, and a plasmid growth selection sequence such as a limited kanamycin bacterial gene or a nonantibiotic selection sequence to maintain stable expression of the plasmid of interest during production.[15,17] The plasmid vaccine is delivered to the skin (intradermally) or muscle by one of several delivery methods. Once inside the cytoplasm of the cell, the plasmid enters the nucleus of transfected local cells (eg, myocytes, keratinocytes, or local resident antigen presenting cells [APCs]).[26–28] Inside these cells, the plasmid-encoded sequences drive host cell transcription, resulting in production of the foreign antigen in vivo. These host-synthesized antigens then become the subject of immune surveillance in the context of both MHC class I and class II molecules of the vaccinated host. There are specific differences between DNA vaccination and live infection. For DNA, antigen delivery remains local after plasmid transfection into cells, as there can be little spreading of antigen expression to other regions of the body. The plasmids themselves are not live but inert; they represent a focused antigen, epitope, or multiple antigens of the pathogen rather than the entire pathogen.

The exact details of DNA vaccine-induced immunity remain a subject of debate. Intramuscular (IM) injection is the most common mode of DNA vaccine delivery and has been shown to induce $CD8^+$ T-cell responses with more limited antibody production.[7] For IM injection, it is likely that myocytes are directly transfected after vaccination. Dendritic cells transfected in muscle have also been shown to efficiently cross-present antigens to activate CTL and may play a role in activating MHC I restricted T cells after intramuscular vaccination.[27] Alternatively, APCs in the muscle can be directly transfected and express antigen via the MHC I pathway to activate CTL.[29] In contrast to IM injection, intradermal (ID) administration has been reported to result in a more robust humoral response consisting of IgG1 antibody production.[4,30] The dermis is rich in APCs such as Langerhans and dendritic cells, which may enhance vaccine-induced immunity. ID vaccination, like IM vaccination, may result in APC transfection and antigen secretion, MHC class I presentation, or MHC class II presentation through cross-representation presentation. APCs are also constantly sampling the environment through endocytosis, resulting in the uptake of secreted antigen and expression, predominantly on MHC class II cells. Additionally, activated APCs express chemokines that may enhance immune cell trafficking of activated antigen-specific cells. Substantial work continues in this area.

Prime-boost emergence

The excitement around DNA vaccine studies in preclinical models quickly led to studies in humans. These first clinical DNA vaccine studies were initiated in the early 1990s. The goals of these studies were to evaluate the safety, tolerability, and immune potency of the platform. These studies examined diverse DNA vaccines for a variety of prophylactic and therapeutic applications, including first HIV-1,[31] influenza, cancer antigens, hepatitis B, and malaria,[32–36] and others.[37] Although the initial DNA vaccine studies in humans demonstrated excellent tolerability and safety,[15,37] the immune responses they elicited were weaker than expected on the basis of preclinical data, stimulating concerns about the technology's immune potency. These concerns drove the development of the important prime-boost field, which combined the immune focus of the DNA platform with the immune expansion power of live recombinant vaccine platforms.[38]

Highly attenuated live recombinant poxviruses, including NYVAC, the modified vaccinia Ankara (MVA), and the ALVAC canarypox-based vector,[39–43] and the important recombinant adenoviral platforms[44–46] were of great interest. Although these viral platforms induced strong antigen-specific cellular responses in preclinical models, preexisting poxvirus or adenovirus immunity has been reported to diminish the immune responses induced by immunization with the matching recombinant vaccine vector.[47–49] This limits the potency of these vectors in multiple-dose regimens. Furthermore, other issues regarding preexisting immunity are also possible and problematic.[50] As the DNA approach could induce a small but focused immune response that could be expanded by the subsequent boost with a recombinant vector encoding the same antigen as the DNA vaccine, this combination was viewed as a boon to the development of focused immune responses.

This strategy was first suggested by studies in a murine model of malaria, in which DNA priming followed by MVA or NYVAC boost induced higher CD8 T-cell responses than either platform delivered alone.[51,52] Schneider and coworkers, using a malaria vaccine, reported that priming with DNA and MVA led to enhanced immunity and greater protective efficacy than that achieved with either vaccine preparation alone.[51] These findings were quickly extended to many other DNA plus recombinant vector combinations.

Important SHIV/SIV (simian-human immunodeficiency virus or simian immunodeficiency virus) model vaccine studies using DNA priming followed by recombinant MVA boosts were reported to induce cell-mediated immune responses of impressive magnitude in nonhuman primates and result in decreased viremia after viral challenge.[53–57] Heterologous DNA prime-boost immunization became popular, as it presented a simple and available option to improve the immune response of two different vaccine platforms, resulting in a more robust level of vaccine-specific cellular and humoral immunity.

An early clinical evaluation of heterologous prime-boost vaccination by McConkey and colleagues is illustrative of these benefits.[58] This study of malaria antigen vaccination tested delivery of the antigen cassette in a plasmid, followed by ID delivery of recombinant modified MVA. The DNA-MVA combination was safe, and it induced cellular immune responses that provided partial protection against an irradiated-sporozoite malaria challenge in humans. DNA prime–pox-viral boosting is one preferred strategy, as well as boosting by adenoviral vectors and by recombinant protein antigens, among others. Studies by GeoVax (a biotechnology company) in collaboration with the HIV Vaccine Trials Network (HVTN) showed that DNA priming followed by MVA boosting in humans can drive high levels of CD4 T-cell responses against HIV antigens and some level of antibody responses, which has moved this vaccine program forward.[59] The NIH Vaccine Research Center (VRC) reported on a phase 1 trial using a DNA prime and a recombinant adenovirus serotype 5 (rAd5) vector as a boost, which demonstrated that this approach induced both cellular and humoral immune responses against HIV antigens.[49,60] Currently, a focused phase 2 study is evaluating this regimen of an HIV-1 DNA prime followed by a rAd5 boost for its ability to prevent infection or to reduce viral loads in study participants who become infected after vaccination.[61]

A possible advantage of a heterologous prime/boost regimen is induction of responses that differ from those induced by repeated dosing of either vaccine modality. Specifically, a report by Cox and colleagues[62] showed that the cellular responses to an HIV vaccine expressing Gag, Pol, and Nef in a heterologous DNA/rAd5 regimen induced a greater Gag-specific CD4 T-cell response than that induced by the homologous rAd5/rAd5 regimen in humans.[63] Furthermore, studies by Schneider and coworkers[64] and Robinson and colleagues[65] of heterologous DNA/poxvirus prime-boost immunization strategies found that T-cell responses generated with a heterologous DNA/poxvirus strategy produce immune responses 10 times higher than either platform given separately.[58,66,67] Taken together, these studies established that heterologous DNA prime-boost immunizations elicit immune responses of greater breadth than can be achieved by priming and boosting with the same vector. Overall, this strategy now has a central role in many vaccine efforts targeting diverse difficult pathogens.

Improved immune potency of the DNA platform

Many approaches have been taken to improve the immune responses induced by the DNA vaccine platform (Table 62-3). These include optimization of the promoter region and transcriptional elements in the plasmid backbone with the aim of improving antigen expression levels,[68–78] improved leader sequences,[79–85] and optimization of the plasmid backbone itself; development of improved gene sequences;[26,28,86] inclusion of molecular adjuvants in the formulation or as immune modulators; and development of a variety of next-generation delivery approaches.[87–98] Many of these areas have been reviewed,[15,99] and some are highlighted later.

DNA technology is highly malleable, which has contributed to systematically attacking basic platform questions, recently yielding a much-improved DNA vaccine immune profile. Major areas that have been addressed are plasmid construction and design, including the optimization of promoters and enhancer elements; polyadenylation;[68–78] incorporation of leader sequences;[79–85] vector design; antibiotic resistance and selection sequences; origin of replication choices for production; and efficient slimmer backbone designs deleted of extraneous DNA sequences, all of which can contribute to improved platform performance. An important consideration for increasing plasmid-driven immune potency involves sequence optimization.[18–25] Bacterial RNA is rich in AU sequences, whereas mammalian DNA is rich in GC. Therefore, the pool of tRNAs needed

Table 62-3 Some Major Approaches* in Plasmid Vaccine Optimization

Plasmid modification	Sequence modifications	Gene adjuvants and formulations	Delivery enhancement
• Promoter choice	• Modification of GC/AT content	• Molecular adjuvants	• Electroporation
• Backbone size	• Species codon optimization	• Cytokines	• Jet injector
• Enhancer elements	• RNA optimizations	• Chemokines	• Gene gun
• Transactivation sequences	• Strong Kozack start sequence	• Toll-like receptors	• Skin abrasion
• Internal termination sequence	• Leader sequence	• HSP	• Microneedle
• Poly AAA tract	• Termination sequence	• Costimulatory genes	• Topical patch
• Optimized ORI for production	• Localization sequences	• Transcription factors	• Needle-free systems
• Antibiotic selection sequence for stable production	• Glycosylation sequences	• Adhesion molecules	• Hydrodynamic delivery
	• Immunogen sequence optimization	• Formulations	
	• Epitope strings	• Alum	
	• Consensus	• Saponin	
	• Mosaic	• Nanoparticles	
	• Center-of-tree	• Liposomes	
	• Matrix immunogens	• Polymers	
	• Polyvalency or particle formation		
	• Localization sequences		
	• Designer immunogens		

*Alternative approaches to modification of plasmids, coding and noncoding sequence changes, formulation or adjuvanting, and delivery methods that can improve insert expression or immunogenicity are indicated.
HSP, heat shock proteins; ORI, origin of replication (site where DNA replication is initiated).

for translation in human cells is favored for sequences enriched in GC. Because of the redundancy in codon usage, unique transcriptional differences exist between bacteria, diverse viruses, parasites, and even host tumor antigens, which may benefit from attention to genetic design. DNA sequences can be codon-optimized to favor tRNA pools available in human cells, allowing the encoded mRNA to be more efficiently translated.

Perhaps an even more important modification is RNA optimization, where changes are made to the RNA sequence that do not affect the amino acid sequence of the final vaccine antigen. For example, sequences rich in GC are more likely to form secondary structures and slow translation, lowering in vivo protein production. RNA optimization also involves removing internal *cis*-acting motifs such as TATA boxes, repeat sequences that can cause instability, cryptic splice sites, and unwanted ribosomal binding sites. A combination of these and other gene optimization strategies can have a dramatic positive effect on protein expression and vaccine immunogenicity.[100–102] More recent studies have examined some of these improved approaches in the clinic. For example, a study of a West Nile virus vaccine DNA using an improved promoter induced relevant antibody responses in most of the 30 trial participants.[267] This study extended the findings reported in trials of severe acute respiratory syndrome (SARS) or Ebola plasmid vaccines, which resulted in positive serology induced by the vaccine, with lower T-cell responses.[103,104]

A major advantage of the DNA platform is the ability to rapidly customize the vaccine antigens with high precision to address specific vaccine design limitations. For example, the diversity in the HIV envelope sequence can reach greater than 15% within a subtype and greater than 30% between clades, and similar diversity issues plague influenza, hepatitis C virus (HCV), and malaria vaccine development, among others. Therefore, vaccination with a single viral sequence is unlikely to drive the diversity of responses necessary for cross-protection from the variety of sequences circulating in the population. Approaches to overcome these limitations combine computer predictions for immunogen design with synthetic chemistry to generate vaccine antigens that improve on nature. Important approaches include consensus antigen, ancestor gene, and center-of-tree designs;[105–108] mosaic antigens;[109–111] or epitope string approaches.[112–117] All of these approaches seek to focus the immune response induced by a synthetic gene cassette to specific epitopes or regions of a native antigen or an antigen predicted by computer analysis to be more conserved or invariant in the population. These strategies seek to maximize cross-reactivity of the T-cell responses induced against divergent strains of the pathogen. Consensus antigens, mosaic antigens, and epitope strings are currently being studied in the clinic, and these studies should be highly informative.

Molecular adjuvants

DNA vaccine approaches are particularly suited to deliver gene-encoded adjuvants to modulate the resulting immune response. As these adjuvants are derived from host genes with known biology, they allow an unprecedented level of insight into adjuvant choice. Unlike traditional adjuvants, molecular adjuvants are delivered as plasmid-encoded vectors as part of, or along with, the antigen-encoded vector.[83,118–128] On vaccination, the molecular adjuvant vector transduces cells at the site of vaccination that can then secrete the adjuvant molecule locally, thus coordinately and temporally targeting the same regional APCs and draining lymph nodes.

Granulocyte-macrophage colony-stimulating factor (GM-CSF), a white blood cell growth factor with considerable adjuvant properties, was one of the first gene adjuvants to clearly demonstrate that a cytokine plasmid could modulate DNA vaccine-induced immunity.[118] In mice vaccinated with a rabies virus antigen DNA, the addition of GM-CSF increased antibody production, $CD4^+$ T-cell responses, and protection after lethal challenge. Accordingly, GM-CSF became a widely studied DNA molecular adjuvant that has been examined in macaques and in human clinical studies of a malaria vaccine.[129] However, in human studies, the adjuvant effect was not as clear as in the initial animal studies. It remains under investigation as part of a new delivery format in prime-boost.[128]

Another cytokine gene, interleukin (IL)-12, has also received a great deal of attention as a DNA vaccine adjuvant.[120,124] IL-12 is a strong T-helper cell type 1 adjuvant that expands T-cell immunity, including $CD8^+$ T-cell function. In mouse models, IL-12 increased $CD8^+$ T-cell lysis of target cells 4.5 fold.[120] HIV-1 vaccination of nonhuman primates with IL-12 demonstrated increased cellular responses that corresponded with control of viremia and improved clinical outcomes after a SHIV98.6P challenge. The ability of IL-12 vector adjuvant to increase HIV-1-specific responses against an HIV-1 DNA vaccine is currently being studied in the clinic by the HVTN, where the adjuvant effect of plasmid-codelivered IL-12 with enhanced delivery appears promising.[125] Additionally, IL-2-Ig, a T-cell growth expansion factor, has shown positive results in mouse and macaque model systems and has been moved to human testing.[123] The ease and specificity of such combination adjuvant approaches in the DNA vaccine arena has generated an enormous amount of study of important cytokine genes, costimulatory molecules, chemokine genes, heat shock antigens, and other immune modulating molecules. It is likely that this area will continue to receive a great deal of attention, particularly on the basis of the early positive effects that are being reported in the clinic.

Enhancing in vivo delivery

As DNA vaccines are delivered without the benefits of a viral vector coat to facilitate host cell attachment and entry, they suffer intrinsically in their limited transfection efficiency, which compounds their similar lack of replication and spreading potential. Therefore, a major area of research has focused on improving DNA entry into target cells in vivo. To some extent, the efficacy of DNA vaccination can be improved by increasing the dosage of plasmid in humans. Whereas early studies in humans used dosages of 1 mg or less, dosages up to 8 mg have been used and shown to increase the consistency and frequency of antibody production.[103] Recently, more-concentrated formulations, with dosages as high as 12 mg, together with cytokine expression vectors and electroporation, have stimulated increased T-cell responses[130] and are promising approaches.

Formulations including novel transfection reagents were a major focus in other early studies. In addition, physical delivery has become an important area of research. An important area for DNA delivery is the formulation of DNA in or on biodegradable polymeric microparticles (see a review[95]) as well as in liposomes.[87,91,98] The applications of microparticle- and liposome-based delivery systems and DNA vaccine technology are well matched: their utility for delivery and enhanced immunogenicity in several different host and antigenic vaccine platforms has been shown in small animals[89,90,93,96] and nonhuman primates[87,94] and is being studied in humans in CMV and influenza applications. These compounds can have dual roles, facilitating plasmid entry into the cell as well as providing an adjuvant effect. Polyethyleneimine, amine-functionalized polymethacrylates, cationic poly (β-amino) esters, poloxamers, and polyvinylpyrrolidone polymers are some important examples of molecules that can enhance DNA vaccine immune potency in specific systems.[93,96] The poloxamer CRL1005 has demonstrated improved immune potency in preclinical models for simian HIV vaccines.[87,98] In fact, Vaxfectin, a related delivery molecule, has exhibited

enhancement of antibody responses to a DNA vaccine for influenza in recent clinical studies. In addition to polymers, ongoing studies have shown that liposome vehicles can improve DNA vaccine-induced immune responses. Studies in general support a greater effect on antibody responses than on improved T-cell responses. As liposomes have structural versatility with regard to the resulting vesicle surface charge (both cationic and anionic liposomes can be made), size, lipid content, and codelivery with other adjuvants, they offer the ability to be customized for specific DNA applications.[89,90,93–96] The formulation of DNA vaccines in polyamine gels or nanoparticles has also been reported to increase the uptake of plasmid vectors and increase antigen expression in vivo. There is a great deal of basic research in these areas. However, these approaches trail the liposome and polymer approaches in their clinical development trajectories.

A major area of research that is starting to bear fruit is combining DNA vaccines with physical delivery methods. The concept is simple—physically forcing more plasmid DNA into cells will improve expression. Many studies support this idea. Improvements in immune responses have been generated by several simple delivery devices such as the gene gun or biolistic jet injection.[131–140] Jet injection involves using high pressure to deliver a liquid formulation of DNA millimeters to centimeters below the skin's surface. The major limitations to clinical use of jet injection are the requirement for large amounts of DNA, and the DNA degradation from the high-pressure delivery. However, it is a relatively simple technique that has shown improved immune responses compared with needle and syringe in experimental model systems. Uniquely, the gene gun uses high pressure to deliver DNA-coated gold nanoparticles to the dermis. Although the gene gun has demonstrated high transfection efficiency and enhanced antibody responses in several species including humans, it has had relatively little effect toward improving cellular immunity.

Electroporation (EP) has been used to transfect cells in vitro for three decades and has more recently been used in vivo to increase the transfection efficiency of DNA vaccines.[17] EP involves applying a small electric field across the site of injection to cause temporary membrane instability and create an electric gradient, which increases plasmid uptake by cells in the field.[18,24] This technique has been studied for two decades as a method to improve delivery of chemotherapy drugs to kill specific tumor cells.[132–134] It has been studied in many animal species (e.g., dogs, pigs, cattle, nonhuman primates) for delivery of genes that encode a variety of hormones, cytokines, enzymes, or antigens.[131,135–137,141] However, the conditions required for EP were considered too harsh for adoption as a prophylactic modality. Over the past 10 years, EP technology has been much developed, leading to devices capable of performing intramuscular, intradermal, and microneedle transfection in vivo.[20,21] One exciting development is that in large-animal models, EP has increased both cellular and humoral immune responses,[22,23] and when combined with other optimization approaches, the magnitude of the immune responses generated by the combined DNA approach has increased more than a log.[100,141–147] EP technology can also be fine tuned to a particular DNA vaccine modality, as devices that control different current, voltage, and timing settings are being developed. Combinations with molecular adjuvants look highly promising,[125,148,149] and delivery to the skin and muscle is also being tested. Importantly, EP has been safely studied in the clinic for many years. More work is needed in this area, but the data in the primate models and the early reports from the clinic appear very encouraging.

Clinical studies

The initial movement of DNA vaccines into the clinic demonstrated the safety and manufacturing advantages of the platform, but the limitations of the platform for induction of stand-alone immune responses were also apparent. Many initial safety concerns have now been addressed. It appears that the risks associated with DNA vaccines are relatively low compared with those of some other approaches. An initial fear of DNA vaccines was the risk of integration and activation of oncogenes or inactivation of tumor suppressor genes. However, studies have demonstrated that the rate of DNA integration in vivo is actually three times lower than the rate of spontaneous mutagenesis. In addition, no negative effects such as induction of autoimmunity or transfer of antibiotic resistance markers have been observed. With the renewed clinical interest in this approach, thousands of volunteers have received DNA vaccines without a single significant adverse event having been reported.

A slew of technologic improvements as well as prime-boost approaches have reinvigorated clinical studies of DNA vaccine technology. In fact, the number of DNA vaccines being tested in clinical studies compared with all other recombinant platforms has increased from just 4% 10 years ago to almost 20% of all such trials as of January 2012. Some of these important trials and their immune targets are highlighted in Table 62-4. One area that can serve as a barometer of the relevance of an experimental platform is HIV vaccine trials. As of January 2012, 11 phase 1 and 2 clinical trials sponsored by the HVTN are ongoing to assess the ability of DNA to elicit immune responses and ultimately prevent HIV infection, either alone or in combination with multiple viral vectors. One of the most prominent trials, HVTN 505, is a phase 2 study enrolling 2,200 subjects to

Table 62-4 Current DNA Vaccine Clinical Trials as of January 2012

Phase	Approach	Trials (*N*)	Vaccine targets
1	DNA alone	20	Cancers (breast, ovarian, cervical, lymphoma), human immunodeficiency virus (HIV) treatment, malaria *(Plasmodium falciparum)*, hepatitis B, influenza
	Prime/boost*	7	HIV prevention, influenza
1/2	DNA alone	5	Cancers (prostate, melanoma, colorectal), hepatitis B, hepatitis C
	Prime/boost†	1	Malaria *(P. falciparum)*
2	DNA alone	8	Cancers (cervical, prostate, leukemia/lymphoma), HIV treatment, hepatitis C, cytomegalovirus
	Prime/boost‡	3	HIV treatment, HIV prevention

*In phase 1 clinical trials for HIV vaccines, the DNA prime was followed by a boost using one of several viral vectors: Ad5, Ad35, or modified vaccinia Ankara (MVA). The influenza vaccine approach incorporates a DNA prime and an inactivated virus boost.
†In the phase 1/2 malaria vaccine trial, the DNA prime is followed by an Ad5 boost.
‡In phase 2 clinical trials investigating HIV vaccine approaches, the DNA prime is followed by an Ad5 or MVA boost.

test the safety and efficacy of a DNA vector prime followed by an adenovirus serotype 5 (Ad5) boost vaccination strategy developed by the VRC. Another important phase 2 study is being conducted by GeoVax through the HVTN to evaluate the efficacy of a DNA prime followed by a MVA virus boost.[59] An alternative clinical approach uses a DNA prime to develop T-cell responses as well as to prime for B-cell immunity, followed by a recombinant protein boost to generate more-potent antibody responses. Preliminary data from these studies are encouraging. An important HIV study of a stand-alone DNA product is being tested under protocol HVTN 080. This study of multiply optimized DNA plus an IL-12 adjuvant is testing a particular formulation (Pennvax) of DNA. Preliminary data from this study have shown clear induction of T-cell immunity in the absence of a viral vector boost. It will be important to see if the trend showing improved immune responses in the clinic induced by EP strategies continues, which would expand the applications of the DNA platform.

In addition to research on HIV, there is active research into the study of DNA vaccine technology for both the prevention and immune therapy of cancer, HCV, and CMV infections. In the United States, there are currently more than 20 active DNA vaccine studies in humans for various forms of cancer, including one phase 3 immunotherapy trial of Allovectin-7, a DNA/liposome complex encoding an allogeneic MHC gene[150] in metastatic melanoma, and at least four trials advancing into phase 2 studies. The phase 2 studies include two analyses of therapeutic vaccination in men with prostate cancer, one for melanoma, and one for colorectal cancer. In two of the four studies, intradermal electroporation is being used to increase immunogenicity. An important phase 2 study is being conducted to determine the ability of a therapeutic CMV vaccine, Transvax, a poloxamer-formulated, bivalent DNA vaccine that contains plasmids encoding hCMV tegument phosphoprotein 65 and the major hCMV surface glycoprotein B, to produce safe immune responses and provide clinical benefit in CMV-seropositive recipients undergoing allogeneic, hematopoietic cell transplant. Results from these studies will have a broad impact on the field.

Although a successful human clinical product has yet to be achieved, it is clear from the preceding discussion that the technologies that are driving the field, as well as interest in the field, are once again increasing (see Table 62-4). The progress of clinical trials will have to be closely watched, as they will present an exciting picture over the next few years as the first efficacy trials of the DNA approach come to fruition.

Another relevant area where there has been commercial success with DNA technology is animal health. Products licensed for veterinary use[151–154] include a dog melanoma immune therapy, a porcine recombinant growth hormone, a vaccine for prevention of rhabdovirus disease in fish, and West Nile virus vaccine for horses based on successful field trials of DNA vaccines. The risks associated with DNA vaccinations appear low, both conceptually and now in practice. Thousands of clinical volunteers have received DNA vaccines without a significant adverse event having been reported. The important safety record, growing consistency of immune responses in the clinic, and the successful licensure of veterinary products suggest that DNA vaccination is poised to become an important and safe platform for continued vaccine development.

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 advanced to preclinical and clinical studies (see Figure 62-1). Depending on their ability to target APCs, their ability to develop packaging lines, the inherent immunogenicity of both the vector and insert, and other factors (see 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 have now demonstrated immunogenicity and protective immunity in a variety of animal models. These viruses have been genetically modified so that they can deliver and express specific recombinant gene products but are unable to grow on their own and are hence replication-defective. Like DNA vaccines, these vectors transduce cells that can synthesize native gene products, and they appear to be quite potent in their ability to induce not only helper but specifically CTL immunity. The majority of clinical vectors have been derived from Ad5, although more than 51 human serotypes in six subfamilies (A-F) are known. Ad5 is derived from the C subfamily and is the most 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.

Preexisting anti-Ad5 immunity may inhibit the response to rAd5 vaccine immunization, so alternative serotypes and chimeric vectors have been developed to circumvent this. The attraction to rAd5 for immunization has followed from its success with a variety of preclinical animal models and with human trials in phase 1 or 2. With respect to animal models, the replication-defective adenovirus has been shown to elicit potent immune responses and protection against Ebola virus, administered either alone as a single injection, or in prime-boost combinations.[155,156] The prime-boost approach induces a more potent and durable immunity, desirable for a preventive vaccine in routine use, whereas a single rAd vaccination induces a more rapid response that is sufficient for immediate protection (Figure 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 rAd5 vaccines and DNA prime/rAd5 boost combinations have been shown to confer partial protection in rhesus macaques against multiple HIV isolates, including SHIV-89.6P,[31,157] SIVmac239,[158] and SIVmac251.[159–161] Replication-defective adenovirus has also been used in a variety of additional animal models of infectious disease, including plague, anthrax, influenza, and malaria.[45]

Phase 1 and 2 clinical studies with replication-defective adenoviral vectors for HIV-1 have been developed independently by the Merck research laboratories and the NIH VRC in the National Institute of Allergy and Infectious Diseases. The Merck vaccine used only rAd5 vectors and encoded the *Gag, Pol,* and *Nef* genes of HIV-1. A clinical efficacy study of this product, called the STEP trial, began in 2005; it evaluated the effect of vaccine-induced T-cell responses to these gene products internal to the virus on controlling viral load. Despite the immunogenicity of this vaccine, no reduction in acquisition or long-term control of postinfection viremia was observed.[162] Further analyses revealed that persons with specific human lymphocyte antigen (HLA) types, as well as those who developed a $CD8^+$ T-cell response to certain Gag and Nef HIV epitopes, selected against viruses that contained the vaccine epitope in vivo.[163] There was also an unexpected association between infection in vaccine recipients and those who were both uncircumcised and immune to Ad5 before vaccination. The rate of HIV infection during the first 18 months after the immunization regimen was higher in this subgroup. This association waned over time, and the mechanism and broader clinical significance of this finding remain unknown.

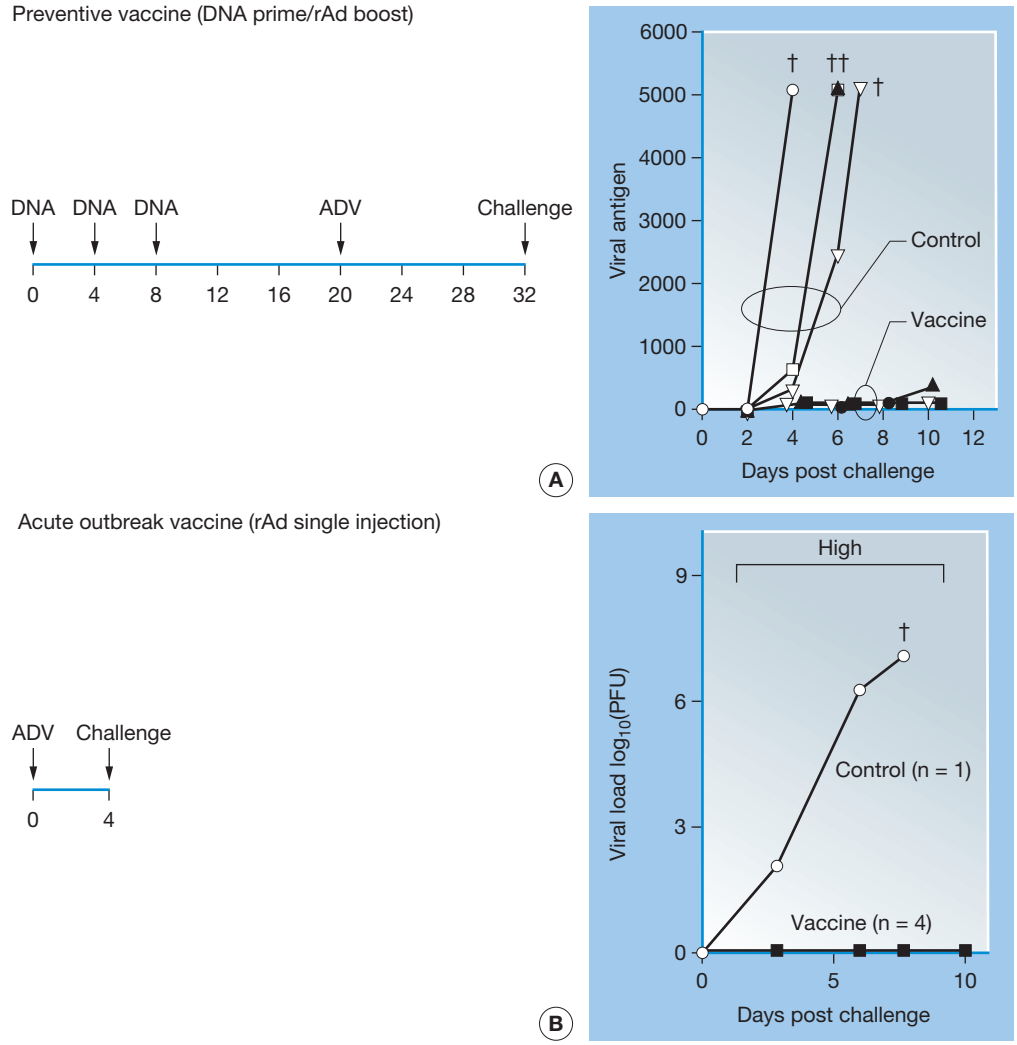

Figure 62-2 Prime-boost versus single shot in models of an Ebola virus vaccine. Alternative approaches for vaccination can be used depending on the intended use of the vaccine. A, In the nonhuman primate challenge model, a DNA prime with recombinant adenoviral vector (rAd) boost of GP and NP genes confers protection in a lethal challenge model 8 months and longer after the initial immunization. B, In contrast, a single shot of an rAd vector encoding these genes stimulates a less potent immune response, but this rapid vaccination produces sufficient immunity to be useful during an acute outbreak. Symbols indicate individual subjects from experimental vaccine and control groups as labeled. The time frame shows the week of vaccination with DNA or ADV vector and subsequent challenge (left). (Adapted from refs. 155 and 156.)

The clinical utility of the DNA prime/rAd5 boost vaccines has yet to be defined, although they have proved more efficacious in animal models of SIV challenge. In addition, the current vaccine contains Env constituents not included in the Merck Ad5 vaccine, and these clinical vaccine candidates differ in multiple ways. The ongoing phase 2B HVTN 505 clinical trial will provide further insight into its potential efficacy.

The effect of preexisting 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 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. Although both cellular and humoral immune responses contribute to anti-Ad5 immunity, it is likely that the Ad5 neutralizing antibodies play a major role in suppressing rAd5-induced immunogenicity, and such immune responses have been observed in humans. This preexisting immunity can reduce the immunogenicity of Ad vaccines in mice,[164,165] rhesus monkeys,[166] and potentially in humans,[167,168] but it is not clear that preexisting immunity in humans will completely 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 reduce its immunosuppressive effects, too.[164,165] 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.[169] However, the safety of this approach, particularly for intranasal delivery, has yet to be determined.[170] In addition, several investigators have explored the possibility of coating rAd5 particles with chemicals such as polyethylene glycol, which may block access of antibodies to the viral surface.

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,[171–174] or through the use of hexon chimeras, which appear to be the targets of the major neutralizing antibody response.[175,176] 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.[177–179] 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[180] 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.[48,181] The usefulness of these vectors has been compared with rAd5. Although some of the alternative vectors show less seropositivity, they are often also less immunogenic in preclinical animal studies. There also appear to be regional differences in seropositivity to diverse "rare" serotype rAd vectors. For example, although the rAd26 and rAd28 B serotypes have shown promise in early clinical trials and their seroprevalence is low in North America, seropositivity to these viruses approaches 80% in parts of Africa,[182,183] complicating development and regulatory issues for such vectors.

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.[184,185] These vaccines not only offer 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, macaques, and chimpanzees.[186–195] 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 usefulness 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 best documented examples of vaccination against infectious diseases. On the basis of safety issues recognized from the experience of using vaccinia strains against smallpox,[196–199] 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 smallpox vectors. These strains are summarized in Box 62-1. 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 been explored extensively in a variety of infectious disease models.

Box 62-1: Poxvirus strains used as immunization vehicles

- Vaccinia virus
- New York vaccinia strain (NYVAC) (18 ORFs deleted)
- Modified vaccinia Ankara (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.
From Sandhu DS, Tartaglia J. Poxviruses as immunization vehicles. In Plotkin SA, Orenstein WA, Offitt PA, eds. Vaccines. Philadelphia, PA: WB Saunders; 2004.

One of the two major attenuated strains of poxvirus is modified vaccinia Ankara, developed by repeated passaging of the Ankara strain on primary chicken embryo fibroblasts (CEFs), originally developed as a safer alternative to vaccinia virus as a vaccine against smallpox. This resulted in the ability of the virus to replicate efficiently on a variety of nonavian cell types because of multiple genetic changes, which facilitates its propagation and use as a vector. A second 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.[200–203] NYVAC, like MVA, is attenuated in animal models and shows favorable safety and immunogenicity in animals and humans.[201,204,205] 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 CEFs.

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.[206] ALVAC is able to express inserted transgenes and has been shown to be immunogenic in both animal and early clinical trials.[204,205,207–210] These vectors have been evaluated both alone and in prime-boost combinations in a variety of infectious disease and cancer models (see review[204]). 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 proved useful in a variety of infectious disease models, including rabies, measles, SIV, canine distemper, respiratory syncytial virus, malaria,[51,211] and influenza.[212] In addition, these vectors have been studied in a variety of HIV challenge models, both in preclinical studies and in humans,[39,213–217] and human studies have been undertaken with vaccinia,[218–226] NYVAC,[227–230] and ALVAC.[227,228,230–235]

These vectors have advanced into efficacy studies in humans. The ALVAC-EnvGag/Pol(clade B and AE) was evaluated in combination with gp120 protein boosting in a phase 3 study, RV144, in Thailand. The RV144 trial demonstrated a 31% reduction in the frequency of acquisition of HIV infection among vaccinated heterosexual men and women compared with the control placebo group.[236] The RV144 study represented a landmark trial, providing a proof of concept that a vaccine could prevent HIV-1 infection, although the degree of efficacy was modest and requires improvement to attain significant public health benefits.

Such poxvirus vectors have also been evaluated in cancer immunotherapy protocols. Although attenuated poxvectors have been evaluated in a variety of human studies, it is clear that developing these vaccines for use in humans has been challenging. In part, this may be because 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, as seen with rAd, the concern of antivector immunity remains for this virus as well, although it may be a lesser concern for canarypox vectors.

Poxvirus vectors show thermostability, an ability to incorporate a large foreign transgene, a lack of persistence or genomic integration, and a demonstrable success in smallpox eradication. However, the difficulties in manufacturing virus in high yields from primary CEFs, as well as their antigenic complexity, reactogenicity and poor immunogenicity, have limited their usefulness 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 (AAVs) 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 an insert size of approximately 5 kb. Like 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,[237] and an HIV vaccine expressed in AAV2 has been analyzed in phase 1 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. These vectors have also been used recently to deliver recombinant antibody genes that protect against viral infection,[238] raising the intriguing possibility that gene-based antibody delivery might be used to generate protective immunity.

Vectors in development

Alphaviruses are negative-stranded RNA viruses that can be modified to express foreign recombinant genes without producing pathogenic infections often seen with prototypes such as Venezuelan equine encephalitis virus,[239,240] Sindbis virus,[241,242] and Semliki Forest virus. Replication-defective herpes simplex virus (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 to be vectors against HSV itself, including both HSV1 and HSV2.[243] More recently, vesicular stomatitis virus, dengue virus type 4, yellow fever virus, and alphavirus have been modified to express heterologous viral genes for vaccines for infectious disease targets including HIV, West Nile virus, filoviruses, CMV, and other pathogens.[244–251]

Cell substrates

The progress of more recent viral vectors has depended on 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 proved invaluable in facilitating this effort. For recombinant adenoviral production, the PERC6 and GV11 cell lines have supported production of clinical-grade adenovirus type 5, and these have progressed into trials for HIV and are under study for other infectious agents, such as Ebola virus, Marburg virus, tuberculosis, and malaria. 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, Venezuelan equine encephalitis 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 CEFs, represents a major advance in vaccine production technology, largely because these 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 these lines has taken years to implement because of regulatory concerns regarding adventitious agents, tumorigenicity, and other safety and consistency considerations. Oversight and evaluation of the strengths and limitations of these cell substrates continues,[252] based on guidelines created several years ago,[253,254] 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 both on their ability to induce mucosal IgA responses and on 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 in 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[255–257] and are summarized briefly here.

Among the live bacterial vectors used for antigen delivery, there are mucosal pathogens that have been attenuated, including strains of *Listeria monocytogenes, Salmonella, Vibrio cholera, Shigella, Mycobacteria bovis, Yersinia enterocolitica,* and *Bacillus anthracis.* In addition, there are commensal strains such as *Streptococcus gordonii,* lactobacilli, and staphylococci that have been used for the induction of humoral and cellular responses. For gene-based vaccination, *L. monocytogenes* has been a particular focus of research. This gram-positive intracellular pathogen has been studied as a model for understanding class I MHC-restricted immune responses. These responses are normally seen against the bacterial proteins or coexpressed antigens. This microorganism uses 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.[258] 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, *M. bovis* Calmette-Guérin (BCG) has been a widely used bacterial vaccine; for example, this organism has been used to express HIV antigens.[259,260] In some instances, expression of mammalian genes has required modification of codons more consistent with the host cell type, which has improved immunogenicity. At present, however, the ability of such microorganisms to induce cellular immunity is 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; organisms such as *Shigella* and *Listeria* have been used for this purpose.[261,262] In addition, attenuated *Salmonella* has been evaluated for these purposes and has shown some promise in both infectious disease and tumor models in experimental animals.[263–265]

Although 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, and the possibility that preexisting 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 will be necessary to improve the efficacy of this approach, which remains limited in its present form.

Clinical applications of gene-based vector technology

Although 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, MVA, and NYVAC, which have been evaluated in various phase 1 to 3 human studies. Because the production technology for poxviruses is well known, and good-manufacturing-practice 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 MVA, which has been evaluated both as a vaccine for HIV (alone and in prime-boost combinations) and as a potentially safer next-generation vaccine for smallpox.

Additionally, DNA vaccines have undergone phase 1 testing for a variety of infectious diseases, including Ebola virus, West Nile virus, the SARS coronavirus, and influenza virus. Proof-of-concept efficacy studies with these viruses have been performed first in animal models with either DNA or in prime-boost combinations. In such studies, impressive protection has been demonstrated.[155,266] Based on these findings, several phase 1 trials have been completed for Ebola, SARS, and West Nile virus disease targets.[103,104,267]

In the case of influenza, both naked DNA and DNA adjuvanted with gold microparticles (by biolistics) have advanced into clinical testing. Of particular interest is the development of prime-boost strategies to stimulate the production of broadly neutralizing antibodies to influenza viruses, demonstrated initially in mice, ferrets, and monkeys.[268] Phase 1 studies testing this concept in humans have revealed that even a single injection of a DNA vaccine can prime for an effective traditional vaccine boost against the H5N1 virus. This regimen also showed that more broadly neutralizing anti-stem antibodies can be elicited by vaccination in humans.[269]

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,[270] and a DNA vaccine for infectious hematopoietic necrosis virus, developed by Merieux 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.[271] A recombinant yellow fever vaccine has advanced into efficacy studies as well.[272] 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.

Access the complete reference list online at http://www.expertconsult.com

3. Tang DC, DeVit M, Johnston SA. Genetic immunization is a simple method for eliciting an immune response. Nature 1992;356:152–4.
5. Ulmer JB, Donnelly JJ, Parker SE, et al. Heterologous protection against influenza by injection of DNA encoding a viral protein. Science 1993;259:1745–9.
6. Wang B, Ugen KE, Srikantan V, et al. Gene inoculation generates immune responses against human immunodeficiency virus type 1. Proc Natl Acad Sci U S A 1993;90:4156–60.
58. McConkey SJ, Reece WH, Moorthy VS, et al. Enhanced T-cell immunogenicity of plasmid DNA vaccines boosted by recombinant modified vaccinia virus Ankara in humans. Nat Med 2003;9:729–35.
87. Nabel GJ, Nabel EG, Yang Z-Y, et al. Direct gene transfer with DNA-liposome complexes in melanoma: expression, biologic activity, and lack of toxicity in humans. Proc Natl Acad Sci U S A 1993;90:11307–11.
150. Plautz GE, Yang Z, Wu B, et al. Immunotherapy of malignancy by in vivo gene transfer into tumors. Proc Natl Acad Sci U S A 1993;90:4645–9.
160. Letvin NL, Mascola JR, Sun Y, et al. Preserved CD4 + central memory T cells and survival in vaccinated SIV-challenged monkeys. Science 2006;312:1530–3.
162. Buchbinder SP, Mehrotra DV, Duerr A, et al. Efficacy assessment of a cell-mediated immunity HIV-1 vaccine (the STEP Study): a double-blind, randomised, placebo-controlled, test-of-concept trial. Lancet 2008;372:1881–93.
166. Casimiro DR, Chen L, Fu TM, et al. Comparative immunogenicity in rhesus monkeys of DNA plasmid, recombinant vaccinia virus, and replication-defective adenovirus vectors expressing a human immunodeficiency virus type 1 gag gene. J Virol 2003;77:6305–13.
236. Rerks-Ngarm S, Pitisuttithum P, Nitayaphan S, et al. Vaccination with ALVAC and AIDSVAX to prevent HIV-1 infection in Thailand. N Engl J Med 2009;361:2209–20.
269. Ledgerwood JE, Wei CJ, Hu Z, et al. VRC 306 Study Team. DNA priming improves influenza vaccine immunogenicity: two phase 1 open label randomised clinical trials. Lancet Infect Dis 2011;11:916–24.