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67 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 as well as in eliminating pathogenic cells. 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 modulate the induction and expansion of cytotoxic T lymphocytes (CTLs). CTLs recognize processed antigen in combination with major histocompatibility complex (MHC) molecules and release cytokines that can influence pathogen replication as well as lyse infected cells. In addition, T-regulatory cells are induced as part of an immune response and can play a negative role in the clearance of chronic infectious agents and in preventing clearance of pathogenic cells.

Both humoral and cellular immunity are elicited by vaccines, each with their own effector functions that can inactivate pathogens in different ways (Box 67.1). 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 CTLs have provided the foundation for understanding MHC restriction and its importance in protection against viral infection.[1,2] Cellular immune responses help control infectious diseases, in concert with antibody responses or when it is difficult to generate neutralizing antibodies, as in patients with AIDS, malaria, hepatitis C virus, or tuberculosis. Humoral immunity is readily induced by protein antigen preparations or inactivated viruses together with appropriate adjuvants. Gene-based vaccines, the engineering of nonviral RNA- or DNA-based systems, or viral recombinant systems to express foreign antigens when delivered to a host 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, or through enhanced delivery and formulation. A variety of vectors, nonviral and viral, have been developed for these purposes (Fig. 67.1), the most studied with a focus of being in or entering the clinic are reviewed here.

The majority of conventional adjuvants that have been previously used in vaccine development affect humoral immunity and 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 in clinical trials are discussed, together with the advantages and disadvantages of the individual vectors and their influence on the different effector arms of the immune system. Although there is considerable experience with inactivated viruses and protein-based vaccines, there is less experience with gene-based vaccine vectors as they are more recent developments. Their ability to induce both humoral and cellular immunity and their safety and mode of antigen presentation are attractive features that must be balanced with the limitations in knowledge about clinical efficacy, production methodology, and concerns about antivector immunity (Box 67.2). However, there is a growing clinical experience that is guiding the analysis of these approaches as well as recent compelling vaccine efficacy data. Based on their early successes and advantages in conceptual development, it is likely that these vectors will make important future contributions to vaccinology.

NONVIRAL VECTORS AND DNA VACCINES

Vaccination with plasmid DNA generated excitement in the early 1990s when four independent groups simultaneously reported that plasmid gene delivery resulted 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 into mice.[3] The animals generated antibody to growth hormone with this approach. Other groups also attempted 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 demonstrated the generation of antigen-specific immunity.[4,5] In addition, David Weiner (University of Pennsylvania) reported that formulations of plasmids containing either HIV envelope or tumor antigens were capable of stimulating both cellular and humoral immune responses in mice or in the case of the tumor antigen formulations, impacting tumor growth.[6] These novel results, combined with the simplicity and ease of manufacturing, spawned research into DNA vaccines for a plethora of viral, bacterial, parasitic, and cancer targets.[7–12]

At this time the DNA vaccine platform was perceived by the scientific community to be an important new approach, 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, it combines the simplicity of synthetic chemistry or bacterial production with the power of genomics, and it allows the rapid design and construction of multiple potential vaccines by removing entirely the need to develop vaccines using pathogen-derived materials. In addition, DNA vectors are extremely stable, reducing the need for a cold chain and increasing product shelf life. Therefore, the ease of production, stability, and cost-effectiveness of this platform made it ideal for producing vaccines for the developing world. DNA vectors themselves are not immunogenic, which allows repeat administration without developing immune interference. Importantly, DNA vaccines combine the appeal of live replicating vaccines that induce broad cellular and humoral immune responses with the safety and ease of manufacturing of a nonlive, nonspreading platform.[15,16] As DNA vaccines are nonreplicating, they eliminate the risk of attenuation reversions in the host, dissemination in the recipient, the unintended consequences of secondary infections by transfer to unvaccinated populations, and they can be delivered to high-risk groups, including immunocompromised subjects. In the clinic, DNA vaccines

BOX 67.1 Mechanisms of Immune Protection by the Adaptive Immune Response

CELLULAR

- 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

HUMORAL

- 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 immunoglobulin A to facilitate mucosal elimination of pathogens

BOX 67.2 Advantages and Limitations of Gene-Based Vectors for Vaccines

ADVANTAGES

- Potent immunogenicity in animal and human infectious diseases
- Ability to induce cellular immunity with or without humoral immunity
- Relative ease of production for many viral and nonviral vectors
- Ease of analysis and screening in the laboratory
- Favorable safety profile and lack of persistence in vivo
- Efficient transduction of cells and reasonable production capability
- Many potential prime-boost combinations

LIMITATIONS

- High level of immunity to some vectors in humans
- Need for qualified packaging cell lines
- Induction of antivector immunity after initial injection of viral vaccines, limiting efficacy of homologous boost
- Potential complexity with multiple vectors in prime-boost
- Limited long-term safety data
- Need to develop large-scale manufacturing processes

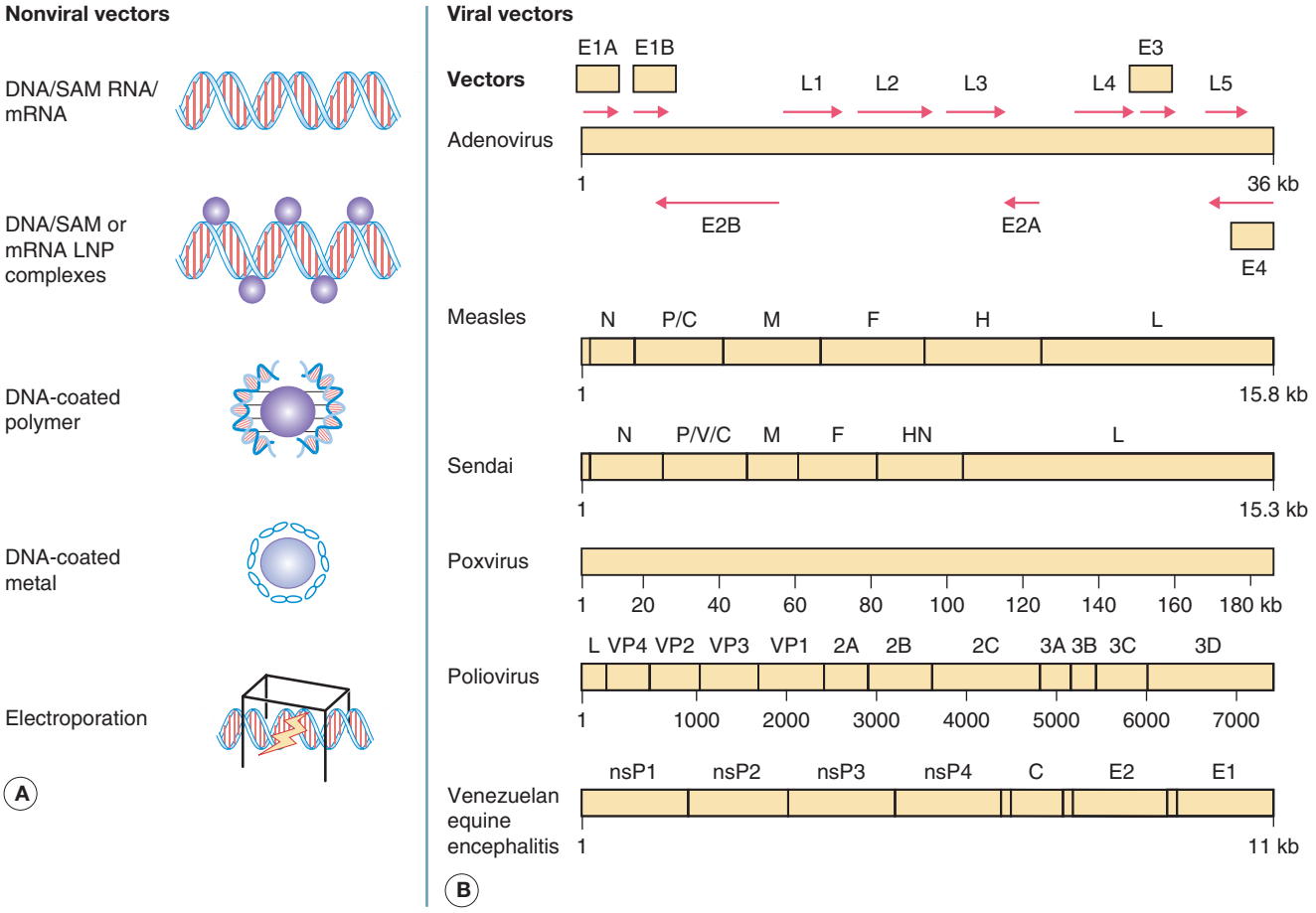

Figure 67.1. Representative vector platforms for gene-based vaccines that have advanced into clinical trials. Vaccination by gene delivery with nonviral **(A)** and replication-defective recombinant viral gene-based **(B)** vectors are shown. **B** shows the genetic organization and virus structure of the natural replication-competent virus.

have provided an unparalleled safety profile over the past decade and a half of study.[17] With some constraints, DNA vaccine products can be developed in repeat-use manufacturing facilities, thus providing enormous savings in product development and manufacturing.

Mechanism of Action

The mechanisms surrounding the generation of antigen-specific immunity with gene-based vaccines are important to understand. An antigen sequence of interest is designed to target a particular antigen or set of antigens of a pathogen or tumor antigen, potentially subjected to additional modifications (as described below), and inserted into a mammalian expression plasmid vector.[18–25] For use in humans, such vectors often share common features, including a high-copy-number origin of replication to enhance production, a human cytomegalovirus immediate-early promoter to drive in vivo expression, an RNA polyadenylation sequence that can be derived from bovine growth hormone or created synthetically to facilitate ribosome function, and a plasmid growth selection sequence such as a limited kanamycin bacterial gene or a nonantibiotic selection sequence to monitor the expression of the plasmid of interest during production.[15,17] The plasmid vaccine is then delivered to the skin by intradermal injection or intramuscularly by one of several delivery methods. Once inside the cytoplasm of the cell, the plasmid enters the nucleus of transfected cells (e.g., myocytes, keratinocytes, or local resident antigen-presenting cells [APCs]),[26–28] where the plasmid-encoded sequences drive host cell transcription, producing the foreign antigen in vivo. The 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 very specific differences between DNA vaccination and infection with the pathogen that the vaccine is intended to prevent. With DNA vaccines, antigen delivery remains local after plasmid transfection into cells, with little spreading of antigen expression to other regions of the body. The plasmids themselves represent a focused antigen, epitope, or multiple antigens of the pathogen and not the entire pathogen capable of dissemination.

The exact details of DNA vaccine-induced immunity remain a subject of debate. IM injection of the DNA vaccine has been shown to induce $CD8^+$ T-cell responses with more limited antibody production.[7] After IM injection, myocytes are likely transfected and dendritic cells present in the transfected muscle efficiently cross-present antigens to activate MHC class I–restricted T cells.[27] Alternatively, APCs in the muscle could be directly transfected and express antigen via the MHC class I pathway to activate CTL.[29] In contrast, intradermal (ID) administration has been reported to result in a more robust humoral response consisting of immunoglobulin (Ig) G_1 antibody production.[4,30] Because the dermis is rich in APCs, such as Langerhans and dendritic cells, ID vaccination, could result in APC transfection and antigen secretion, with either MHC class I or MHC class II presentation. APCs are also constantly sampling the environment through endocytosis, resulting in the uptake of secreted antigen predominant expression on MHC class II cells. It is thought that plasmid-encoded DNA triggers immune activation through stimulation of innate immune sensors, which include PAMPS (pathogen-associated molecular patterns) or the STING (stimulator of interferon genes)–TBK1 (TANK-binding kinase 1) pathways.[31,32] Such activated APCs express chemokines and cytokines that enhance immune cell trafficking and inflammation.

Animal Studies

Several DNA vaccines have been licensed for use in animals,[33–36] including dog melanoma immunotherapy, porcine recombinant growth hormone, vaccine for the prevention of rhabdovirus disease in fish, and West Nile virus vaccine for horses. These promising outcomes have yet to be translated to humans.

Prime-Boost Emergence

The success in preclinical models by DNA vaccines led to clinical studies in humans, initiated in the early 1990s. The goals of these human studies were to evaluate the safety, tolerability, and immune potency of the platform. Diverse DNA vaccines for a variety of prophylactic and therapeutic applications were studied, including HIV-1,[37] influenza, cancer antigens, hepatitis B, malaria,[38–42] and others.[43] Although the initial DNA vaccine studies in humans demonstrated excellent tolerability and safety,[15,43] the immune responses they elicited were much weaker than expected based on the preclinical data. Concerns about the ability of the technology to stimulate robust immune responses led to the development of the prime-boost strategy which sought to take advantage of properties of both DNA as well as recombinant viral vectors when combined resulting in focused immunity to the transgene (see below).[44]

Highly attenuated live recombinant poxviruses, including NYVAC, the modified vaccinia Ankara (MVA), the ALVAC canarypox-based vector,[45–49] and recombinant adenoviral platforms[50–52] were of great interest. Although these viral platforms induced strong antigen-specific cellular responses in preclinical models, preexisting poxvirus or adenovirus immunity diminished the immune responses induced by immunization with these recombinant vaccine vectors,[53–55] limiting the potency of the vectors in multidose regimens. Furthermore, other issues regarding preexisting immunity are also possible and problematic; these are further discussed below.[56]

Early DNA approaches induced small but focused immune responses that could be expanded with subsequent boosting with a recombinant vector encoding the same antigen as the DNA vaccine, with the prime boost strategy providing a more robust immune response.

This strategy was first suggested by studies in a murine model of malaria, in which DNA priming followed by MVA or NYVAC boost consisting of specific preerythrocytic antigens of *Plasmodium berghei*, induced higher CD8 T-cell responses than either platform delivered alone.[57,58] Schneider and coworkers, using a malaria vaccine, reported that priming with DNA and boosting with MVA vectors encoding the same antigen, led to enhanced immunity and greater protective efficacy than that achieved with either vaccine preparation alone.[57] These findings were quickly extended to many other DNA and recombinant vector combinations.

Important SHIV (simian-human immunodeficiency virus)/SIV (simian immunodeficiency virus) model vaccine studies using DNA priming followed by recombinant MVA boosts were reported to induce cell-mediated immune responses of improved magnitude in nonhuman primates and result in decreased viremia after viral challenge.[59–63] Accordingly, these heterologous-DNA-prime-followed-by-viral-vector-boosting for immunization became popular, as they 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.[64] This study of malaria antigen vaccination tested vaccination with a plasmid antigen cassette encoding

a preerythrocytic malaria antigen, thrombospondin-related adhesion protein (TRAP), followed by ID delivery of recombinant modified MVA containing the TRAP antigen as well. The DNA–MVA combination was safe, and induced cellular immune responses that provided partial protection against an irradiated-sporozoite malaria challenge in humans.

Studies by GeoVax (a biotechnology company) using HIV antigen cassettes showed that DNA priming followed by MVA boosting can be attractive as a combined vaccine modality.[65] The authors studied DNA and recombinant MVA HIV antigen vaccines which encode Gag, protease, reverse transcriptase, and the native, membrane-bound trimeric forms of envelope (Env) as vaccine antigens. They reported that DNA priming followed by recombinant poxviral boosting resulted in superior CD4 and CD8 T-cell immunity than poxviral vaccination alone. A follow-up study by these same investigators compared the data from an HIV gp120 env protein vaccination protocol where the protein vaccine was administered three times (group 1) versus a second group (group 2) which received four administrations of the immunizations with the HIV poxviral vector followed by two administrations of the HIV protein vaccination, versus a group (group 3) which received three administrations of the immunizations with an HIV env-encoding plasmid vaccine followed by boosting with gp120 protein twice.[66] The studies reported significant antibody response differences in the three arms. Group 2 exhibited the lowest neutralizing antibody titers, but a high level of binding antibody responses. Group 1 exhibited high neutralizing titer antibodies as did group 3. Interesting, the DNA prime–protein boost group (group 3) exhibited the highest level of broadly neutralizing titers observed suggesting a unique benefit of the DNA prime in the context of protein boosting.

The National Institutes of Health (NIH) Vaccine Research Center reported on several Phase I trials with the goal of developing a DNA prime followed by a recombinant adenovirus serotype 5 (rAd5) vector boost protocol for HIV.[67] For these studies both vector systems, the plasmid and the adenovirus serotype 5 (Ad5) contained similar inserts. The VRC-HIVDNA009-00-VP vaccine consisted of a four-construct mixture of plasmids encoding subtype B Gag-Pol-Nef fusion protein and three modified Env constructs from subtypes A, B, and C. Plasmid doses of 4 or 8 mg were studied but these appeared similar. The adenoviral vectors were delivered at 10^{10} particle units (PU) in a 1-mL volume, in the deltoid and as a single immunization in the prime-boost study. They observed that while each platform was capable of inducing immune responses as a standalone platform,[55,67,68] when the platforms were combined (DNA prime administered three times followed by Ad5 boost) improved immunogenicity was observed. The sequential DNA/rAd5 administration resulted in 7-fold higher magnitude Env-biased HIV-1-specific $CD8^+$ T-cell responses and 100-fold greater antibody binding titers measured by enzyme-linked immunosorbent assay.

An additional possible advantage of a heterologous prime-boost regimen is induction of responses that differed from those induced by repeated dosing of either vaccine modality. Specifically, a report by Cox and colleagues[69] 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.[70] Furthermore, studies by Schneider and coworkers[71] and Robinson and colleagues[72] 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.[64,73,74] Together these studies established that heterologous DNA prime-boost immunizations elicited immune responses of greater breadth than could be achieved by priming and boosting with the same vector (Fig. 67.2).

Specific studies, however, have provided unexpected and less-positive results. A focused Phase II study evaluated the regimen of an HIV-1 DNA prime followed by a rAd5 boost, to prevent infection or to reduce viral loads in study participants who became naturally infected after vaccination.[75] This study was designed after an earlier study entitled STEP, which tested an rAd5 vector that encoded gag pol and nef antigens of HIV. The STEP trial was ended early because of futility and a possible concern that persons in the vaccine arm acquired HIV infection more often than those in the control arm. The new trial was specifically designed to avoid potential risks observed in the STEP study, such as inclusion of env antigens in the 505 study as part of the vaccine. However, this trial also ended early because of futility,[76] supporting the conclusion that this prime-boost approach with these vaccine platforms was not effective. It has been speculated that rAd5 may not be the best genetic vaccine platform for HIV as its strong immune cell activation may work against the vaccine during HIV field challenge. An outcome of this study is a shift to other nonhuman adenoviral vectors, such as chimpanzee adenoviral vectors.[77] Overall, the prime boost strategy continues to have a central role in many vaccine efforts targeting diverse difficult pathogens, but as illustrated above, many challenges remain, and there is a major interest in further improving the immune potency of these approaches.

Improved Immune Potency of the DNA Platform

Many approaches have been taken to improve the immune responses induced by the DNA vaccine platform (Table 67.1). These start from reengineering the plasmid vector to better deliver and express antigens in vivo and include optimization of the promoter region and transcriptional elements to enhance antigen expression levels,[78–88] improved leader sequences,[89–95] and optimization of the plasmid backbone itself; development of improved gene sequences[26,28,96]; inclusion of molecular adjuvants in the formulation or as immune modulators; and development of a variety of next-generation delivery approaches.[97–108] Many of these areas have been reviewed,[15,109] and some are highlighted below in more detail.

DNA technology is highly malleable and a much-improved DNA vaccine immune profile has been described. Major areas of improvement are plasmid construction and design, including the optimization of promoters and enhancer elements; polyadenylation[78–88]; incorporation of novel leader sequences[89–95]; vector design; antibiotic resistance and selection sequences; origin of replication choices for production; and efficient and slimmer backbone designs deleted of extraneous DNA sequences, all of which may contribute to improved platform performance. An important consideration for increasing plasmid-driven immune potency involves sequence optimization.[18–26,28,96] Bacterial RNA is rich in AU sequences, whereas mammalian DNA is rich in GC. Therefore, the pool of transfer RNAs needed 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 the transfer RNA pools available in human cells, allowing the encoded messenger RNA (mRNA) to be more efficiently translated.

An important modification for increased immune potency is RNA optimization, where changes are made to the RNA sequence that do not affect the amino acid sequence of the

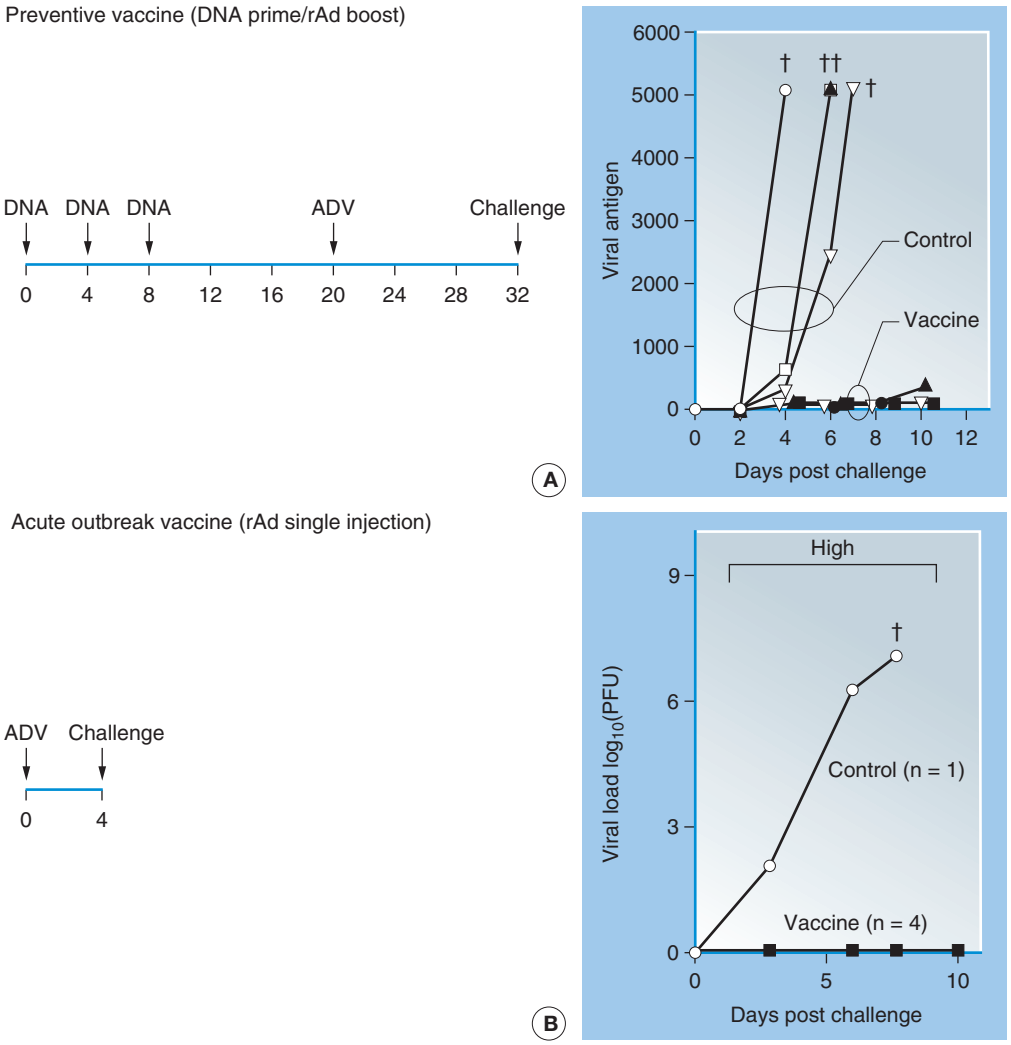

Figure 67.2. Prime-boost versus single shot in models of an Ebolavirus vaccine. Alternative approaches for vaccination can be used depending on the intended use of the vaccine. **A,** In the nonhuman primate challenge model, a DNA prime with recombinant adenoviral vector (rAd) boost of *GP* and *NP* 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)*. *(Modified from Sullivan NJ, Sanchez A, Rollin PE, et al. Development of a preventive vaccine for Ebolavirus infection in primates.* Nature*. 2000;408:605–609; and Sullivan NJ, Geisbert TW, Geisbert JB, et al. Accelerated vaccination for Ebolavirus haemorrhagic fever in non-human primates.* Nature*. 2003;424:681–684.)*

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.[110–112] A recent study of a West Nile virus vaccine DNA using an improved promoter induced relevant antibody responses in most of the 30 trial participants.[113] This study extended the findings reported in trials of severe acute respiratory syndrome (SARS) and Ebola plasmid vaccines, which resulted in low but positive serology induced by the vaccine, with however low T-cell responses suggesting more improvement is still important for this platform to be stand alone.[114,115]

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 HIV's Env sequence can reach greater than 15% within a subtype and greater than 30% between clades. Similar diversity issues plague influenza, hepatitis C virus, 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[116–119]; mosaic antigens[120–122]; and epitope string approaches.[123–128] All these approaches seek to focus the immune response induced by a synthetic gene cassette to

TABLE 67.1 Some Major Approaches[a] in Plasmid Vaccine Optimization

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

[a]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).

specific epitopes or regions of a native antigen or an antigen predicted by computer analysis to be more conserved or invariant in the population of divergent viruses. Such targeting would, in theory, preferentially expand the most desired T- and B-cell responses that nature would not drive. 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 for hepatitis C, hepatitis B, and HIV, among others. Data from HIV as well as human papillomavirus (HPV) are discussed further below.

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.[93,129–139] 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[28,29] and draining lymph nodes as the vaccine antigen.

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.[129] In mice vaccinated with a rabies virus antigen DNA, the addition of GM-CSF delivered as DNA 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.[140] 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 a prime-boost protocol.[139]

Another cytokine gene, interleukin (IL)-12, has also received a great deal of attention as a DNA vaccine adjuvant.[131,135] 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.[131] Using an HIV-1 DNA vaccination of nonhuman primates (NHPs) along with plasmid encoded IL-12 demonstrated increased cellular responses that corresponded with control of viremia and improved clinical outcomes from a chimeric SHIV virus. The NHP challenge virus consists of SIV core antigens as well as an HIV-1 Env antigen and is designated SHIV98.6P. The ability of plasmid IL-12 vector to increase HIV-1–specific responses against an HIV-1 DNA vaccine is currently being studied in humans, where the adjuvant effect of the plasmid codelivered with IL-12 appears to significantly enhance the immune response, expanding both the CD4 and CD8 T-cell responses induced by the vaccine.[136] Additionally, IL-2–Ig, a T-cell growth expansion factor, administered with an HIV antigen DNA vaccine showed positive results in mouse and macaque model systems and was moved to human testing.[134] In the study of 70 persons which compared an HIV DNA vaccine alone to the HIV DNA vaccine given with IL-2–Ig either with the vaccine or 2 days later, the group receiving the IL-2–Ig adjuvant 2 days later exhibited improved enzyme-linked immunosorbent spot (ELISPOT) results compared to either of the other groups.[141] In a study of a DNA vaccine in the SIV model using a checkpoint inhibitor, anti–CTLA-4 or 41BB Ig adjuvants were reported.[142] This study showed that the 41BB adjuvanted groups showed superior CD8 T-cell responses to DNA alone or DNA + CTLA-4 adjuvanted groups. Furthermore, when the macaques were challenged with a highly pathogenic SIVmac251 monkey virus in the 41BB arm, seven of 14 animals showed several logs lower peak viremia and significant control of infection. The ease and specificity of such combination adjuvant approaches in the DNA vaccine arena has generated an enormous amount of data regarding vaccine effects 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.

Increased Vaccine Dose

As DNA vaccines are delivered without the benefits of a viral vector coat to facilitate host cell attachment and entry, they suffer intrinsically with a limited transfection efficiency, which compounds their 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 in humans can be improved by increasing the dosage of plasmid delivered. Early studies in humans used doses of 1 mg or less but newer studies use doses of 8 mg, which increase the consistency and frequency of antibody production.[114] Formulations with doses as high as 12 mg, together with cytokine expression vectors and electroporation, have stimulated increased T-cell responses[143] and are promising approaches.

Novel Transfection Reagents

Formulations including novel transfection reagents were a major focus in early studies. In addition, physical delivery has become an important area of research. An important area for DNA delivery is the formulation of DNA into or on biodegradable polymeric microparticles (see a review[105]) as well as contained in liposomes.[97,101,108] Microparticle- and liposome-based DNA vaccine delivery systems are being studied for their utility of delivery and enhanced immunogenicity in several different host and antigenic vaccine platforms in small animals,[99,100,103,106] NHPs[97,104] and in humans in cytomegalovirus (CMV) and influenza DNA vaccines. 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 additional examples of molecules that can enhance DNA vaccine immune potency in specific systems.[103,106] The poloxamer CRL1005 has demonstrated improved immune potency in preclinical models for simian HIV vaccines.[97,108] In addition, ongoing studies show that liposome vehicles can improve DNA vaccine-induced immune responses. Studies with liposomes, in general, support an improved, but still modest, impact on antibody responses and lesser impact on 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.[99,100,103–106]

The formulation of DNA vaccines in polyamine gels or nanoparticles[106,144,145] has also been reported to increase the uptake of plasmid vectors and increase antigen expression in vivo. Although there is increasing research in this area, they are not as well studied as the liposome and polymer approaches in their clinical development trajectories. However, alone none of these formulations appear capable of inducing immune responses from the DNA platform that rival the responses induced by viral vectors.

Vaccine Delivery Methods

Another major area of productive research has been in combining DNA vaccines with physical delivery methods. By physically forcing more plasmid DNA into cells, as well as increasing the number of transfected cells, expression levels should improve. Improvements in immune responses have been reported with physical delivery devices such as the gene gun or biolistic jet injection.[146–155] Jet injection involves using high pressure to deliver a liquid formulation of DNA just centimeters below the skin 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 when compared with IM injection in experimental model systems. The gene gun uses high pressure to deliver DNA-coated gold nanoparticles to the dermis and has demonstrated high transfection efficiency and enhanced antibody responses in several species including humans.[152,153] In contrast, it has had relatively little effect on improving cellular immunity.

Electroporation (EP) has been used to transfect cells in vitro for 3 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 2 decades as a method to improve delivery of chemotherapy drugs to kill specific tumor cells[147–149] and in several animal species (e.g., dogs, pigs, cattle, NHPs) to deliver genes that encode a variety of hormones, cytokines, enzymes, or antigens.[146,150–152,156] However, the conditions required for EP were initially considered too harsh for adoption into vaccination strategies. Yet, over the past 10 years, EP technology has developed more benign delivery devices that are computer controlled and capable of enhancing delivery through IM, ID, and microneedle transfection.[20,21] These approaches use lower voltage and are more tolerable. An exciting development is that in large-animal models, delivery of DNA vaccine by EP has led to both increased cellular and humoral immune responses,[22,23] rivaling those seen with viral vectors.[157] When these newer EP approaches are combined with other DNA optimization approaches, the magnitude of the immune responses generated by the combined DNA approach has increased more than a log.[110,156,158–164] EP technology can also be tailored to a particular DNA vaccine, using devices that control current, voltage, and timing settings. Combinations with molecular adjuvants also look highly promising,[136,165,166] and delivery to the skin, muscle, and the mucosal has been reported. Data from multiple primate challenge models have demonstrated much improved immunogenicity and efficacy against challenge by such delivery of plasmid vaccines.[167,168]

Clinical Studies

The initial studies of DNA vaccines in the clinic demonstrated safety and ease of production, but immune responses were not adequate. The initial fear regarding the use of DNA vaccines was the risk of integration into the host chromosome with subsequent 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 significant vaccine-related adverse events being reported. In fact, the number of DNA vaccines being tested in clinical studies compared with all other recombinant platforms has increased from just 4% in 2000 to comprise 18% of all such trials as of December 2016. Table 67.2 summarizes several pivotal trials of DNA vaccines.

One area that can serve as a barometer of the interest in an experimental platform is HIV vaccine trials. Seven of the

TABLE 67.2 Current DNA Vaccine Clinical Trials as of August 2015

Phase	Approach	No. of Trials	Vaccine Targets
I	DNA alone	17	Cancer, infectious disease
	Prime/boost[a]	3	Cancer, infectious disease
II	DNA alone	3	Cancer, infectious disease
	Prime/boost[a]	3	Cancer, infectious disease

[a]Boost studies include poxviral boosts, recombinant protein boosts, adenoviral boosts; seven DNA-only studies using electroporation. Genetic adjuvants, including granulocyte-macrophage colony-stimulating factor and interleukin-12, are found in multiple DNA studies. Both ID and cancer studies are recognized.

18 clinical trials sponsored by the HIV Vaccine Trials Network (HVTN) are evaluating DNA vaccines to elicit immune responses, either alone or in combination with multiple viral vectors as boosts. Additionally, new studies of EP-delivered DNA alone build on the data observed in HVTN 080 and EP DNA prime-boost strategies are being developed. An alternative approach uses a DNA prime to stimulate both T-cell and B-cell responses, followed by a recombinant protein-boost to generate more-potent antibody responses. DNA vaccines are also being studied in non–prime-boost settings using enhanced physical delivery. For example, in HVTN protocol 080, multiple-codon and genetically optimized DNA plus plasmid IL-12 as an adjuvant is delivered by EP to healthy volunteers. This study reported induction of T-cell immunity in 90% of study volunteers. In fact, this three-immunization protocol induced overall T-cell responses as high as responses previously reported to a DNA prime followed by poxviral or adenoviral boosts consisting of five immunizations. Furthermore, induced CD4 and CD8 T cells were still detectable at 6 months after the final immunization.[169] This was the first study to show that the combination of both EP and IL-12 can dramatically improve the T-cell responses in humans.

A Phase I study of a multioptimized (synthetic) consensus DNA-VGX3100 delivered by EP for treatment of early HPV disease was recently conducted in 18 women with precancerous cervical disease (Cin2 or Cin3). Participants were vaccinated with synthetic DNA encoding two different HPV oncogenes (E6 and E7) and two different oncogenic HPV strains (types 16 and 18) at week 1, week 4, and week 12. Three dose groups (0.6 mg, 3.0 mg, and 6.0 mg) of the plasmid vaccine were studied. Subjects were evaluated for induction of antibodies as well as CTL. In contrast to prior DNA vaccine studies, 100% of women in this study seroconverted, irrespective of dose group, greater than 90% after the second dose of vaccine. Antibodies persisted for at least 6 months after the final immunization. Cellular responses were demonstrated in 78% of women by ELISPOT and in more than 80% of women by cytotoxic assays. In the high-dose group six of six women exhibited killing capacity against targets.[167] Based on these encouraging data, a Phase IIB study was conducted in 147 women with cervical disease (CIN2 or CIN3) testing the same schedule as the Phase I study using the highest dose of vaccine. The primary end point was regression of disease, and the secondary end point was regression of disease as well as viral clearance. The study reported that 50% of the study participants had regression of their cervical disease, with 40% completely clearing the HPV infection ($P = .001$). Similar to the Phase I study, close to 100% of women seroconverted to the vaccine antigens, and greater than 90% exhibited strong T-cell responses. Furthermore, T cells were elevated in cervical samples examined from women who cleared their disease.[168]

It will be important to study optimized DNA vaccines delivered by EP in additional settings to extend these findings to additional targets, expanding the applications of the DNA platform. As with the report of the first efficacy data, it is clear from the preceding discussion that improvements in technologies are driving the field (see Table 67.2). The progress of clinical trials will have to be closely watched, as they will present an exciting picture over the next few years as various DNA approaches come to fruition.

Animal Studies

Several DNA vaccines have been licensed for use in animals. Products licensed for veterinary use[33–36] include a dog melanoma immunotherapy, porcine therapy, a porcine recombinant growth hormone, vaccine for the prevention of rhabdovirus disease in fish, and West Nile virus vaccine for horses based on successful field trials of DNA vaccines. The important safety record of this technology in animal health and in humans, the growing database regarding consistency of immune responses in the clinic, the first efficacy data reported in humans now reported, and the successful licensure of veterinary products suggest that DNA vaccination is well positioned to become an important platform for continued vaccine and immune therapeutic development.

RNA VACCINES

The application of nucleic acid encoded genes (nucleic acid vaccines) delivered in vivo adds another novel approach to the vaccine/immunotherapy pipeline. The use of plasmid DNA for in vivo antigen production and mRNA to deliver encoded antigens was first reported more than 2 decades ago.[170,171] Initially, DNA vaccines took center stage because of their ease of production and stability but problems with immunogenicity were soon appreciated. However, RNA-based approaches have reemerged as an important genetic vaccine platform. RNA-based vaccine approaches allow for infinite boosting because they are not subject to neutralization by the host immune response, even in previously seropositive individuals. RNA vaccines are produced, customized in vivo, and processed by the host. They are naturally folded and can be further modified by the endogenous host cell systems. Similar to DNA vaccine approaches the antigens produced in vivo can be presented on endogenous APC and stimulate both CD4 and CD8 T-cell responses, mimicking the immune responses induced by live infection. Mixtures of RNAs were also tested.[172] However, similar to the poor immunogenicity seen with DNA vaccines, the early studies using RNA vaccines were also plagued by low immunogenicity in small animals, as well as manufacturing issues resulting in low production yields and product instability.

Over the last decade, RNA vaccines have improved through ex vivo transfection studies.[173,174] RNA immunization by transfection of patient-derived dendritic cells showed induction of immunity in vivo. Based on these data, there was interest in cancer immune therapy using patient-derived cells, which are modified by transfecting them with either mRNA derived from their own cancer cells or antigen-specific synthesized mRNA to focus the immune response. These approaches were tested in pancreatic cancer, neuroblastoma, melanoma, colorectal cancer, lung cancer, and prostate cancer, among others. The early studies were well tolerated. Additionally, RNA-based approaches are being investigated for delivery of chimeric T-cell receptors for functionally targeting tumor antigens

directly.[175,176] This ex vivo approach is preferred because of difficulties in RNA production and storage, among other difficulties.

As a result of technological advances, the direct injection of RNA for vaccination purposes is growing in importance.[177] This reemergence is the result of separate advances in the field of RNA vaccines. One advance is the ability to stabilize mRNA during production. The second is the existence of more potent self-amplifying RNA vectors based on the alphavirus platform. In a recent study using a synthetic mRNA encoding influenza antigens, optimized for RNA GC content and complexed with protamine, mice or ferrets vaccinated with this formulation seroconverted to influenza antigens and were protected against influenza challenge[178] after a single immunization. The formulation was also immunogenic in animals as large as pigs, and while not fully protective after influenza challenge in pigs, the disease was attenuated. mRNA vaccines are now being tested in humans, with a focus on therapy of cancer, particularly prostatic cancer.[179–182] A prostate cancer mRNA vaccine containing "self-adjuvanted" mRNA encoding antigens of relevance to prostate disease, including prostate specific antigen, prostate stem cell antigen, prostate specific membrane antigen, and six transmembrane epithelial antigens of the prostate, was evaluated for immunogenicity and safety in 44 patients with advanced castration-resistant prostate cancer. These mRNA-based vaccines contained free and protamine-complexed mRNA to support the stability and delivery of the mRNA. In mice, the vaccine was immunogenic and suggested that these vaccines drove innate immune stimulation that was mediated in part via Toll-like receptor 7 activation. In clinical studies, the mRNA vaccines were administered with five ID injections at a recommended dose of 1280 µg. Results showed that immune responses could be detected against at least one vaccine antigen in 26 of 33 evaluable patients, with 15 of 33 patients responding to more than one of the vaccine antigens. Treatment-related adverse events were experienced by 39 (89%) patients, including injection-site erythema and injection-site reactions, fatigue (18%), pyrexia (16%), chills (11%), and influenza-like illness (11%). Although most of these reactions were considered mild in nature, additional safety studies are needed. The outcome results were interesting as the immune responders showed a trend toward increased survival compared to the non-immune responders. There was also a trend to better clinical outcome based on induction of more antigenic responses; patients who responded to three antigens had better outcomes than those who responded to only one or two antigens. Antibody responses induced by the vaccine were not as robust as the T-cell responses as only four of the patients showed increased titers to tested vaccine antigens. Additional studies in the mRNA vaccine area will be important to follow.

An additional area of importance in the RNA vaccine field is the reemergence of the alphavirus system as a nonviral RNA vaccine delivery platform.[183,184] For example, studies reported from Novartis have described this self-amplifying RNA technology.[185] In this approach the alphavirus genes encoding the RNA replication machinery along with the recombinant viral target antigens are synthesized in the laboratory. Users of this technology to vaccinate mice against respiratory syncytial virus F protein have reported rapid induction of potent antibody responses. This group also reported responses in an NHP study of HIV immunogenicity.[186] The self-amplifying HIV vaccine induced both T cells, measured by ELISPOT, and antibody responses, and were further boosted by MF59 adjuvanted HIV env antigen. Overall, the data clearly illustrate that this platform can induce immune responses. As with mRNA vaccine it will be important to monitor self-amplifying vaccines as they enter clinical evaluation.

RNA approaches have conceptual advantages as they are simple focused immunogens and do not require nuclear localization to generate expression. The replicating vectors such as self-amplifying vectors expand mRNA copies by providing the replication machinery from alphaviruses to maximize expression. The newer stabilized mRNA approaches exhibit a longer half-life compared to earlier-generation mRNA vaccines. These advances improve delivery and expression. Furthermore, RNA approaches strongly stimulate the host innate defense system in part through activation of the Toll-like receptor 3 and Toll-like receptor 7/8 pathways.[187] Innate activation may be an advantage for immune priming that needs further investigation. Overall, even though the RNA field has to catch up to other genetic vaccine platforms, this next generation of RNA vaccines presents interesting opportunities as a standalone platform or as part of prime-boost protocols.

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 Fig. 67.1). Depending on their ability to target APCs, the ease of developing packaging lines, the inherent immunogenicity of both the vector and insert, and other factors (see Box 67.2), these viral vectors are helping to improve vaccine efficacy in a variety of infectious disease models. The properties of promising vectors and current progress in their development are summarized in the following sections.

Replication-Defective Adenoviral Vectors

Among the viral vectors that have been studied, recombinant adenoviral vectors have demonstrated immunogenicity and protective immunity in a variety of animal models. These vectors have been genetically modified so that they can deliver and express specific recombinant gene products but are unable to grow on their own and thus are 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.[188] The majority of clinical vectors have been derived from Ad5, although more than 51 human serotypes in six subfamilies (A to 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.[189]

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 I or Phase II. With respect to animal models, the replication-defective adenovirus elicits potent immune responses and protection against Ebolavirus, administered either alone as a single injection, or in prime-boost combinations (see Fig. 67.2).[190,191] The prime-boost approach induces a more potent and durable immunity, desirable for a preventive vaccine in routine use, whereas a single rAd5 vaccination induces a more rapid response that is sufficient for immediate protection (see Fig. 67.2). This rAd5 approach may be useful in containing acute outbreaks of Ebola infection and could be applicable to other pathogens.[39] In addition, both rAd5 vaccines and DNA prime/rAd5 boost combinations

confer partial protection in rhesus macaques against multiple HIV isolates, including SHIV-89.6P,[37,192] SIVmac239,[193] and SIVmac251.[194–196] Replication-defective adenovirus has also been used in a variety of additional animal models of infectious disease, including plague, anthrax, influenza, and malaria.[51]

Phase I and Phase II clinical studies with replication-defective adenoviral vectors for HIV-1 have been conducted by several groups. The STEP trial, a clinical efficacy study of an rAd5 vector encoding *Gag*, *Pol*, and *Nef* genes of HIV-1, evaluated the effect of vaccine-induced T-cell responses on controlling viral load. Although the vaccine was immunogenic, there was no reduction in HIV acquisition or long-term control of postinfection viremia.[197] Further analyses revealed that persons with specific human lymphocyte antigen 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.[198] 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 appeared higher in this subgroup, although this result remains controversial.

The clinical utility of the 505 DNA prime/rAd5 boost vaccines is as discussed above in the prime-boost section. The 505 trial was similar to the Merck STEP study, which also was ended early because of futility. The results support that improvement to Ad5 vector approaches must be considered for improving the outcome in the clinic. This could be a vector issue as the inclusion of env antigens did not impact positively on efficacy in the 505 trial. The serology issues associated with Ad5 vectors, as well as the controversy regarding whether there is an increased risk of HIV infection,[199] have driven interest in other non-Ad5 adenoviral vectors.

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. In certain regions of Africa, the Ad5 seroprevalence is greater than 90% with a high degree of neutralizing antibody, limiting the use of this vector. 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, as seen in humans. This preexisting immunity has been shown to reduce the immunogenicity of Ad vaccines in mice,[200,201] rhesus monkeys,[202] and potentially in humans,[203,204] but it is not clear that preexisting immunity in humans will completely block vaccine immunogenicity.

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.[204–206] Ad boosting after DNA priming may potentially reduce its immunosuppressive effects, too, although this was not seen in the described HVTN 505 study.[200,201] Finally, the administration of Ad5 vectors through mucosal routes may help circumvent this problem.[207] However, the safety of this approach, particularly for intranasal delivery, has yet to be determined.[208] 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,[209–212] or through the use of hexon chimeras, which appear to be the targets of the major neutralizing antibody response.[213,214] 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 NHPs. Recombinant Ad vectors from human serotypes have been well described.[215–217] 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,[218] and thus may 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.[54,219] Some of the alternative vectors show less-potent antibody responses than seen with rAd5. 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,[220,221] complicating development and regulatory issues for such vectors.

In addition to these replication-incompetent Ad vectors, attenuated replication-competent vectors from Ad4 and Ad7 have been used as vaccine vectors to prevent adenoviral mediated disease in the military where there is a high incidence of this disease among recruits. These live vaccines appear well tolerated and highly effective against Ad4 and Ad7.[222,223] These serotypes have also been developed as recombinant vectors platforms—for example, against HIV.[222,224] These vaccines not only offer the potential of alternative serotypes but when used orally can deliver immune stimulus to the gut mucosa, which may have potentially desirable effects in protection against some viral challenges. Finally, recombinant Ad vectors have been developed from alternative species, including sheep, pigs, cows, macaques, and chimpanzees.[190,225–233] In particular such chimp adenoviral vectors appear to be gaining traction for clinical evaluation. Specifically, the Ad5 Ebola vaccine was superseded by a new chimp adenoviral vaccine.[234] A chimpanzee-derived replication-defective adenovirus (chAd) vaccine induced uniform protection against acute lethal Ebolavirus challenge in macaques. However, the protection was short lived. When chAd3 was boosted with a MVA Ebola vaccine, much more durable protection against lethal Ebolavirus challenge was generated. In human studies, the vaccine induced antibody responses were detected in 68% of vaccine recipients in the highest dose group studied (5×10^{-10vp}) with 100% of vaccinated persons responding in ELISPOT assays.[235] Overall, the data were encouraging. Surprisingly, preexisting neutralizing antibodies were observed against chimp adenoviral vectors in sub-Saharan Africa suggesting that at some level chimp adenoviruses or related viruses have crossed into the human population with some frequency. This finding has consequences beyond HIV vaccines as the chAd3-EBO-Z viral vaccine developed by the NIH is among the important new vaccine candidates being studied in the context of the recent Ebola outbreak in West Africa. The impact of preexisting immunity on this vaccine or others being developed will require additional investigation.[235,236]

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. The concerns

over preexisting Ad5 immunity has generated a number of new approaches for Ad vectors. Novel delivery vectors, molecularly engineered rAd5, and alternative Ad serotypes from other species provide a number of options for clinical study in a variety of infectious disease as well as cancer immune therapy settings.

Poxvirus Vectors for Immunization

The efficacy of vaccinia virus against smallpox represents one of the best examples of the impact of vaccination on infectious disease. However, safety issues using vaccinia strains against smallpox were substantial,[237–240] and a number of alternative vaccinia virus strains have been developed as immunization vehicles (summarized in Box 67.3). These attenuated vaccinia viruses have also been used as delivery vectors for gene products against specific pathogens other than smallpox.

One of the two major attenuated strains of poxvirus is MVA, developed by repeated passaging of the Ankara vaccinia strain on primary chicken embryo fibroblasts (CEFs), as a safer alternative as a vaccine against smallpox. This resulted in multiple genetic changes that enabled the virus to replicate efficiently on a variety of nonavian cell types. A second alternative attenuated strain, the New York vaccinia strain (NYVAC), was developed by genetic modification including the deletion of 18 open reading frames associated with virulence and host range in the Copenhagen strain.[241–244] NYVAC, like MVA, is attenuated in animal models and shows favorable safety and immunogenicity in animals and humans.[242,245,246] NYVAC has a block at an early stage of replication, although it is able to replicate productively in African green monkey kidney cells and primary CEFs.

ALVAC, derived from a plaque-purified virus isolated from an existing canarypox strain, canapox,[247] is able to express inserted transgenes and is immunogenic in both animal and early clinical trials.[245,246,248–251] Additional avipox vectors include fowlpox and canarypox. These vectors have been evaluated both alone and in prime-boost combinations in a variety of infectious disease and cancer models (see review[245]).

In general, 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,[57,252] and influenza.[253] In addition, these vectors have been studied in a variety of HIV challenge models in animals. Human studies have also been conducted [45,254–258] with vaccinia,[259–267] NYVAC,[268–271] and ALVAC,[268,269,271–276] and 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 III study in Thailand and demonstrated a 31% reduction in the frequency of acquisition of HIV infection among vaccinated heterosexual men and women when compared with placebo recipients.[277] This 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. 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, because the 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, antivector immunity remains problematic, although to a lesser degree with 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, possibly impacts their effect in multiple immunization regimens. A poxviral vaccine was tested in a human malaria challenge model.[278] The vaccine expressed a polyprotein insert which consisted of a string of six preerythrocytic antigens designed from *Plasmodium falciparum*. Following safety assessment of single doses, 15 volunteers received a heterologous prime-boost vaccination regime involving two difference poxvirus malaria subunit vaccines containing the insert, FP9-PP and MVA-PP. Following immunization the subjects underwent malaria sporozoite challenge. The vaccines were safe; however, T-cell interferon-γ ELISPOT responses were low and there was no vaccine efficacy observed. A novel MVA vaccine expressing the antigen 85A of *Mycobacterium tuberculosis* was evaluated in a placebo-controlled Phase IIb trial in South Africa in infants.[279] Infants who had previously received bacille Calmette-Guérin (BCG) vaccination were randomized to receive either the MVA85A or placebo intradermally and then followed for immune responses and protection against tuberculosis. Although the vaccine was safe and the immunogenicity was modest, there was no efficacy against tuberculosis infection or disease. Both of these studies with malaria and tuberculosis suggest that much more potent immune responses will be needed to impact these two infections. Thus, prime-boost strategies are being pursued.

In addition to studies in the infectious disease arena, poxviral vectors have been important tools in multiple cancer immune therapy protocols[280] targeting diverse tumor types. In these studies, T cells have been induced; however, the poxviral vectors appear to induce modest antibody responses. The first positive efficacy outcome has been reported in a Phase IIb study using a recombinant poxviral vector. Prostvac-V/F is a candidate, anticancer immunotherapy for therapy of prostate cancer which was originally championed by the National Cancer Institute. It was developed for men with asymptomatic or minimally symptomatic metastatic castration-resistant prostate cancer. Therapy consists of priming with a vaccinia recombinant poxviral vector followed by up to five monthly immunizations with a fowlpox vector. The vectors contain the identical gene inserts. The inserts include a gene sequence encoding a modified immune stimulatory prostate-specific antigen cassette along with additional gene cassettes for three human immunostimulatory molecules (known as TRICOM), leukocyte function–associated antigen-3, intercellular adhesion molecule-1, and B7.1. In clinical trials, the treatment generated clear anti–prostate-specific antigen CTL responses in up to 57% of vaccinees, but almost no antibody responses.[281]

BOX 67.3 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.

These CTLs were shown to have the ability to lyse tumor targets.[282] In a randomized, placebo-controlled, double-blind, Phase II clinical trial (NCT00078585), Prostvac-V/F increased overall survival in patients with metastatic castration-resistant prostate cancer by 8.5 months which translated to a 44% reduction in risk of death.[283] Bavarian Nordic in conjunction with the National Cancer Institute is currently testing Prostvac with or without a GM-CSF adjuvant in a global, double-blind, randomized, Phase III efficacy trial dubbed PROSPECT (NCT01322490). It will be interesting to clinically evaluate this immune therapeutic vaccine in the context of checkpoint inhibitor therapy as well. Further improvement in poxviral immunogenicity remains important.

Adeno-Associated Viruses

The adeno-associated viruses (AAVs) were defined initially 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, AAV's genome sizes are 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,[284] and an HIV vaccine expressed in AAV2 has been evaluated in Phase I human studies, with poor immunogenicity results. Alternative serotypes, including AAV1, are currently under development and may be assessed both alone and in prime-boost combinations for efficacy in humans. An entirely unique use of these vectors has also been described recently. Recombinant adeno-associated virus vectors have been studied as a platform to deliver recombinant antibody genes for direct in vivo production of antibodies. This strategy, vector immune prophylaxis, allows previously identified rare neutralizing antibodies to be engineered into an Recombinant adeno-associated virus vector, which, upon infection, results in in vivo antibody production of protective antibodies. Vector immune prophylaxis also allows for production of protective antibodies in vivo that have not been achieved with vaccination.[285] Several studies have demonstrated the effectiveness of this delivery strategy in protecting NHPs against SIV,[285,286] humanized mice against HIV,[287,288] and mice and ferrets against influenza.[289,290] The first vector immune prophylaxis approach has reached the clinic to inhibit HIV infection. A similar approach has been described using DNA vectors delivered by EP. This DNA monoclonal antibody delivery approach showed in vivo production of broadly neutralizing HIV antibodies,[291] as well as antibodies that can protect against Dengue challenge.[292] Collectively the vector immune prophylaxis and related platforms are clearly exciting, even though they are early and have many hurdles to overcome. They illustrate how gene vectors are revolutionizing the way we think about traditional vaccination for protection against infectious disease.

VECTORS IN DEVELOPMENT

Alphaviruses are negative-stranded RNA viruses that can be modified to express foreign recombinant genes without producing pathogenic infections. Prototypes include Venezuelan equine encephalitis virus,[293,294] Sindbis virus,[295,296] 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.[297] 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.[298–305]

Although not yet in the clinic, interesting data have been generated in NHP challenge models by a novel recombinant replicating simian CMV vector (RhCMV/SIV). This is engineered to contain SIV antigens env, gag, pol, vpr/vpx in four separate RhCMV vectors. The concept is that CMV infection is a potent T-cell memory driving infection, therefore recombinant antigens expressed from this vector may represent a novel CTL-inducing vaccine platform.[306] In an illustrative example, animals were vaccinated twice at 98-day intervals with the RhCMV/SIV vectors, then rested for 545 days and subjected to repeat low-dose pathogenic SIVmac[293] challenge. Over time, 50% of the vaccinated challenged animals exhibited close to complete control over the SIV challenge.[307] Surprisingly, CD8 T-cell responses were not responsible for this impressive control, rather it appears that CD8 T-cell killing is redirected in the context of MHC II molecules.[308] Important issues, such as development of a more field-ready vaccine protocol, understanding why protection is observed in specifically 50% of the animals, understanding the role of the observed CD8 class II killing and its relevance to humans, development of similar acting human appropriate vectors for study, as well as the unique differences between simian CMV and human CMV vectors will require more investigation. However, the interesting impact in the difficult SIV challenge model and the associated control and immune-based clearance is an important avenue for research for control of HIV and other chronic infections.

An unique vector that has moved quickly into the clinic is based on the vesicular stomatitis platform (VSV). The VSV Ebolavirus vaccine (rVSV-ZEBOV) was developed by the Public Health Agency of Canada and consists of a recombinant, replication-competent VSV engineered to express the surface glycoprotein from the Zaire strain of Ebolavirus (Fig. 67.3). Preclinical testing in mice and NHPs demonstrated that IM injections of rVSV-ZEBOV induced neutralizing antibodies that were capable of protecting the animals from a challenge with a lethal dose of Ebolavirus.[309] Phase I clinical trials studying the safety of this vaccine reported that vaccination with rVSV-ZEBOV at doses ranging from 3×10^5 to 50×10^6 plaque forming units (PFU) resulted in no serious adverse events with the only reported side effects being mild fever, joint pains, and vesicular dermatitis in some vaccines.[310] Importantly, vaccination resulted in a transient systemic infection that generated antibodies against Ebolavirus that were capable of neutralizing the virus in vitro (described more fully in the Chapter 20). This vaccine was deployed during the current outbreak. A Phase III study was designed using a ring vaccination strategy and a single immunization protocol. Excitingly, the preliminary results of this Phase III clinical trial of rVSV-ZEBOV in Guinea revealed that the vaccine was 100% effective in protecting close contacts of Ebolavirus patients from becoming infected when the vaccine was administered immediately as opposed to when it was delivered 3 weeks following the patient's diagnosis.[311] Based on these encouraging preliminary data, the World Health Organization approved the continuation of the trial with the elimination of the 3-week postexposure vaccination arm of the study. Further testing is needed to qualify and quantify the anti-Ebolavirus immune response(s) generated in vaccinees, to determine the longevity of protection, and to evaluate the protection generated against different strains of Ebolavirus. Nonetheless, these data are impressive and important for in the context of Ebola and other pandemic outbreaks.

The VSV Ebola is an example of pressing into service a recombinant platform resulting from the urgency of a serious

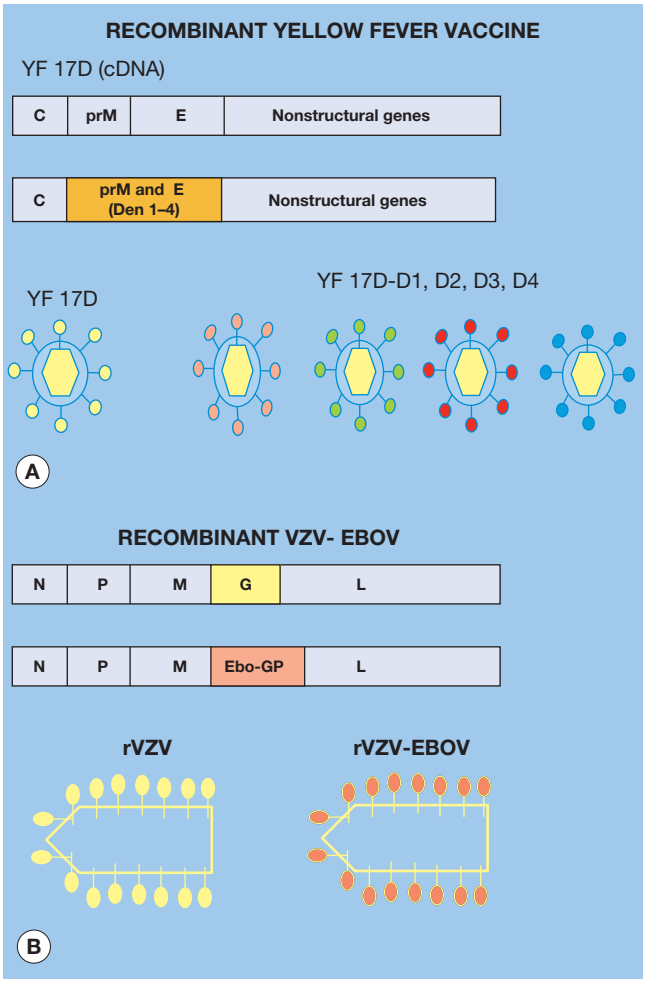

Figure 67.3. A, Recombinant chimeric dengue vaccine. The figure depicts the genome of the attenuated yellow fever vaccine *(top)* versus the chimeric vaccines genome, which has the *prM* and *E* genes *(in orange)* inserted in their place, one each in four different backbones. The *prM* and *E* genes associate to form a complex for env transport and correct surface orientation allowing attachment of the E antigen allowing infection and replication of the attenuated vaccine. The E antigen contains the important immune targets for protection. As there are four different Dengue viral serotypes *(Den 1-4)*, a different chimeric virus was constructed to contain each serotype. This is depicted in the schematic on the bottom of the figure to the left of the attenuated YF17D viral particle cartoon. **B,** Varicella zoster virus–Ebolavirus (VZV-EBOV). The figure depicts the genome of attenuated VZV *(top)* versus the genome of the attenuated VZV with the Ebola glycoprotein inserted upstream of the L antigen. *Yellow*, glycoprotein from VZV; *orange*, glycoprotein from Ebola strain. The glycoprotein switch between the attenuated vesicular stomatitis backbone and the constructed VZV-Ebolavirus vaccine as a viral particle is shown in the schematic below. To construct the vaccine the gene for the native envelope of VZV was deleted. The glycoprotein from the Ebolavirus Zaire-Kikwit 1995 strain is inserted into the recombinant viral genome.

life-threating emerging disease. In contrast, the development of chimeric attenuated yellow fever virus vector expressing dengue viral antigens is a story reminiscent of the development of recombinant poxviral vaccine vectors. The live attenuated 17D strain of yellow fever vaccine is considered one of the world's most effective and safest vaccines. It has been deployed since the 1950s with millions of doses administered. Originally developed by Max Theiler in 1937 through passage, a single dose of the vaccine can confer lifelong immunity. This passed virus has been used as the basis for construction of the new chimeric tetravalent dengue vaccine (CYD-TDV or ChimeriVax) which consists of four recombinant, live, attenuated yellow fever virus 17D vaccine backbones in which the surface Env and premembrane genes were swapped out for those of one of the four serotypes of dengue virus (see Fig. 67.3).[312] The administered vaccine dose consists of 1×10^5 CCID50 (median end point of the cell culture's infectious dose) of each chimeric vector, and each vaccinee receives three doses of vaccine, 6 months apart. Phase IIb clinical trials reported that the vaccine was 30% effective at preventing any dengue infection, and that the vaccine primarily provided protection from dengue serotypes 1, 3, and 4, and offered no protection from serotype 2.[313,314] In two Phase III trials in dengue endemic regions ChimeriVax was found to be 56% to 60% effective in preventing dengue virus infections including infections with dengue serotype 2. Additional testing and research are needed to determine the immune correlates of protection for the vaccine, and a Phase IV clinical trial will likely be needed after licensure to monitor the continued safety, efficacy, and feasibility of large scale vaccination with ChimeriVax. An important fact is that development of this vaccine cost more than $1.5 billion and more than 20 years of research to get to this milestone. Importantly, ChimeriVax is poised to be one of the first examples of successful licensure of a recombinant gene-based vaccine.

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 Ad5, and these have progressed into trials for HIV and are under study for other infectious agents, such as Ebolavirus, Marburg virus, tuberculosis, and malaria. Once approved, these cell lines can be used for diverse vectors. The PERC6 cell line has 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 Fig. 67.1B) include recombinant Ad, poxviruses, measles, Venezuelan equine encephalitis virus, and AAV, all of which have progressed into human trials. The development of transformed cell lines that are capable of propagation, in contrast to the previous standard, avian leukosis–free primary CEFs, represents a major advance in vaccine production technology. These cell lines facilitate the production of replication-defective viral vectors in stably transfected cell lines and 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,[315] based on guidelines created several years ago,[316,317] 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 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[318–320] 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.[321] 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 (bacille Calmette-Guérin) has been a widely used bacterial vaccine; for example, this organism has been used to express HIV antigens.[322,323] In some instances, expression of mammalian genes has required modification of codons more consistent with the host cell type, which has improved immunogenicity.[18,324] At present, however, the ability of such microorganisms to induce cellular immunity at levels similar to adenoviral or poxviral vectors in humans is still under investigation.

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.[325,326] 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.[327–329]

Although the use of such bacterial vectors is attractive in theory, it is 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 if additional improvements will be necessary to improve the efficacy of this approach remains to be established in the clinic.

CLINICAL APPLICATIONS OF GENE-BASED VECTOR TECHNOLOGY

There has been substantial work in animal models of genetic vaccine efficacy. Until very recently there were no reported successes in human studies for guidance; rather there were several failures that brought into question the potential of the technology. However, this status is rapidly changing. Some of the oldest of the recombinant vector platforms have been in clinical evaluation for close to 2 decades. Several trials using the poxvirus technology have been studied in efficacy trials. These include canarypox, MVA, and NYVAC, which have been evaluated in various Phase I to III human studies. As the production technology for poxviruses is well researched, and good-manufacturing-practice procedures for amplification of these viruses followed protocols similar to those developed for vaccinia virus, the path toward clinical studies appeared relatively straightforward. Over the past 3 decades efficacy data were elusive, but recently there has been some positive news. The "Thai" trail of a canarypox vector engineered to carry HIV antigens in which patients were boosted with gp120 antigen provided a possible glimpse of efficacy in a prime-boost platform study, with approximately 31% protection from infection reported.[277] Subsequently, the ProstaVac immune therapy efficacy trial using two different poxviral backbones in prime-boost fashion demonstrated overall increased survival in patients with metastatic castration-resistant prostate cancer of 8.5 months. These important results are supportive of potential impact of these vectors in other targets in the future. It is also important to remember that studies of recombinant MVA technology have given rise to a potentially safer next-generation vaccine for smallpox.[31]

Similarly, DNA vaccines have undergone Phase I testing targeting a diverse collection of infectious diseases, including Ebolavirus, West Nile virus, the SARS coronavirus, MERS, Zika, and influenza virus as well as in cancer studies. Proof-of-concept studies against these viruses have been performed first in animal models with either DNA or in prime-boost combinations or using newer synthetic EP systems. In such studies, impressive protection has been demonstrated in animals.[75,330] Based on these findings, several Phase I trials were completed for Ebola, SARS, and West Nile virus disease targets.[113–115]

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.[331] Phase I studies testing this concept in humans 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.[332]

A Phase IIb efficacy trial testing a synthetic DNA delivered by EP (VGX-3100) in cervical disease reported that 50% of the women in the vaccine arm regressed disease, and 40% of them cured their underlying infection, providing the first efficacy outcome for the DNA vaccine field.[333] It is likely that additional studies building off these more potent approaches will

be important to study. As previously discussed, DNA vaccines have been approved for veterinary use, including a DNA vaccine for West Nile virus in horses[334] and a DNA vaccine for infectious hematopoietic necrosis virus for protection of 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.[335]

Among many research and early clinical accomplishments, the efficacy data reported in multiple gene-based studies is a refreshing change. The protection reported for rVSV-ZEBOV vaccine studies which prevented Ebola transmission in West Africa, the efficacy of tetravalent ChimeriVax lowering hospitalizations and improving outcome in dengue endemic regions, the impact of ProstaVac for extending life of cancer patients, and the ability of VGX 3100 to regress and clear some women with neoplastic cervical disease serve as major milestones for gene based vaccine technologies. These efficacy outcomes have come to fruition only in the last few years suggesting that specific gene based platforms appear to be coming of age. The precedent set by these studies provide 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 as well as providing new therapies for cancer.

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