Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Continued improvements in vaccination technologies have led to remarkable progress in the control of human infectious diseases. Our fundamental view of the nature of a vaccine was changed with the discovery of DNA immunization in the early 1990s when it was determined that the genetic material that encodes for antigens, rather than the actual antigens themselves, can be effective in eliciting an immune response. Given the ever increasing threat of emerging and reemerging infectious diseases and a renewed concern regarding the use of biological agents for bioterrorism purposes, the opportunities that DNA vaccine technology provides could not have come at a more critical time in history. Since its inception, DNA vaccination technology has undergone significant advancements and many candidate human vaccine formulations have already been developed. Improved modes of administration, the use of codon and antigen gene optimization, and the implementation of vaccination DNA prime/boost regimens have led to the quick progression of DNA vaccines from research laboratory benches to human clinical trials. Significant progress has been made in developing DNA vaccines against various biodefense and emerging infectious disease targets, such as HIV-1, influenza, severe acute respiratory syndrome associated
coronavirus (SARS-CoV), Ebola, the viral encephalitides, anthrax, plague, and botulism, among others with some already moving into early phase clinical trials with promising results. Having the ability to respond to a potential bioterrorism threat or to some other emerging infectious disease outbreak is crucial and the advancements associated with DNA vaccination technology will allow us to do so in a prompt and rational manner.

INTRODUCTION

In the early 1990s, several research groups discovered, independently, that the direct inoculation of a DNA plasmid coding for a specific protein antigen could elicit an immune response against that antigen (Tang et al., 1992; Robinson et al., 1993; Ulmer et al., 1993; Wang et al., 1993; Lu et al., 1995). DNA or nucleic acid vaccination is now considered to be the fourth generation of vaccines after live attenuated vaccines, inactivated vaccines, and recombinant protein-based vaccines. This new vaccine technology could not have come at a more important time in history given the increasing threat of emerging and reemerging infectious diseases and a renewed concern regarding the use of biological agents for bioterrorism purposes. The need for immunological protection for large populations would require the production or stockpiling of large quantities of vaccines. Future vaccines need to be effective while maintaining a high safety profile. The designs of vaccines have to be flexible to counter antigen alterations and to protect people from more than one type of pathogen. DNA vaccines are an ideal vaccine system to employ in the face of such challenges.

DNA vaccines utilize a eukaryotic expression vector to express a gene product or multiple gene products in vivo. It is this expression system that serves as a delivery system to produce either secreted or cell-associated antigens with the goal of eliciting optimized antigen-specific humoral and cell-mediated immune responses in the host. Over the past 15 years, DNA vaccine technology has gone through significant improvements. The focus of research has gradually shifted from proof-of-concept studies in animal models to the demonstration of immunogenicity in humans. DNA vaccine technology provided researchers with a unique platform that is effective in achieving three important objectives in the development of the next generation of vaccines against emerging infectious diseases and pathogens that have the potential to be used as bioterrorism agents. First, DNA vaccines are a simple alternative option against those pathogens to which there were effective vaccines in the past but the original manufacturing process is no longer considered safe. Second, DNA vaccines are able to elicit improved cell-mediated immune responses in addition to protective antibody responses, which dominate the design of previous generations of vaccines. Finally, DNA vaccines are an ideal tool to screen for new or optimal protective antigens (PA) to formulate new or improved vaccines against emerging and neglected infectious diseases or bioterrorism.

ADVANCEMENTS IN DNA VACCINE TECHNOLOGY

Since the discovery of DNA vaccines in the early 1990s, it is well recognized that this novel immunization technology possesses the following key strengths: (1) the ease of construction and production of the constructs; (2) the native conformation of in vivo expressed protein antigens including any associated post-translational processing of newly synthesized proteins, such as glycosylation; (3) the flexibility of modifying antigen sequences to match with mutated pathogen genes or to produce optimized “designer’s antigens”; (4) the ability to elicit both cell-mediated and humoral immune responses; (5) overall good safety profile; and (6) the potential to deliver multiple antigen genes in one construct or as a mixture of multiple plasmids.

Over the past 15 years, the immunogenicity of DNA vaccines has been further improved through the utilization of several key technological advancements including antigen gene codon and coding sequence optimizations, various DNA delivery methods, and the adoption of “prime-boost” regimens.

Codon and Antigen Gene Optimizations

The critical step for DNA vaccination is to establish a high level of gene expression in a mammalian host. One way to improve the immunogenicity of DNA vaccines is to alter the nucleic acid sequences of the viral DNA so that they better complement the genetic makeup of the mammalian host while keeping the same amino acid—a process called codon optimization. The basis for using codon optimization in DNA vaccine design stems from the idea that many amino acids can be coded by more than one codon and that the frequency by which a mammalian host may use a particular codon may differ from usage by
a virus. Various studies have used this knowledge in order to improve the ability of mammalian cells to express viral proteins (Haas et al., 1996; Andre et al., 1998; Kotsopoulou et al., 2000; Liu et al., 2004; Apt et al., 2006; Wang et al., 2006a; Wang et al., 2006c). It is this method of altering the genetic sequence that has proven to be effective in increasing the immunogenicity of various DNA vaccines against human immunodeficiency virus (HIV) (Haas et al., 1996; Andre et al., 1998; Kotsopoulou et al., 2000; Liu et al., 2004; Wang et al., 2006a), influenza virus (Wang et al., 2006c), and dengue virus (Apt et al., 2006) due to increased overall antigen production as a result of enhanced mRNA stability (Wang et al., 2006a) and better utilization of host-cell tRNAs (Haas et al., 1996). Due to the adaptation of codon optimization, an otherwise low efficient DNA expression vector can achieve high-level antigen expression (Wang et al., 2006a).

The efficacy of DNA vaccines can be further optimized if the antigen genes are modified. The full-length gene sequences of some candidate antigens cannot be directly used as the antigen gene inserts of the DNA vaccine and may require modification in order to achieve a high-level expression of functional antigenic proteins. For example, an HIV DNA vaccine is not efficient in expressing the full-length glycoprotein (Env) of HIV-1, a gp160 kDa protein; however, two truncated versions of Env, the gp120 and gp140 proteins, that only express the extracellular portion of Env are capable of producing large amounts of Env antigens and eliciting high Env-specific antibody responses (Lu et al., 1998). Sometimes a signal peptide sequence different from the natural leader sequence may also improve antigen expression and immunogenicity of DNA vaccines (Wang et al., 2006a). This process of modifying the original antigen coding sequence is called “antigen engineering” (Lu et al., 1999).

Mode of Administration

The route of DNA vaccine inoculation can greatly alter the immunogenicity of DNA vaccines. Various methods to deliver DNA into the hosts have been developed, using various administration methods, with or without adjuvants. The delivery methodology for DNA vaccines started with the intramuscular injection of so-called “naked DNA” into the host allowing for the uncoated DNA to be taken up into the cells, transcribed, the antigen produced and finally, an immune response elicited. Further development of DNA vaccine technology include facilitated DNA vaccines that employ the use of carriers made of various chemical materials (e.g., lipid polymers and cationic poly(lactide-coglycolide) [PLG] microparticles) (Feltsner and Ringold 1989; Wheeler et al., 1996; Stopeck et al., 1998; Herrmann et al., 1999; Klavinska et al., 1999; O’Hagan et al., 2001; Otten et al., 2005). DNA vaccines, naked or formulated with the above carriers, can be delivered intradermally, intramuscularly, and subcutaneously by a conventional needle injection. Mucosal delivery of DNA vaccines has also been tested including intranasal route; however, in some studies, an emulsion-mediated technique was required in order to induce immunity (Kim et al., 2006).

Other physical delivery methods (such as “gene gun” and electroporation) can improve the efficiency of DNA vaccine uptake by cells and/or increase activation of the immune system. While significant immune responses have been observed in small mammals using the more traditional routes of administration (i.e., intramuscular or intradermal needle injections), these inoculation methods have proven less successful in larger animals and in human studies. On the other hand, the physical delivery approach using particle mediated epidermal delivery (PMED) device has proven to be effective in eliciting immune responses in humans against hepatitis B virus (Tacket et al., 1999; Roy et al., 2000; Rottinghaus et al., 2003), influenza (Drape et al., 2003), and malaria (McConkey et al., 2003).

Prime and Boost Approach

Since the most successful DNA vaccine inoculation method (PMED) for eliciting immune responses in nonhuman primates and in humans is not commercially available for clinical use, it has become important to utilize other techniques and methods to increase the immunogenicity of DNA vaccines that are currently in development. One such method is the prime-boost approach. The rationale underlying this strategy is that gene-based vaccines, presented by a recombinant viral vector or as DNA plasmids, elicit immune responses by producing antigens in vivo; however, these immune responses have not been strong, particularly in humans. In order to increase the levels of immune responses, in particular the cell-mediated immune responses, strategies have been developed to prime the immune system with the DNA vaccine and boost with a recombinant viral vector (Pancholi et al., 2000; Ramshaw and Ramsay, 2000; McShane et al., 2001; Schneider et al., 2001; Gonzalo et al., 2002; Mellquist-Riemenschneider et al., 2003; Woodland, 2004; Seaman et al., 2005; Wu et al., 2005; Perkins et al., 2005), or an attenuated virus (Yuan et al., 2005). This DNA-viral vector combination has proven effective in eliciting high-level cell-mediated immune
cells, which in turn becomes the source of continuing HIV-1 integrates into the genome of host dividing be induced by a vaccine is uncertain. As a retrovirus, HIV-1 infection; hence, the correlate of protection to be natural adaptive immune protection against innovative AIDS vaccine has been proven more challeng-

ing than initially anticipated. There does not appear to be natural adaptive immune protection against HIV-1 infection; hence, the correlate of protection to be induced by a vaccine is uncertain. As a retrovirus, HIV-1 integrates into the genome of host dividing cells, which in turn becomes the source of continuing infection. Its proteins go through constant mutations due to the lack of proofreading function of retroviral polymerases. The structure of envelope glycoprotein (Env), the immunodominant antigen of HIV-1, is very complicated and highly variable to escape from antibody responses.

The recombinant protein HIV-1 vaccines in the form of gp120 antigens have failed to protect a high-risk population in two phase III efficacy trials (Flynn et al., 2005; Gilbert et al., 2005; Pitisuttithum et al., 2006). While the live attenuated vaccination approach was successful in simian immunodeficiency virus (SIV) studies in nonhuman primates (Desrosiers et al., 1989; Daniel et al., 1990), due to safety concerns this approach has little hope of being tested in humans. HIV was among the first pathogens for which the DNA vaccine approach was explored (Wang et al., 1993; Lu et al., 1995) and the HIV vaccine field quickly became the major testing ground for the optimization and application of DNA vaccines. DNA vaccines have been used to express many key HIV-1 antigens including Env, Gag, Pol, Nef, Rev, and Tat for the induction of both antibody- and cell-mediated immune responses. Currently, DNA vaccines are one of the key components in almost every major HIV vaccine development program. DNA vaccines are used mainly as priming immunizations and are typically combined with either a viral vector boost (Mwau et al., 2004; Graham et al., 2006; Tavel et al., 2007) or a protein boost (Wang et al., 2008a). More human studies have been planned or are ongoing. The results from these studies will provide important guidance for the future development of DNA vaccines for other emerging infectious disease and biodefense applications.

**Influenza Viruses**

Influenza virus infection remains a major health threat to both humans and animals. Influenza A viruses infects both humans and other animal species, including swine, horses, and avian. The predominant human influenza virus vaccine is the trivalent inactivated influenza vaccine (TIV), which incorporates the predominant viruses that are predicted to circulate during the next influenza season. The vaccine typically includes an H1 subtype and an H3 subtype from influenza A plus an influenza B virus. The immunogenicity of these vaccines is relatively low and requires annual booster immunizations to maintain the levels of protective immune responses and to develop new protective antibodies against mutated viral antigens that occasionally emerge. A newly developed cold-adapted live influenza virus vaccine has much improved immunogenicity but it

**II. FUNDAMENTAL ASPECTS OF VACCINOLOGY**

**REPRESENTATIVE DNA VACCINES FOR BIODEFENSE AND EMERGING INFECTIOUS DISEASES**

A large number of studies, using various animal models, have been conducted over the past 15 years to examine DNA vaccine immunogenicity against a wide range of pathogens including viruses, bacteria, and parasites. Contrary to the popular belief, DNA vaccines are more complicated than simply inserting a randomly selected pathogen gene into a DNA vaccine vector. The process of developing a successful DNA vaccine is no different from any scientific discovery: trial and error. In the following sections, results from selected DNA vaccine studies are presented to illustrate various aspects of the development of DNA vaccines against many important biodefense and emerging infectious disease targets over the past decade.

**DNA Vaccines against Viral Agents**

**Human Immunodeficiency Virus Type 1 (HIV-1)**

Acquired immunodeficiency syndrome (AIDS) is the number one emerging infectious disease in the 20th century. It continues to be a worldwide pandemic in the new millennium. The development of an effective AIDS vaccine has been proven more challenging than initially anticipated. There does not appear to be natural adaptive immune protection against HIV-1 infection; hence, the correlate of protection to be induced by a vaccine is uncertain. As a retrovirus, HIV-1 integrates into the genome of host dividing cells, which in turn becomes the source of continuing
cannot be used for young children and older populations due to a lack of safety data in these groups. This restriction greatly reduces the utility of this new vaccine because young children and older people are particularly vulnerable to influenza infection.

The emerging threat of avian influenza as a potential cause of a future pandemic has raised issues of vaccine efficacy, production, and stockpiling. The old influenza vaccine technologies may not be sufficient in facing new challenges.

Influenza DNA vaccine development was another early key area for the field. Studies demonstrated the immunogenicity of DNA vaccines using either nucleocapsid (NP) or hemagglutinin (HA) antigens (Ulmer et al., 1993; Robinson et al., 1993; Fynan et al., 1993). A landmark study in the early 1990s showed that a DNA vaccine expressing NP was effective in eliciting NP-specific CTL and protection in mice from a subsequent challenge with a heterologous strain of influenza A virus, as measured by decreased viral lung titers, inhibition of mass loss, and increased survival (Ulmer et al., 1993). Subsequent studies from this and other groups confirmed the protective immune responses induced by NP DNA vaccines (Ulmer et al., 1998; Cox et al., 2002) or by the combination of DNA prime-adenoviral vector boost vaccines (Epstein et al., 2005), but there were also reports showing that NP DNA vaccine alone was not effective in protecting pigs against challenges with homologous virus (Macklin et al., 1998). However, later studies in ferrets and non-human primates demonstrated the efficacy of a DNA vaccine encoding three proteins, NP, HA, and M1 antigens (Donnelly et al., 1995; 1997). DNA vaccine expressing other influenza viral antigens have also been reported including the co-expression of both M1 and M2 antigens (Okuda et al., 2001), the co-delivery of NP and M2 DNA vaccines plus a low dose of HA-expressing DNA vaccines (Jimenez et al., 2007), and the use of NA-expressing DNA vaccines (Chen et al., 1998, 2000). Various levels of protection with these influenza DNA vaccines were achieved in mouse models. Protection against influenza B virus infection was also achieved with DNA vaccines expressing HA or NA antigens but not with NB or NP DNA vaccines (Chen et al., 2001).

The HA-expressing DNA vaccines appear to be the most protective influenza DNA vaccine and protection elicited by HA DNA vaccines correlates with titers of neutralizing antibody (Robinson et al., 1997). However, recent studies suggested that the HA antigens from H1 and H3 serotypes of influenza A viruses require different antigen designs for the induction of optimal protective antibody responses (Wang et al., 2006c). This may further affect the selection of HA antigens from avian influenza viruses. Until now, HA DNA vaccines have successfully elicited antibody responses and protection against several avian influenza viruses including H5N1 (Kodihalli et al., 1999), H5N2 (Kodihalli et al., 1997; Jiang et al., 2007), and H9N2 (Qiu et al., 2006). The H5 HA DNA vaccine has been shown to induce immune protection against not only a homologous, lethal H5 virus but also two antigenic variants in chickens (Kodihalli et al., 1997). The level of protection may vary in different animal models. Despite the success of inducing protective immunity against heterologous viral challenge in chickens, an HA DNA vaccine encoding for heterologous viruses did not prevent infection in mice. However, this HA DNA vaccine did prevent death associated with infection (Kodihalli et al., 1999).

New DNA vaccine technologies have also been incorporated into the design of improved influenza DNA vaccine designs. Codon optimization was shown to improve the immunogenicity and protective antibody responses (both HI and microneutralization antibodies) of HA DNA vaccines (Wang et al., 2006c; Jiang et al., 2007). Consensus-based DNA vaccines against H5N1 avian influenza viruses have been produced with initial results showing the possibility of inducing cross-reactive cellular immunity (Laddy et al., 2007). However, whether such consensus antigens can induce protective immune responses against different H5N1 viruses remains unconfirmed.

One of the key contributions of influenza DNA vaccine research was the demonstration that an HA DNA vaccine could induce HA-specific antibody responses induced in humans. Using a particle-mediated epidermal delivery (PMED) device, 3 groups of 12 healthy adult subjects received a single dose of 1, 2, or 4 μg of an HA DNA vaccine. The PMED influenza DNA vaccine elicited serum hemagglutination-inhibition antibody responses at all three dose levels with the highest and most consistent responses in subjects vaccinated with the highest dose. On day 56, sera HA-specific antibody responses in both 2 and 4 μg groups reached the levels required for influenza vaccine approval established by the Committee for Proprietary Medical Products (CPMP) in the European Union (Drape et al., 2006). This result was important because it demonstrated that a DNA vaccine was capable of eliciting protective immune response levels. One notable factor in this study was the use of the “gene gun” delivery method, which has proven to be more effective than needle injection of DNA vaccines in inducing immune responses in humans. The availability of the gene gun or other epidermal delivery methods will be important in the successful development of DNA vaccines for biodefense and emerging and neglected diseases.

II. FUNDAMENTAL ASPECTS OF VACCINOLOGY
Smallpox

The original smallpox vaccine, based on the vaccinia virus (VACV), facilitated the worldwide eradication of smallpox with the last case of natural smallpox infection reported in Somalia in 1977 (WHO, 1980). Despite the elimination of naturally occurring smallpox infection, the threat of its use as a biological weapon is compelling enough to develop a vaccine strategy to protect the large percentage of the population that no longer has immunity against this disease. Poxviruses are large viruses with a genome that encodes about 200 proteins. The size of the virus has complicated efforts to delineate the major PAs. Furthermore, the relative importance of humoral and cellular immunity in vaccine-induced protective immunity is uncertain.

In well-designed animal studies a DNA vaccination approach has recently identified several protective pox antigens. There are two forms of infectious poxvirus: the intracellular mature virus (IMV) and the extracellular enveloped virus (EEV). Studies have shown that vaccinia virus IMV-specific antigens, A27, L1, and D8, and EEV-specific antigens, A33 and B5, are immunogenic and protective, albeit variably, against VACV infection in animal models (Galmiche et al., 1999; Hooper et al., 2000, 2004; Fogg et al., 2004; Pulford et al., 2004; Sakhatksky et al., 2006, 2008; Xiao et al., 2007). These antigens induced high-titer serum antibodies and/or neutralizing antibodies against the vaccinia virus (Galmiche et al., 1999; Hooper et al., 2000, 2003, 2004, 2007; Héraud et al., 2006; Sakhatksky et al., 2006, 2008). A four–gene combination DNA vaccine that encodes two IMV (A27 and L1) and two EEV (A33 and B5) antigens was protective against VACV challenge in mice and against monkeypox virus challenge in nonhuman primates (Hooper et al., 2003). In a separate study, IMV antigen D8 was shown to be a highly immunogenic antigen that induced high-level protection, alone or in combination with the other pox antigens (Sakhatksky et al., 2006, 2008). However, these pox DNA vaccines, as well as the recombinant protein-based pox subunit vaccines, were not as protective as the live-attenuated vaccinia virus vaccine unless polyvalent formulations and/or multiple immunizations are used (Hooper et al., 2000, 2003; Fogg et al., 2004; Pulford et al., 2004; Sakhatksky et al., 2006, 2008).

Only limited studies have measured cell-mediated immune responses following immunization with various DNA vaccines against VACV challenge (Otero et al., 2006). A recent study demonstrated that mice immunized with the DNA plasmid encoding for the B5R antigen of VACV elicited strong EEV-induced IFN-γ responses (Pulford et al., 2004). Interestingly, contrary to previous studies, this study did not observe increased antibody responses in vaccinated mice but rather found that cell-mediated responses correlated with protection. In a DNA prime-protein boost study, rhesus macaques were immunized with a four-antigen formulation (L1R, A27L, A33R, and B5R) in the form of either DNA vaccines alone, Escherichia coli produced recombinant protein vaccines alone, or a combination of both vaccines. Animals that received the prime-boost immunization achieved the best protection (Héraud et al., 2006).

The DNA vaccination approach also provides a unique technical advantage in developing vaccines that are more closely related to smallpox antigens. It can permit the direct cloning and expression of antigen from variola major (Sakhatksky et al., 2008; Aldaz-Carroll et al., 2007) since the protective antigen genes can be chemically synthesized, even codon optimized, without directly working with the highly pathogenic variola major viruses, which are no longer available for research applications. In summary, DNA vaccination is particularly useful for the next generation of smallpox vaccines based on subunit antigens. It can deliver multiple protective antigens in one mixed formulation, elicit both antibody- and cell-mediated immune responses, and optimize the antigen sequences and designs for the protective antigens. Optimized genes can also be used to produce recombinant protein-based subunit vaccines.

Severe Acute Respiratory Syndrome Associated Coronavirus (SARS-CoV)

Severe acute respiratory syndrome (SARS), an acute respiratory disease cause by a newly recognized human coronavirus (SARS-CoV), was first reported in Asia in 2003. During the 2003 outbreak, approximately 8000 people worldwide became ill with 774 fatalities before the outbreak was finally contained (CDC, 2005). SARS is the first major emerging infectious disease in the 21st century. Although no final human vaccine has been developed, several candidate antigens have been discovered and some have been tested as DNA vaccines against SARS infection. Both the nucleocapsid protein (N) and the envelope spike protein (S) have been examined for their ability to induce protective immunity against SARS-CoV in mice.

DNA plasmids, encoding the N protein, have been shown effective in eliciting both humoral and cellular immunity in mice after two or three immunizations (Zhu et al., 2004; Zhao et al., 2005). Since the N antigen is not known to be a target for antibody-mediated protection, the majority of SARS-CoV DNA vaccines have incorporated the S protein as the key protective

II. FUNDAMENTAL ASPECTS OF VACCINOLOGY
antigen. No matter whether the entire S protein or fragments of the S protein are incorporated into the DNA vector for immunization, significant levels of S-specific immune responses were elicited including high-titer binding antibody (Yang et al., 2004; Kong et al., 2005; Zakhartchouk et al., 2005; Huang et al., 2006; Zhao et al., 2006; Hu et al., 2007; Wang et al., 2005b) or neutralizing antibody responses (Yang et al., 2004; Woo et al., 2005; Zakhartchouk et al., 2005; Wang et al., 2005b). In addition, S-specific cellular immune responses including both CD4+ and CD8+ T cell responses were detected with S DNA vaccines (Yang et al., 2004; Huang et al., 2006; Zhao et al., 2006; Hu et al., 2007). Even greater levels of immunity were observed when these S DNA vaccines were included as part of a prime-boost regimen (Kong et al., 2005; Woo et al., 2005; Zakhartchouk et al., 2005) or when a cytokine (IL-2) was included as the molecular adjuvant (Hu et al., 2007).

Although it has not been possible to determine the efficacy of most SARS DNA vaccines due to the limited availability of animal testing facilities capable of working with the SARS-CoV, it has been shown that some S DNA vaccines (i.e., deleted transmembrane domain or a truncated cytoplasmic domain) were effective in significantly reducing the viral load in the lungs of infected mice (Yang et al., 2004). However, a limitation of the SARS-CoV mouse model is its lack of clinical endpoints (e.g., disease or death) and the field is further limited by the lack of a generally accepted nonhuman primate model.

Results have been promising in the development of a DNA vaccine against SARS-CoV. However, more studies are needed, using better animal models that can assess the ability of the experimental vaccines to protect against infection and disease. It is important to note that use of the DNA vaccine platform proved to be nimble in facilitating the rapid development of highly immunogenic candidate vaccines against the sudden emergence of a new human infectious disease.

The Filoviruses: Ebola and Marburg

Infection with either Ebola or Marburg typically results in severe hemorrhagic fever in both humans and nonhuman primates. Four species of Ebola have been identified (i.e., Zaire, Sudan, Reston, and Ivory Coast) with the first known case occurring in Zaire in 1976. Marburg virus was identified in 1967 and named after the site where the infection occurred, Marburg, Germany. There is no effective prophylaxis or treatment for infection with either virus. While filoviruses are uncommon causes of human infection, the development of filovirus vaccines are warranted based on their remarkable lethality with mortality rates ranging from 23% (Marburg) to 90% (Ebola). There has been limited work on the development of live attenuated Ebola virus vaccines, but safety issues make this approach somewhat problematic.

Significant progress has been made in the development of DNA vaccines for both Ebola and Marburg viruses including the completion of a recent phase I human trial for an Ebola DNA vaccine. These vaccine strategies have targeted various viral proteins including both the secreted (sGP) and transmembrane (GP) forms of the glycoprotein and the nucleoprotein (NP). These DNA vaccines were tested in various animal models including mice, guinea pigs, and nonhuman primates, and in humans with promising immunogenicity results. In an early study, all three viral proteins were tested to determine their immunogenicity in mice and their protective efficacy in guinea pigs (Xu et al., 1998). Three 50μg injections of the NP and sGP DNA plasmids were effective, although to varying levels, in eliciting an antibody response while the GP DNA did not elicit a neutralizing response. However, the NP construct induced only minimal CTL responses, whereas the sGP and GP plasmids induced strong CTL responses. Furthermore, guinea pigs challenged approximately 2 months after the first immunization showed nearly complete protection: GP (6/6), sGP (5/6), and NP (4/4). Similar results were observed in mouse protection studies with the GP protein although as many as four immunizations were required (Vanderzanden et al., 1998; Riemenschnieder et al., 2003). Likewise a GP DNA prime-recombinant baculovirus-derived GP protein boost regimen afforded guinea pigs good protection. A separate study showed that DNA immunization and subsequent boost with adenovirus vectors that encoded either GP, NP, or a combination of the two conferred 100% protection in guinea pigs (Sullivan et al., 2000). Furthermore, antibody titers were increased in mice that received the GP DNA prime plus adenovirus vector boost when compared to DNA alone. Since the Ebola virus has to be modified in order to produce disease in rodents, primate models of infection would more closely emulate infection in humans. After three injections of naked GP DNA vaccines, several months of rest, and recombinant adenovirus based vaccine boost, antibody titers were increased 10- to 20-fold when compared to DNA immunization alone and there was 9- to 20-fold increase in cellular immune responses that appear to be driven by CD4+ T cells. This vaccination regimen was able to confer protection in four out of four monkeys and sterilizing immunity was observed in three out of four.

Recently, a phase I clinical trial was conducted to determine the safety and immunogenicity of a DNA vaccine for the Ebola virus in healthy volunteers.

II. FUNDAMENTAL ASPECTS OF VACCINOLOGY
(Martin et al., 2006). The DNA vaccine contained three plasmids in equal concentrations for the NP (Zaire strain), and two GP (one from the Zaire strain and the other from the Sudan/Gulu strain). This vaccine formulation was safe and well tolerated. DNA vaccine formulation elicited antigen-specific antibody responses to at least one of the three antigens in 100% of vaccinees and 19 out of 20 vaccines showed an antibody response to either GP antigen at one or more time points during the vaccination schedule. Similar to what was observed for monkey (Sullivan et al., 2000), CD4+ T cell responses were more robust than CD8+ responses with the GP antigens producing the greatest response when compared to NP.

The DNA vaccine platform has also been used to develop a vaccine against the Marburg virus although few studies have been conducted to date. Marburg virus GP-expressing DNA vaccine was administered to guinea pigs and serum antibodies were detected after three immunizations. This vaccine prevented death in 8 out of 10 animals that exhibited a 10-fold or higher increase in anti-Marburg virus antibodies 1 month after infection. However, a combination DNA prime plus baculovirus-expressed recombinant GP boost in adjuvant (one DNA prime and two protein boosts) conferred complete protection whereas neither vaccine modality did so when administered alone (Hevey et al., 2001). A second study observed complete protection in guinea pigs and protection in two of three monkeys vaccinated with a Marburg virus GP DNA plasmid (Riemenschneider et al., 2003). The antibody responses observed in the two monkeys that survived challenge were not significantly different from those in the monkey that did not survive, indicating that antibody levels alone do not necessarily predict protection against Marburg infection.

Results from the filovirus vaccine studies are promising in that they all indicate that vaccination with related DNA vaccines which encode for various Ebola or Marburg antigens can induce both cellular and humoral immune responses and that these vaccines can confer some level of protection from infection. More human trials will determine the efficacy of these vaccines.

Flaviviruses: West Nile Virus, Japanese and Tick-Borne Encephalitis Viruses, Dengue Virus

West Nile Virus  At the end of the summer of 1999, there was an outbreak of viral encephalitis in the New York City and surrounding areas, which was later determined to be caused by West Nile virus (WNV). This outbreak continued in the subsequent years in the United States in humans and in horses. No human or veterinary vaccines existed for WNV at the time of the initial outbreak. Different versions of DNA vaccines were developed and they all showed good immunogenicity. One version expresses the WNV capsid protein, and in vivo study in mice showed Th1 type immune responses in immunized animals (Yang et al., 2001). Another used a novel approach to express a full-length infectious Kunjin virus RNA. Kunjin virus is a genetically stable Australian flavivirus originally shown to be genetically and antigenically very closely related to several WNV strains, which later was reclassified as a WNV (Heinz et al., 2000). Sequence analysis revealed 98–99% amino acid homology between Kunjin and NY99, the WNV causing the initial 1999 New York outbreak. By using a further modified molecular clone with mutated NS1 gene, an attenuated Kunjin strain was produced and used as a vaccine. The use of DNA vaccine directing in vivo transcription of the full-length attenuated but infectious KUN viral RNA in mice led to full protection against the WNV NY strain (Hall et al., 2003).

Scientists at US CDC and Fort Dodge Laboratories, Inc., a division of Wyeth, developed the pCBWN DNA vaccine that expresses the WNV prM and E proteins (Davis et al., 2001). A single intramuscular injection of pCBWN DNA vaccine induced protective immunity, preventing WNV infection in both mice and horses (Davis et al., 2001). This vaccine was licensed by the US Department of Agriculture on July 18, 2005, becoming the first DNA vaccine approved by a regulatory agency. In granting full licensure, USDA’s Center for Veterinary Biologics determined that the vaccine’s safety and efficacy have been satisfactorily demonstrated.

In April of 2006, a DNA vaccine targeting WNV, developed by scientists at the NIH’s Vaccine Research Center (VRC) in collaboration with Vical, Inc., entered into a phase I clinical trial with a targeted completion date of October of 2007 (NIH, 2007). This experimental vaccine encodes two key WNV surface proteins, the precursor transmembrane (prM) protein and the envelope (E) protein. The vaccine was administered via a Biojector 2000 device. Participants received three injections (i.m., 1 ml of vaccine per immunization for a total of 3 ml) at study days 0, 28, and 56. The primary study endpoint is vaccine safety, and secondary immunogenicity endpoints include ELISA and neutralizing antibody responses and ELISPOT and ICS staining assays for WNV-specific T cell responses. Results of the phase I clinical trial are not yet available (Martin et al., in press).

Japanese and Tick-Borne Encephalitis  The viral encephalitides, in particular, Japanese encephalitis (JE)
and Tick-borne encephalitis (TBE), are transmitted to humans and animals by infected mosquitoes or ticks. Infection with these viruses results in various symptoms ranging from fever to encephalitis. While there are inactivated vaccines against these infections, the immunization regimens require multiple doses without producing long-term immunity. Repeated immunizations increase the cost of vaccination campaigns in the developing countries. Some of these inactivated vaccines are not licensed in the United States. Efforts have been made to utilize DNA vaccine technology in order to produce a vaccine against these encephalitides with improved efficacy, greater ease, and lower cost of production and administration.

JEVAX boost regimen induced long-lasting immunity, in inducing neutralizing antibody. The DNA prime-boost regimens have proven to be superior to other methods of maximizing vaccine immunogenicity. Two studies showed that boosting an E/prM DNA vaccine formulation with either sub-viral extracellular particles (Imoto and Konishi, 2005) or inactivated commercially available JEVAX (Konishi et al., 2003b, 2003c) was superior to either vaccine modality alone in inducing neutralizing antibody. The DNA prime-JEVAX boost regimen induced long-lasting immunity, as titers were still 1:80 to 1:160, as of 21 weeks following the last immunization (Imoto and Konishi, 2005).

Furthermore, DNA vaccines encoding for prM/E viral proteins afforded greater cross protection against heterologous JE viral strains when compared to inactivated virus vaccines (Wu et al., 2003a).

TBE is most frequently caused by Russian spring summer encephalitis (RSSE) and Central European encephalitis (CEE). DNA vaccines developed against TBE have been successful in animal models in eliciting protective immune responses against viral challenge. As with the other flaviviruses, the most effective vaccines have encoded the prM and E genes. DNA vaccines expressing the prM and E genes of RSSE and CEE, delivered by a gene gun, have been shown to protect both mice (Schmaljohn et al., 1997) and monkeys (Schmaljohn et al., 1999) against homologous and heterologous viral challenge while inducing long-lasting neutralizing antibody responses. Protection appears to be mediated by neutralizing antibody based on passive transfer studies in mice using high neutralizing titer antibodies generated in monkeys by RSSE or CEE DNA immunization (Schmaljohn et al., 1999).

Dengue Viruses Currently, there is no approved vaccine against dengue viruses (DEN). These viruses, spread by the Aedes aegypti mosquito, are endemic throughout tropical and sub-tropical regions of the world. There are approximately 100 million infections per year, resulting in both dengue fever and the more serious, dengue hemorrhagic fever (DHF). Although various vaccine approaches including live attenuated, inactivated whole virion and recombinant protein-based subunit vaccines have been tested, the presence of four serotypes of DEN and complicated immunopathologic mechanisms involved in DHF have greatly reduced the speed of developing an effective DEN vaccine. Several DNA vaccine formulations have been developed against all four serotypes (DEN-1, DEN-2, DEN-3, and DEN-4), and in some cases, a tetravalent formulation has induced memory immune responses against both homologous and heterologous viral challenges (Konishi et al., 2006). Typically, the DNA vaccines that most effectively elicit both humoral and cell-mediated immune responses contain the DEN premembrane/membrane (prM) and envelope (E) genes. Immunization with plasmids encoding these genes have been shown to engender antigen-specific antibody and neutralizing antibody responses and protection against DEN-1 in mice (Raviprakash et al., 2000a; Konishi et al., 2003b, 2003c) and nonhuman primates (Kochel et al., 2000; Raviprakash et al., 2000b) and against DEN-2 in mice (Konishi et al., 2000, 2003b, 2003c; Putnak et al., 2003) and nonhuman
primates (Putnak et al., 2003). Additional studies have examined the ability of a tetravalent DNA vaccine formulation, encoding the prM and E genes of all four dengue serotypes, to induce immune responses and found that antibodies specific for each serotype could be induced following immunization (Mota et al., 2005; Apt et al., 2006; Konishi et al., 2006; Raviprakash et al., 2006). Furthermore, immunization with such a tetravalent DNA vaccine was able to elicit neutralizing antibody responses 4 days following the viral challenge against both the homologous virus used for the challenge assay and against the heterologous viruses (i.e., not used for challenge) (Konishi et al., 2006).

The ability of the dengue virus nonstructural protein 1 (NS1) to elicit immune response has also been examined with contradictory results. In one study, a DNA vaccine encoding for the NS1 failed to induce antigen-specific antibodies after one to three immunizations; however, greater responses were observed following challenge with DEN-2 in vaccinated mice when compared to the unvaccinated group (Wu et al., 2003b). In contrast, mice vaccinated with a DNA vaccine based on the NS1 protein from DEN-2 displayed high levels of antibodies (Costa et al., 2006). While it appears as though these two DNA vaccines encoded the same antigen from the DEN-2 virus, slight differences in immunization schedule and/or dosing may have led to the different results. Despite the fact that antibodies were not detected after one to three immunizations with the NS-1 from DEN-2 (Wu et al., 2003b), the NS-1 DNA vaccine conferred protection against lethal challenge in both studies. Furthermore, newborn mice born to dams that were either infected with the DEN-2 virus (100% of litters) or immunized with the NS-1 DNA vaccine (75% of litters) were also protected against the dengue virus following challenge (Wu et al., 2003b).

**Alphaviruses: The Equine Encephalitides**

The equine encephalitides, including Venezuelan equine encephalitis (WEE) and western equine encephalitis (WEE), are mosquito-borne viruses that can be transmitted to birds, horses, and humans. The clinical findings of these infections range from mild flu-like symptoms to encephalitis, coma, and death. While equine vaccines against some of these viruses have been developed, no human vaccines are currently available. DNA vaccine technology has been used to develop vaccines against VEE and WEE, which is of particular importance as these viruses have the potential to be used as biological warfare agents. In a first study, DNA immunization with a plasmid encoding the major structural proteins of WEE (capsid, E3, E2, 6K, and E1) afforded protection to 50–100% of mice challenged with a lethal dose of one of three strains of WEE. Furthermore, immunized mice showed a strong T-cell proliferation response to purified E2 protein and a partial response to E1 when compared to controls (Nagata et al., 2005). A DNA vaccine has also been developed against VEE. In this study, a replication-defective adenovirus (Ad5) was used to boost the immune responses following the delivery of the DNA vaccine that expressed a region encoding for the E3, E2, and 6K structural proteins (Perkins et al., 2006). Following DNA immunization, no difference was observed in VEE virus-specific IgG response; however, following one intranasal boost with a homologous Ad-based vaccine a significant increase in VEE virus-specific IgG was observed. The prime-boost strategy also significantly enhanced protection against aerosol VEE challenge.

**Rabies**

Infection with the rabies virus causes acute encephalitis with an extremely high fatality rate. Both inactivated and live-attenuated human rabies vaccines exist but each has its own limitations. DNA vaccination has proven successful against rabies infection in various animal models. In a first series of studies, mice immunized with the rabies glycoprotein displayed rabies virus glycoprotein-specific cell-mediated and humoral immune responses, in addition to complete protection against viral challenge (Xiang et al., 1994, 1995). Subsequent studies showed that a DNA vaccine encoding for a rabies glycoprotein induced a 176-fold increase in geometric mean neutralizing antibody titers following a boost 190 days after the primary immunization in nonhuman primates (Lodmell et al., 1998a, 1998b). Additional studies by this group showed that gene gun administration afforded mice protection against rabies virus challenge and that protective levels of antibodies persisted for over 300 days (Lodmell et al., 1998a, 1998b). They also showed that different prime-boost regimens elicited varying levels of neutralizing antibody responses: priming with a DNA vaccine, a recombinant vaccinia virus vaccine (RVV), or the commercially available inactivated rabies vaccine followed by boosting with either the DNA or inactivated vaccines resulted in rapid and long-lasting antibody responses (Lodmell and Ewalt, 2000). This group also demonstrated that DNA vaccination could be used as an effective post-exposure vaccine (Lodmell and Ewalt, 2001; Lodmell et al., 2002).

**Arenaviruses**

The arenaviruses are a family of viruses that are usually transmitted from rodents to humans and
include viruses such as lymphocytic choriomeningitis (LCM), Lassa virus, Junin virus, and Machupo virus. Infection with these viruses presents as a wide range of symptoms including fever and flu-like symptoms, meningitis, encephalitis and severe, fatal hemorrhagic fever. Few studies have been conducted in the development of DNA vaccines against these viral agents. In one study, a single immunization with a DNA plasmid encoding the full-length Lassa nucleoprotein was able to induce CD8+ T cell responses in mice and afforded protection against two arenaviruses, LCMV and the Pichinde virus (Rodriguez-Carreno et al., 2005). Unfortunately, DNA immunization against LCMV in another study did not show favorable results following intracranial viral challenge. Not only were some vaccinated mice not afforded protection against viral challenge but they also displayed greater immunopathology instead as evidenced by clinical symptoms of encephalitis and eventual death (Zarozinski et al., 1995). This study represented a rare example of where a DNA vaccine may worsen the outcome of viral infection. Although it is believed that such unexpected results are part of the unique immunopathogenesis of LCMV infection, careful selection of PAs may also be important for the development of DNA vaccines.

**DNA Vaccines against Bacterial Agents**

**Anthrax**

Anthrax is one of the early model pathogens used to assess the feasibility of applying DNA vaccine technology to the development of a vaccine against bacterial infection. During the early days of DNA vaccine research, many considered DNA vaccine as a useful tool for the induction of cell-mediated immune responses but did not appreciate the potential of DNA vaccine to induce high-quality protective antibody responses. Because antibody plays a central role in the control of most of the bacterial infections, initially there were only limited DNA vaccine studies for bacterial agents, and the detailed analysis of DNA vaccine against anthrax provided an important milestone in this area.

Anthrax infection, caused by Bacillus anthracis, is a disease with high mortality. It attracted great attention in the United States following intentional release of the agent in 2001. Although a licensed vaccine against anthrax has been developed (Bioport Corporation, Lansing, MI), multiple immunizations are required in order to maintain protective levels of immunity, and safety of previous manufacturing process has been an issue. In a first study to establish the possibility of using a DNA vaccine to induce a protective immune response against anthrax, Gu et al. (1999) developed a DNA vaccine that contained a portion of the protective antigen (PA), the protein responsible for initial binding of the bacteria to the target cell. Antibodies against the PA have been confirmed to protect guinea pigs and rabbits against anthrax challenge (Little et al., 1997; Kobiler et al., 2002; Riemenschneider et al., 2003; Mohamed et al., 2005). After multiple inoculations with the PA-expressing DNA plasmid, anti-PA IgG antibodies were observed in all immunized mice, levels of which increased with subsequent boosting. Using an in vitro neutralization assay, this study showed that pooled serum from twice-boosted immunized mice was 100% protective against anthrax at a 1:20 dilution while 50% protection was observed at a 1:100 dilution. In an in vivo protection assay, the authors found that mice immunized with the plasmid PA DNA were completely protected against challenge while no control mice survived. Subsequent studies showed that plasmid DNA prime encoding for the PA antigen followed by a recombinant protein (rPA) boost significantly increased antibody levels and protective efficacy against anthrax challenge in both mice (Williamson et al., 2002) and rabbits (Galloway et al., 2004). However, increases in antibody titers and protective efficacy were also observed in rabbits without the recombinant protein boost (DNA alone) and these effects were long-lasting (Hermanson et al., 2004). In an examination of passive immunotherapy of anthrax infection, rabbits were immunized with a codon-optimized plasmid DNA vaccine encoding PA, with the most effective immunization strategy being the twice DNA prime + one time protein boost regimen. Immune rabbit sera, shown to contain ELISA and neutralization antibodies, were transferred to mice either 1 h prior to or 1 h after lethal anthrax challenge. All mice that received the hyperimmune rabbit sera 1 h prior to anthrax challenge survived 14 days after challenge (end of observation period), while in mice that received the sera 1 h after challenge, only one out of 5 mice died (2 days after challenge), indicating that protection against anthrax could be achieved through passive administration of antibodies that resulted from DNA immunization (Herrmann et al., 2006). Together, these studies indicate that DNA immunization was effective in producing high-titer antibody responses, which conferred protection against aerosolized anthrax challenge in both mice and rabbits.

**Plague**

While Yersinia pestis (Y. pestis), the causative agent of plague, is endemic in many rodent populations throughout the world, it is of great concern due to...
its potential as a biological weapon. Plague can be transmitted by flea bite causing bubonic plague or through aerosolization to cause pneumonic plague. It is pneumonic plague that is the greatest concern for biodefense as this form of the disease progresses very rapidly, is highly transmissible, and requires therapy shortly after infection. Without early intervention, pneumonic plague infection is most certainly fatal. These features of pneumonic plague along with its possible use as a biological weapon present a strong argument for the development of an effective vaccine.

Currently, there is no known effective clinical vaccine against pneumonic plague. While a variety of whole-cell, killed plague vaccines were developed, their efficacy in protection against pneumonic plague is limited in small animal models (Williamson et al., 1997, 2000). DNA vaccine technology offers advantages in establishing a safe and effective platform by which protection against a variety of plague antigens can be achieved. A first study compared two DNA vaccine delivery methods (i.e., gene gun delivery of 0.6 or 4 μg plasmid DNA versus intramuscular or intradermal delivery of 50 μg plasmid DNA) for the administration of a plasmid DNA encoding for the V antigen of \( Y. pestis \). Antibody responses, obtained at 21 days post vaccination, were observed in all vaccinated groups. However, the highest geometric mean titers were observed in mice that were vaccinated with 4 μg DNA via gene gun, with the least effective being intradermal administration of 50 μg DNA (Bennett et al., 1999). Very little protection was afforded in mice inoculated (i.m. or gene gun) with this “optimized” V antigen-expressing DNA vaccine (addition of a CMV promoter) as only two out of six mice (gene gun) survived a subcutaneous challenge with \( Y. pestis \) (Garmory et al., 2004).

Greater success was achieved when mice were administered a DNA vaccine with a modified V antigen (LcrV) using a human tissue plasminogen activator (tPA) signal sequence (Wang et al., 2004b). In this study, not only were strong V antigen-specific antibodies elicited but mice were also protected against an intranasal challenge with \( Y. pestis \). This is of particular importance as this model reflects infection with the more deadly mucosal challenge of plague. The tPA-V DNA vaccine was able to induce excellent secreted V antigens in oligomeric form, which has been hypothesized to be important in eliciting protective antibody responses.

In addition to the V antigen, immunity to the F1 capsular protein has been shown to afford protection against plague and F1-expressing DNA vaccines have been reported effective in eliciting protection. Grosfeld et al. (2003) compared three F1 DNA vaccines that carried different signals for cellular localization for their ability to induce antibodies and protection against \( Y. pestis \). Results showed that although the full-length form of the F1 antigen was a poor immunogen, one altered plasmid in particular (deF1, containing the coding sequence of the F1 protein but a 21-amino acid long sequence that codes for a putative bacterial signal peptide is missing) induced substantial antibody titers and protective immunity (38 out of 39 mice) against a subcutaneous \( Y. pestis \) challenge. It was determined that mice that received a gene gun inoculation of deF1 were protected against 4000 LD\(_{50}\) \( Y. pestis \) challenge and 10 out of 10 animals survived this challenge.

By using the DNA vaccine technology, a group of “2nd line” antigens from \( Y. pestis \) have been studied for their ability in eliciting protective antibody responses (Wang et al., 2008b). \( Y. pestis \) outer proteins (YopB, YopD, and YopO), plasminogen activator protease (Pla), and Yop secretion protein F (YscF) are necessary for the full virulence of \( Y. pestis \). They have been proposed as potential PAs for vaccines. DNA immunization was used as a tool to study the relative protective immunity of these individual antigens with a standardized intranasal challenge system in BALB/c mice. The natural full-length gene sequences for most of these \( Y. pestis \) proteins did not display a good level of protein expression in vitro when delivered by a DNA vaccine vector into mammalian cells. As a result, the overall immunogenicity of these wild-type gene DNA vaccines was low in eliciting antigen-specific antibody responses. However, gene sequence modifications, including the removal of hydrophobic regions and the addition of signal peptide sequences with high secretion potential, significantly improved the level of protein expression, as well as the immunogenicity of these antigens in eliciting high-level antigen-specific antibody responses in mice. Modified YopD, YopO, and YscF antigens were able to protect immunized mice, to varying degrees, against the lethal challenge of \( Y. pestis \) Kim strain (5000 cfu) by intranasal inoculation while no protection was observed with either the YopB or Pla antigens (Wang et al., 2008b). Results of this study demonstrate that DNA immunization is useful in screening, optimizing, and comparing the antigen design and immunogenicity of candidate antigens for the development of a subunit-based plague vaccine. This approach will be useful to expand the search for additional protective antigens against highly virulent \( Y. pestis \) infection.

Results from these studies indicate that protection against both subcutaneous and intranasal plague, indicative of bubonic and pneumonic plague, respectively, can be conferred through administration of DNA vaccines encoding different \( Y. pestis \) antigens. The development of broadly active vaccines against plague is important to defend against any ill use of this...
pathogen as a biological weapon, with or without any intentional modification of the antigens to escape from the protection elicited by the original vaccination.

**Botulism**

Botulism is a very rare but serious paralytic disease caused by the bacterium, *Clostridium botulinum*. Historically, three natural forms of botulism existed: food-borne, wound, and infant. However, there also exits a man-made, inhalation form of botulism that is lethal in the microgram dose range and hence represents a highly dangerous potential biothreat. Seven types of non-cross-reactive botulinum neurotoxin exist (A through G) and a licensed trivalent antitoxin, available from the CDC, contains neutralizing antibodies against types A, B, and E, for treatment against botulism toxin poisoning (CDC, 2007). An additional investigational heptavalent antitoxin has been shown to be effective against all known strains of botulism. It is this heptavalent formulation that has been contracted into the Strategic National Stockpile beginning in 2007 (HHS, 2006). However, it has been shown that some strains of botulism are capable of producing mixtures of two toxin types (Cordoba et al., 1995). It is also now known that there are subtypes under each of the serotypes, indicating that the understanding of cross protection and the development of appropriate vaccine strategies will remain a great challenge.

Few studies have examined the feasibility of using DNA vaccines to protect against botulism. In an early study, mice were immunized with a DNA vaccine expressing the carboxyl terminal 50 kDa fragment of the type A toxin, which was placed in two designs: pJT-1, a construct that leads to MHC I presentation, or pJT-2 that leads to MHC II presentation (Clayton and Middlebrook, 2000). After challenge with botulinum serotype A (or in a cross-protection study serotypes B or E), it was observed that the pJT-1 construct was ineffective in inducing protection against challenge with botulism (serotype A 25 LD₅₀). However, mice that received the pJT-2 construct (at least 10 μg DNA) were afforded some level of protection when challenged at week 11. While 50 μg of DNA increased the rate of survival (9 out of 10 challenged with serotype A 25 LD₅₀), a minimum of 11 weeks had to pass prior to challenge as no protection was afforded if challenge was presented at 4 or 7 weeks after immunization. Furthermore, these results showed that no cross protection was afforded by pJT-2 DNA immunization against serotypes B or E, as none of the 10 animals survived the challenge.

Additional studies showed that a DNA vaccine against botulinium subtype E, which is a very rare form of botulism, was found to protect mice against lethal challenge with the same serotype (Bennett et al., 2003; Jathoul et al., 2004). In the first study by this group, a minimum of three i.m. injections of the F subtype-specific DNA vaccine, pABFHc2, which contained a signal sequence after the Hc domain, given over a 4-week period, was sufficient to protect 100% of mice (10 out of 10) against 10⁴ MLD of botulinum toxin subtype F, and a single dose protected 60% of mice when they were challenged no less than 28 days after vaccination (Bennett et al., 2003). Additional results from this study indicated that a relationship may exist between FHc-specific antibody levels and survival in the challenge assay, and that a DNA prime-protein boost regimen induced greater levels of serum antibodies when compared to both DNA alone and protein alone. Based on these findings, DNA vaccine appears useful for the design and optimization of a future botulinum vaccine that can protect from multiple serotype toxins.

**Helicobacter pylori**

Gastritis, peptic ulcers and gastric cancer have all been linked to infection with the *Helicobacter pylori* bacterium. While only two studies have examined the possibility of using DNA vaccine technology to induce antibodies and protection against *H. pylori* infection, the studies were successful (Todoroki et al., 2000; Miyashita et al., 2002). The difference between studies was the DNA vaccine encoding antigen, the enzyme catalase, or the heat shock protein A or B (HspA and HspB, respectively). Both DNA vaccine formulations induced high-titer serum antibodies when compared to the empty DNA vector control. For the DNA vaccine encoding catalase, serum antibody levels were detected beginning 4 weeks after the final immunization with peak levels by 3 months post immunization. Additional analyses included measurement of bacterial colonization in stomach after challenge with *H. pylori*, and all three DNA vaccine formulations (catalase, HspA, and HspB) afforded protection against *H. pylori* replication. The results support the potential use of a DNA vaccine to protect against *H. pylori* infection. As more antibiotic-resistant strains of this bacterium develop, there will be a greater need for the development of an *H. pylori* vaccine.

**Tuberculosis**

The *Mycobacterium tuberculosis* (TB) vaccine Bacille Calmette-Guerin (BCG) is widely used outside the United States in settings of high TB prevalence. Unfortunately, BCG is not completely effective in preventing TB infection, it can complicate interpretation of the tuberculin skin test, and it has not prevented

II. FUNDAMENTAL ASPECTS OF VACCINOLOGY
the emergence of extensively drug-resistant TB strains. Despite its shortcomings, BCG will likely continue to be used in afflicted countries as it does reduce the mortality rates against the more serious forms of childhood TB (e.g., disseminated and meningeal TB) (ACET, 1998). It is clear that more effective TB vaccines are needed and numerous studies have explored the potential of using DNA vaccination technology to facilitate the development of new and more effective vaccines.

The majority of TB DNA vaccine studies have focused on a family of three protein complexes of the Ag complex: Ag85A, Ag85B, and Ag85C. These antigens are prime targets because they are both found on the cell surface and are also secreted from the bacterium (Wiker and Harboe, 1992). An initial study examined the immunogenicity of a DNA vaccine encoding all three forms of the Ag85 complex (Ag85A, B, and C) and observed high-titer antibody responses in immunized mice (Ulmer et al., 1997). A subsequent study showed that the secreted form of Ag85A induced higher levels of serum antibodies at lower DNA doses and conferred protection against a highly virulent strain of M. tuberculosis when compared to the mature form of the protein (Baldwin et al., 1999). DNA vaccines encoding the Ag85A and Ag85B proteins but not Ag85C induced strong Th1-like responses (e.g., increased levels of IL-2, IFN-γ, and TNF-α) towards native Ag85 protein (Lozes et al., 1997), a broader T cell response than observed with natural M. tuberculosis infection and increased CD8+ -mediated cytotoxicity (Denis et al., 1998). In addition to these cellular immune responses, protection against challenge was conferred by DNA vaccines encoding the Ag85A protein in mice (Lozes et al., 1997; Denis et al., 1998) and guinea pigs by intramuscular injection (Baldwin et al., 1998) and gene gun inoculation (Sugawara et al., 2003) and for the Ag85B protein in mice (Lozes et al., 1997).

Additional studies have utilized various “prime-boost” strategies to increase both the immunogenicity and protective efficacy of Ag85-encoded DNA vaccines. One such study primed with a DNA vaccine encoding the Ag85 protein and subsequently boosted with purified Ag85 protein. This “prime-boost” strategy increased IL-2 and IFN-γ responses, increased IFN-γ producing CD4+ cells, increased IgG2a isotype antibody responses, and increased the efficacy of the DNA vaccine against M. tuberculosis challenge (Tanghe et al., 2001). A second study examined the effects of DNA prime and subsequent boost with the current TB vaccine, BCG, and found that the DNA + BCG regimen was more effective than BCG alone in protecting against TB challenge (Feng et al., 2001; Ferraz et al., 2004) and that CD8+ T cell depletion attenuated this protection (Feng et al., 2001).

DNA vaccines have also been developed to target other TB antigens including hsp65 (Tascon et al., 1996; Ferraz et al., 2004), hsp70 (Lowrie et al., 1999; Ferraz et al., 2004), the alanine–proline-rich antigen (Apa) (Ferraz et al., 2004), and MPT-63, MPT-64, MPT-83, ESAT-6, KatG, and other antigens, either alone or in various combinations (Morris et al., 2000; Repique et al., 2002; Cai et al., 2005a, 2005b). High levels of specific IgG antibodies were observed against Ag85A, MTP-64, and MPT-83 and mice immunized with individual DNA vaccines (Cai et al., 2005b) or a construct encoding all the antigens (Cai et al., 2005a) were protected against TB challenge. While individual DNA vaccines encoding the MPT-63 and MPT-83 antigens provided only partial protection against TB challenge, the multivalent MPT-63, MPT-83, ESAT-6, and KatG DNA vaccine produced antigen-specific cell-mediated and humoral immune responses and a stronger protective response against TB challenge when compared to the current BCG vaccine (Morris et al., 2000). Studies examining the efficacy of DNA vaccines that encode for the heat shock proteins of M. tuberculosis found that these proteins were able to decrease the numbers of live bacteria in both the spleen and the lungs of infected mice after only one dose of the hsp65 DNA vaccine, with lower effects observed with the hsp70 and ESAT6 DNA vaccine formulations (Lowrie et al., 1999).

DNA Vaccines against Parasites

Malaria

Malaria is transmitted through mosquitoes and remains a major global public health problem. Each year 350–500 million new cases of malaria occur throughout the world with approximately 1 million deaths occurring mostly in children in sub-Saharan Africa. Although antimalarial drugs are effective in the prevention and treatment of malaria infections, the development of vaccines continues to be the ultimate goal for malaria control.

Significant developments have been made through clinical trials that have tested various designs of DNA vaccines against malaria. In the first set of human DNA vaccine studies, a plasmid DNA vaccine encoding the Plasmodium falciparum circumsporozoite protein (PICSP) produced low CMI responses in human volunteers even with the use of a PMED (Wang et al., 1998, 2001). High DNA dose (2500 μg per immunization) was effective in increasing positive responses, and antigen-specific CTL responses were observed (Wang et al., 1998). Measurable antibody responses were not detected in any of the volunteers who had received this safe and well-tolerated malaria DNA

II. FUNDAMENTAL ASPECTS OF VACCINOLOGY
vaccine. In later studies, a DNA prime-recombinant vaccinia virus Ankara (MVA) boost approach was developed to further enhance the immunogenicity of malaria DNA vaccines [for recent review, see (Gilbert et al., 2006)]. A unique chimeric antigen, ME-TRAP, was produced to express the thrombospondin related adhesion protein (TRAP) coupled with a string of multiple epitopes (ME) (18 T-cell epitopes and 2 B-cell epitopes from various pre-erythrocytic *P. falciparum* antigens) (Moorthy et al., 2003). DNA immunization elicited low-level T-cell responses that were amplified by a boost with MVA ME-TRAP. More significantly, intramuscular DNA priming was more effective than gene gun-mediated priming when combined with a MVA boost. The combination immunization regimen produced partial protection, which manifested as delayed parasitemia after sporozoite challenge with a different strain of *P. falciparum*. Recent studies have further demonstrated that the DNA prime-MVA boost vaccine encoding TRAP produced stronger ex vivo IFN-γ ELISPOT responses than did a similar DNA prime-MVA boost vaccine encoding the circumsporozoite (CS) antigen. Importantly, the latter vaccine could not protect healthy malaria-naïve adults against *P. falciparum* sporozoite challenge as did the TRAP-expressing DNA prime-MVA boost vaccine (Dunachie et al., 2006).

A different type of malaria vaccine prime-boost approach was tested in humans. The combination of a PfCSP DNA vaccine prime with the boost of a known protective recombinant protein RTS,S, which is a fusion protein of the *P. falciparum* PICSP protein and the hepatitis B virus surface antigen (HBsAg). This construct encodes several CD4+ and CD8+ T-cell epitopes. It was administered to volunteers who had received the DNA vaccine (prime) in an earlier trial (Le et al., 2000) or naïve volunteers. Volunteers who were primed with PICSP DNA vaccine had protective CD4+ T-cell and antibody responses (effects of protein boost) in addition to the previously observed CD8+ CTL and IFN-γ responses (effects of DNA prime). The naïve volunteer group exhibited only antibody and CD4+ T-cell responses, and not CD8+ T-cell responses (Epstein et al., 2004; Wang et al., 2004a). This finding suggested that the PfCSP DNA vaccine was not effective for the induction of antibody responses, either alone or as a priming vaccine.

In an attempt to expand the breadth of protective immune responses, volunteers were immunized with a mixture of five malaria DNA vaccines expressing different *P. falciparum* pre-erythrocyte stage antigens in conjunction with an “adjuvant” plasmid encoding human granulocyte-macrophage colony-stimulating factor (hGM-GSF) (Wang et al., 2005a). IFN-γ responses were detected to multiple class I and/or class II restricted T-cell epitopes from all five antigens (CSP, EXP-1, LSA-1, SSP2, and LSA-3) with no statistical difference between the groups. Additional analyses revealed that in volunteers who received DNA alone (i.e. without hGM-CSF) IFN-γ responses to both class I and class II peptides were boosted after challenge with *P. falciparum* parasites while only responses to class II peptides were boosted in volunteers who were vaccinated with DNA and hGM-GSF. Volunteers who received hGM-CSF actually had a reduced frequency of IFN-γ responses to class I peptides. More importantly, none were protected against malaria challenge, further raising the issue of antigen selection, since previous studies have demonstrated that only a DNA prime-MVA boost vaccine expressing ME-TRAP antigen was able to induce partial protection (Gilbert et al., 2006).

**CONCLUSIONS**

Tremendous strides have been made in the last two centuries in the control of human infectious diseases through the development of continuously improved vaccination technologies. The discovery of DNA immunization in 1992–1993 fundamentally changed our view of the nature of a vaccine: genetic material that encodes for antigens, rather than the actual antigens themselves, as the effective components of DNA vaccines. This new vaccine technology could not come at a more important time in history given the increasing threat of emerging and reemerging infectious diseases and a renewed concern regarding the use of biological agents for purposes of bioterrorism. Since its inception, DNA vaccination technology has undergone significant advancements and many candidate human vaccine formulations have already been developed. Improved modes of administration, the use of codon and antigen gene optimization, and the implementation of vaccination DNA prime/boost regimens have led to the quick progression of DNA vaccines from research laboratory benches to human clinical trials. Biodefense and emerging infectious disease targets have made a significant contribution to this important process.

**References**

ACET, A. Development of new vaccines for tuberculosis recommendations of the advisory council for the elimination of tuberculosis (ACET). MMWR 1998; 47:1–6.

Aldaz-Carroll, L., Xiao, Y., Whitbeck, J.C., Ponce de Leon, M., Lou, H., Kim, M., Yu, J., Reinerz, E.L., Isaacs, S.N., Eisenberg, R.J. and Cohen, G.H. Major neutralizing sites on vaccinia virus
glycoprotein B5 are exposed differently on variola virus ortholog Bv. J. Virol. 2007; 81(15):8131–8139.

Andre, S., Seed, B., Eberle, J., Schraut, W., Ballmann, A. and Haas, J. Increased immune response elicited by DNA vaccination with a synthetic gp120 sequence with optimized codon usage. J. Virol. 1998; 72(2):1497–1503.

Apt, D., Raviprakash, K., Brinkman, A., Semyonov, A., Yang, S., Skinner, C., Diehl, L., Lyons, R., Porter, K. and Pumnoben, J. Tetravalent neutralizing antibody response against four dengue serotypes by a single chimeric dengue envelope antigen. Vaccine 2006; 24(3):335–344.

Baldwin, S.L., D’Souza, C., Roberts, A.D., Kelly, B.P., Frank, A.A., Liu, M.A., Ulmer, J.B., Huygen, K., McMurray, D.M. and Orme, I.M. Evaluation of new vaccines in the mouse and guinea pig model of tuberculosis. Infect. Immun. 1998; 66(6):2951–2959.

Baldwin, S.L., D’Souza, C.D., Orme, I.M., Liu, M.A., Huygen, K., Denis, O., Tang, A., Zhu, L., Montgomery, D. and Ulmer, J.B. Immunogenicity and protective efficacy of DNA vaccines encoding secreted and non-secreted forms of Mycobacterium tuberculo-

Barnett, S.W., Rajasekar, S., Legg, H., D’Souza, Y., Matsuo, K., Asanuma, H., Takahashi, H., Chang, G.J., Hunt, A.R. and Davis, B. Prevention. 2007, 11/05/2007. Retrieved 11/05/2007, 2007, from http://www.cdc.gov/ncidod/srp/drugs/formulary.html.

Clayton, J. and Middlebrook, J.L. Vaccination of mice with DNA encoding a large fragment of botulinum neurotoxin serotype A. Vaccine 2000; 18(17):1855–1862.

Cordoba, J.J., Collins, M.D. and East, A.K. Studies on the gene encoding botulinum neurotoxin type A of Clostridium botuli-

Costa, S.M., Paes, M.V., Barreto, D.F., Pinhão, A.T., Barth, O.M., Queiroz, J.L., Armôa, G.R., Freire, M.S. and Alves, A.M. Protection against dengue type 2 virus induced in mice immu-

Cox, R.J., Mykkeltveit, E., Robertson, J. and Haas, L.R. Non-lethal viral challenge of influenza haemagglutinin and nucleoprotein DNA vaccinated mice results in reduced viral replications. Scand. J. Immunol. 2002; 55(1):14–23.

Denis, O., Tanghe, A., Pailliet, K., Jurion, F., van den Berg, T.P., Vanonckelen, A., Ooms, J., Saman, E., Ulmer, J.B., Content, J. and Huygen, K. Vaccination with plasmid DNA encoding mycobac-

Desrosiers, R.C., Wyand, M., Kassler, L.O., Arthur, L.O., Sehgal, S.K., Letvin, N.L., King, N.W. and Daniel, M.D. Vaccine protection against Simian Immunodeficiency Virus Infection. Proc. Natl. Acad. Sci. USA 1989; 86(16):6353–6357.

Donnelly, J.J., Friedman, A., Martinez, D., Montgomery, D.L., Shiver, J.W., Motzel, S.L., Ulmer, J.B. and Liu, M.A. Preclinical efficacy of a prototype DNA vaccine: enhanced protection against antigenic drift in influenza virus. Nat. Med. 1995; 1(6):583–587.

Donnelly, J.J., Friedman, A., Ulmer, J.B. and Liu, M.A. Further protection against antigenic drift of influenza virus in a ferret model by DNA vaccination. Vaccine 1997; 15(8):865–868.

Draper, R.J., Macklin, M.D., Bard, L.J., Jones, S., Haynes, J.R. and Dean, H.J. Epidermal DNA vaccine for influenza is immunogenic in humans. Vaccine 2006; 24(11):4479–4481.

Dunachie, S.J., Walther, M., Epstein, J.E., Keating, S., Berthoud, T., Andrews, L., Andersen, R.F., Bejon, P., Goonetilleke, N., Poulton, L., Webster, D.P., Butcher, G., Watkins, K., Sinden, R.E., Levine, G.L., Richie, T.L., Schneider, J., Kaslow, D., Gilbert, S.C., Carucci, D.J. and Hill, A.V. A DNA prime-modified vaccinia virus ankara boost vaccine encoding thrombospondin-related adhesion protein but not circumsporozoite protein partially protects healthy malaria-naive adults against Plasmodium falciparum sporozoite challenge. Infect. Immun. 2006; 74(10):5923–5942.

Epstein, J.E., Charpentier, Y., Kester, K.E., Wang, R., Newcomer, R., Fitzpatrick, S., Richie, T.L., Tornieporth, N., Heppner, D.G., Ockenhouse, C., Majam, V., Holland, C., Abot, E., Ganeshan, H., Berzins, M., Jones, T., Freyberg, C.N., Ng, J., Norman, J., Carucci, D.J., Cohen, J. and Hoffman, S.L. Safety, tolerability, and antibody responses in humans after sequential immunization
with a PfCSP DNA vaccine followed by the recombinant protein vaccine RTS/AS02A. Vaccine 2004; 22(13-14):1592–1603.

Epstein, S., Kong, W.-P., Mispoln, J., Lo, C.-Y., Tumpey, T., Xu, L. and Nabel, G. Protection against multiple influenza A subtypes by vaccination with highly conserved nucleoprotein. Vaccine 2005; 23(46-47):5404–5410.

Felgner, P.L. and Ringold, G.M. Cationic liposome-mediated transfection. Nature 1989; 337(6209):387–388.

Feng, C.G., Palendira, U., Demangel, C., Spratt, J.M., Malin, A.S. and Britton, W.J. Priming by DNA immunization augments protective efficacy of Mycobacterium bovis Bacille Calmette-Guerin against tuberculosis. Infect. Immun. 2001; 69(6):4174–4176.

Ferraz, J.C., Stavropoulos, E., Yang, M., Coade, S., Espitia, C., Lowrie, D.B., Colston, M.J. and Tacon, R.E. A heterologous DNA priming-Mycobacterium bovis BCG boosting immunization strategy using mycobacterial Hsp70, Hsp65, and Apa antigen improves protection against tuberculosis in mice. Infect. Immun. 2004; 72(12):6945–6950.

Flynn, N.M., Forthal, D.N., Harro, C.D., Judson, F.N., Mayer, K.H. and Para, M.F. Placebo-controlled phase 3 trial of a recombinant glycoprotein 120 vaccine to prevent HIV-1 infection. J. Infect. Dis. 2005; 191(5):654–666.

Fogg, C., Lustig, S., Whitbeck, J.C., Eisenberg, R.J., Cohen, G.H. and Moss, B. Protective immunity to vaccinia virus induced by vaccination with multiple recombinant outer membrane proteins of intracellular and extracellular virions. J. Virol. 2004; 78(19):10230–10237.

Fynan, E.F., Webster, R.G., Fuller, D.H., Haynes, J.R., Santoro, J.C. and Robinson, H.L. DNA vaccines: protective immunizations by parenteral, mucosal, and gene-gun inoculations. Proc. Natl. Acad. Sci. USA 1993; 90(24):11478–11482.

Galloway, D., Liner, A., Legutki, J., Mateczun, A., Barnewall, R. and Espley, J. Genetic immunization against anthrax. Vaccine 2004; 22(13-14):1604–1608.

Galmiche, M.C., Goenaga, J., Wittek, R. and Rindlisbacher, L. Neutralizing and protective antibodies directed against vaccinia virus envelope antigens. Virology 1999; 254(1):71–80.

Garmory, H., Freeman, D., Brown, K. and Tithball, R. Protection against plague afforded by immunisation with DNA vaccines against plague. Proc. Natl. Acad. Sci. USA 2003; 100(18):10460–10464.

Hanka, T., McMichael, A.J. and Dorrell, L. Clinical experience with plasmid DNA- and modified vaccinia virus Ankara-vectored human immunodeficiency virus type 1 clade A vaccine focusing on T-cell induction. J. Gen. Virol. 2007; 88(Pt 1):1–12.

Heinz, F.X., Collett, M.S., Purcell, R.H., Gould, E.A., Howard, C.R., Houghton, M., Moorman, R.J.M., Rice, C.M. and Thiel, H.-J. Eds. 7th Report of the International Committee on Taxonomy of Viruses. San Diego, Academic, 2000.

Heraud, J.M., Edgell-Smith, Y., Ayala, V., Kalisz, I., Parrino, J., Kalyanaraman, V.S., Manischewitz, J., King, L.R., Hrynewicz, A., Trindade, C.J., Hassett, M., Tsai, W.P., Venzon, D., Nalca, A., Vaccari, M., Silvera, P., Bray, M., Graham, B.S., Golden, H., Hooper, J.W. and Franchini, G. Subunit recombinant vaccine protects against monkeypox. J. Infect. Dis. 2006; 197(4):2552–2564.

Hermanson, G., Whitlow, V., Parker, S., Tonsky, K., Rusolov, D., Ferrari, M., Lator, P., Komai, M., Mere, R., Bell, M., Brennan, K., Mateczun, A., Evans, T., Kaslow, D., Galloway, D. and Hobart, P. A recombinant plasmid DNA vaccine confers sustained antibody-mediated protection against aeroallergen. Ann. Allergy Asthma Immunol. 2003; 91(5):418–426.

Herrmann, J.E., Chen, S.C., Jones, D.H., Tinsley-Bown, A., Fynan, E.F., Greenberg, H.B. and Farrar, G.H. Immune responses and protection obtained by oral immunization with rotavirus VP4 and VP7 DNA vaccines encapsulated in microparticles. Virology 1999; 259(1):148–153.

Herrmann, J.E., Wang, S., Zhang, C., Panchal, R.G., Bavari, S., Lyons, C.R., Lovchik, J.A., Golding, B., Shiloach, J. and Lu, S. Passive immunotherapy of Bacillus anthracis pulmonary infection in mice with antisera produced by DNA immunization. Vaccine 2006; 24(31-32):5872–5880.

Hoey, M., Negley, D., VanderZanden, L., Tammarriello, R.F., Geisbert, J., Schmaljohn, C., Smith, J.F., Jahrling, P.B. and Schmaljohn, A.L. Marburg virus vaccines: comparing classical and new approaches. Vaccine 2001; 20(3-4):586–593.

HHS. HHS Awards Bioshield Contract For Botulism Antitoxin. 2006, 06/01/2006. Retrieved 11/05/2007, 2007, from http://www.dhhs.gov/news/press/2006press/20060601.html.

Hooper, J.W., Custer, D.M., Schmaljohn, C.S. and Schmaljohn, A. L. DNA vaccination with vaccinia virus L1R and A33R genes improves protection against tuberculosis in mice. Infect. Immun. 2006; 74(5):2041–2047.

II. FUNDAMENTAL ASPECTS OF VACCINOLOGY
protects mice against a lethal poxvirus challenge. Virology 2000; 266(2):329–339.

Hooper, J.W., Custer, D.M. and Thompson, E. Four-gene-combination DNA vaccine protects mice against a lethal vaccinia virus challenge and elicits appropriate antibody responses in nonhuman primates. Virology 2003; 306(1):181–195.

Hooper, J.W., Golden, J., Ferro, A. and King, A. Smallpox DNA vaccine delivered by novel skin electroporation device protects mice against intranasal poxvirus challenge. Vaccine 2007; 25(10):1814–1823.

Hooper, J.W., Thompson, E., Wilhelmsen, C., Zimmerman, M., Ichou, M., Steffen, S.E., Schmaljohn, C.S., Schmaljohn, A.J. and Jahrling, P.B. Smallpox DNA vaccine protects nonhuman primates against lethal monkeypox. J. Virol. 2004; 78(9):4433–4443.

Hu, H., Lu, X., Tao, L., Bai, B., Zhang, Z., Chen, Y., Zheng, F., Chen, J., Chen, Z. and Wang, H. Induction of specific immune responses by severe acute respiratory syndrome coronavirus spike DNA vaccine with or without interleukin-2 immunization using different vaccination routes in mice. Clin. Vaccine Immunol. 2007; 14(7):894–901.

Huang, J., Ma, R. and Wu, C.-Y. Immunization with SARS-CoV S DNA vaccine generates memory CD4+ and CD8+ T cell immune responses. Vaccine 2006; 24(23):4905–4913.

Imoto, J.-I. and Konishi, E. Needle-free jet injection of a mixture of Japanese encephalitis DNA and protein vaccines: a strategy to effectively enhance immunogenicity of the DNA vaccine in a murine model. Viral Immunol. 2005; 18(1):205–212.

Jimenez, G.S., Planchon, R., Wei, Q., Rusalov, D., Geall, A., Enas, J., Lalar, P., Leamy, V., Vahle, R., Luke, C.J., Rolland, A., Kaslow, D.C. and Smith, R. Virus-specific delayed-type hypersensitivity responses in mice administered DNA vaccine encoding the E2 gene of the hepatitis B virus surface antigen by intranasal DNA vaccination using a cationic emulsion as a mucosal gene carrier. Mol. Cells 2006; 22(2):175–181.

Klavinsks, L.S., Barnfield, C., Gao, L. and Parker, S. Intranasal immunization with plasmid DNA-lipid complexes elicits mucosal immunity in the female genital and rectal tracts. J. Immunol. 1999; 162(1):254–262.

Kohler, D., Gozes, Y., Rosenberg, H., Marcus, D., Reuveny, S. and Altboun, Z. Efficiency of protection of guinea pigs against infection with Bacillus anthracis spores by passive immunization. Infect. Immun. 2002; 70(2):544–560.

Kochel, T.J., Raviprakash, K., Hayes, C.G., Watts, D.M., Russell, K.L., Gozalo, A.S., Phillips, I.A., Ewing, D.F., Murphy, G.S. and Porter, K.R. A dengue virus serotype-1 DNA vaccine induces virus neutralizing antibodies and provides protection from viral challenge in Aotus monkeys. Vaccine 2000; 18(27):3166–3173.

Kodihalli, S., Goto, H., Kobasa, D.L., Krauss, S., Kawaoa, Y. and Webster, R.G. DNA vaccine encoding hemagglutinin provides protective immunity against H5N1 influenza virus infection in mice. J. Virol. 1999; 73(3):2094–2098.

Kodihalli, S., Haynes, J.R., Robinson, H.L. and Webster, R.G. Cross-protection among lethal H5N2 influenza viruses induced by DNA vaccine to the hemagglutinin. J. Virol. 1997; 71(5):3391–3396.

Kong, W.-P., Xu, L., Stadler, K., Ulmer, J., Abrignani, S., Rappuoli, R. and Nabel, G. Modulation of the immune response to the severe acute respiratory syndrome spike glycoprotein by gene-based and inactivated virus immunization. J. Virol. 2005; 79(22):13915–13923.

Konishi, E., Ajiro, N., Nukuzuma, C., Mason, P. and Kurane, I. Comparison of protective efficacies of plasmid DNAs encoding Japanese encephalitis virus proteins that induce neutralizing antibody or cytotoxic T lymphocytes in mice. Vaccine 2003a; 21(25-26):3675–3683.

Konishi, E., Kosugi, S. and Imoto, J.-I. Dengue tetravalent DNA vaccine inducing neutralizing antibody and amnestic responses to four serotypes in mice. Vaccine 2006; 24(12):2200–2207.

Konishi, E., Terazawa, A. and Fujii, A. Evidence for antigen production in muscles by dengue and Japanese encephalitis DNA vaccines and a relation to their immunogenicity in mice. Vaccine 2003b; 21(25-26):3713–3720.

Konishi, E., Terazawa, A. and Imoto, J.-I. Simultaneous immunization with DNA and protein vaccines against Japanese encephalitis or dengue synergistically increases their own abilities to induce neutralizing antibody in mice. Vaccine 2003c; 21(17-18):1826–1832.

Konishi, E., Yamaoka, M., Kurane, I. and Mason, P.W. A DNA vaccine expressing dengue type 2 virus premembrane and envelope genes induces neutralizing antibody and memory B cells in mice. Vaccine 2000; 18(11-12):1133–1139.

Kotsopoulos, E., Kim, V.N., Kingsman, A.J., Kingsman, S.M. and Mitrophanous, K.A. A Rev-independent human immunodeficiency virus type 1 (HIV-1)-based vector that exploits a codon-optimized HIV-1 gag-pol gene. J. Virol. 2000; 74(10):4839–4852.

Laddy, D., Yan, J., Corbett, N., Kobasa, D., Kobinger, G. and Weiner, D. Immunogenicity of novel consensus-based DNA vaccines against avian influenza. Vaccine 2007; 25(16):2984–2989.

Law, M., Cardoso, R.M., Wilson, L.A. and Burton, D.R. Antigenic and Immunogenic Study of Membrane-Proximal External Region-Graded gp120 Antigens by a DNA Prime-Protein Boost Immunization Strategy. J. Virol. 2007; 81(8):4272–4285.

Le, T.P., Cooran, K.M., Hedstrom, R.C., Chanenvert, Y., Sedegah, M., Epstein, J.E., Kumar, S., Wang, R., Doolan, D.L., Maguire, J. D., Parker, S.E., Hobart, P., Norman, J. and Hoffman, S.L. Safety, tolerability and humoral immune responses after intramuscular administration of a malaria DNA vaccine to healthy adult volunteers. Vaccine 2000; 18(18):1893–1901.

Letvin, N.L., Huang, Y., Chakrabarti, B.K., Xu, L., Seaman, M., Beaudry, K., Koroith-Schnitz, B., Yu, F., Rohne, D., Martin, K.L., Miura, A., Kong, W.P., Yang, Z.Y., Gelman, R.S., Golubeva, O.G., Montefiori, D.C., Mascola, J.R. and Nabel, G.J. Heterologous envelope immunogens contribute to AIDS vaccine protection in rhesus monkeys. J. Virol. 2004; 78(14):7490–7497.

Letvin, N.L., Montefiori, D.C., Yasutomi, Y., Perry, H.C., Davies, M.E., Lekutis, C., Alroy, M., Freed, D.C., Lord, C.I., Handt, L.K., Liu, M.A. and Shiver, J.W. Potent, protective anti-HIV immune responses generated by bimodal HIV envelope DNA plus protein vaccination. Proc. Natl. Acad. Sci. USA 1997; 94(17):9378–9383.

Lin, Y.L., Chen, L.K., Liao, C.L., Yeh, C.T., Ma, S.H., Chen, J.L., Huang, Y.L., Chen, S.S. and Chiang, H.Y. DNA immunization with Japanese encephalitis virus nonstructural protein NS1 elicits protective immunity in mice. J. Virol. 1998; 72(1):191–200.

Little, S.F., Ivins, B.E., Fellows, P.F. and Friedlander, A.M. Passive protection by polyclonal antibodies against Bacillus anthracis infection in guinea pigs. Infect. Immun. 1997; 65(12):5171–5175.

II. FUNDAMENTAL ASPECTS OF VACCINOLOGY
CONCLUSIONS

Liu, F., Mboudjeka, I., Shen, S., Chou, T.H., Wang, S., Ross, T.M. and Lu, S. Independent but not synergistic enhancement to the immunogenicity of DNA vaccine expressing HIV-1 gp120 glycoprotein by codon optimization and C3d fusion in a mouse model. Vaccine 2004; 22(13-14):1764–1772.

Lodmell, D.L. and Ewalt, L.C. Rabies vaccination: comparison of neutralizing antibody responses after priming and boosting with different combinations of DNA, inactivated virus, or recombinant vaccinia virus vaccines. Vaccine 2000; 18(22):2394–2398.

Lodmell, D.L. and Ewalt, L.C. Post-exposure DNA vaccination protects mice against rabies virus. Vaccine 2001; 19(17-19):2468–2473.

Lodmell, D., Parnell, M., Bailey, J., Ewalt, L. and Hanlon, C. Rabies DNA vaccination of non-human primates: post-exposure studies using gene gun methodology that accelerates induction of neutralizing antibody and enhances neutralizing antibody titers. Vaccine 2002; 20(17-18):2221–2227.

Lodmell, D.L., Ray, N.B. and Ewalt, L.C. Gene gun particle-mediated vaccination with plasmid DNA confers protective immunity against rabies virus infection. Vaccine 1998a; 16(2-3):115–118.

Lodmell, D.L., Ray, N.B., Parnell, M.J., Ewalt, L.C., Hanlon, C.A., Shaddock, J.H., Sanderlin, D.S. and Rupprecht, C.E. DNA immunization protects nonhuman primates against rabies virus. Nat. Med. 1998b; 4(8):949–952.

Lowrie, D.B., Tacon, R.E., Bonato, V.L., Lima, V.M., Faccioli, L.H., Stavropoulos, E., Colston, M.J., Hewinson, R.G., Moelling, K. and Silva, C.L. Therapy of tuberculosis in mice by DNA vaccination. Nature 1994; 369(6471):269–271.

Lozes, E., Huygen, K., Content, J., Denis, O., Montgomery, D.L., Yawman, A.M., Vandenbusche, P., Van Vooren, J.P., Drowart, A., Ulmer, J.B. and Liu, M.A. Immunogenicity and efficacy of a tuberculosis DNA vaccine encoding the components of the secreted antigen 85 complex. Vaccine 1997; 15(8):830–833.

Lu, S. Combination DNA plus protein HIV vaccines. Springer Sem. Immunopathol. 2006; 28(3):255–265.

Lu, S., Manning, S. and Arthos, J. Antigen engineering in DNA immunization. In: Methods in Molecular Medicine DNA Vaccines (D.B. Lowrie and R. Whalen, Eds.), pp. 355–374. Secaucus, NJ: Humana Press, 1999.

Lu, S., Santoro, J.C., Fuller, D.H., Haynes, J.R. and Robinson, H.L. Use of DNAs expressing HIV-1 Env and noninfectious HIV-1 particles to raise antibody responses in mice. Virology 1995; 209(1):147–154.

Lu, S., Wyatt, R., Richardson, J., Mustafa, F., Wang, S., Weng, J., Montefiori, D.C., Sodroski, J. and Robinson, H.L. Immunogenicity of DNA vaccines expressing human immunodeficiency virus type 1 envelope glycoprotein with and without deletions in the V1/2 and V3 regions. AIDS Res. Hum. Retroviruses 1998; 14(2):151–155.

Macklin, M.D., McCabe, D., McGregor, M.W., Neumann, V., Meyer, T., Callan, R., Hinchaw, V.S. and Swain, W.F. Immunization of pigs with a particle-mediated DNA vaccine to influenza A virus protects against challenge with homologous virus. J. Virol. 1998; 72(2):1491–1496.

Martin, J., Sullivan, N., Enama, M., Gordon, I., Roederer, M., Koup, R., Bailer, R., Chakrabarti, B., Bailey, M., Gomez, P., Andrews, C., Moodie, Z., Gu, L., Stein, J., Nabel, G. and Graham, B. A DNA vaccine for Ebola virus is safe and immunogenic in a phase 1 clinical trial. Clin. Vacc. Immunod. 2006; 13(11):1267–1277.

Martin, J.E., Pierson, T.C., Hubka, S., Rucker, S., Gordon, I., Enama, M.E., Andrews, C.A., Xu, Q., Davis, B.S., Nason, M., Fay, M., Koup, R.A., Roederer, M., Bailer, R.T., Gomez, P.L., Mascella, J. R., Chang, C.J., Nabel, G.J. and Graham, B.S. A West Nile Virus DNA Vaccine Induces Neutralizing Antibody in Healthy Adults in a Phase I Clinical Trials. J. Infect. Dis. 2007; 196(2):1732–1740.

McConkey, S.J., Reece, W.H., Moorthy, V.S., Webster, D., Dunachie, S., Butcher, C., Vuola, J.M., Blanchard, T.J., Gothard, P., Watkins, K., Hannan, C.M., Everaere, S., Brown, K., Kester, K.E., Cummings, J., Williams, J., Heppner, D.G., Pathan, A., Flanagan, K., Arulanantham, N., Roberts, M.T., Roy, M., Smith, G.L., Schneider, J., Peto, T., Sinden, R.E., Gilbert, S.C. and Hill, A. V. Enhanced T-cell immunogenicity of plasmid DNA vaccines boosted by recombinant modified vaccinia virus Ankara in humans. Nat. Med. 2003; 9(6):729–735.

McShane, H., Brooks, R., Gilbert, S.C. and Hill, A.V. Enhanced immunogenicity of CD4(+) T-cell responses and protective efficacy of a DNA-modified vaccinia virus Ankara prime-boost vaccination regimen for murine tuberculosis. Infect. Immun. 2001; 69(2):681–686.

Mellquist-Riemenschneider, J.L., Garrison, A.R., Geisbert, J.B., Saikh, K.U., Heidebrink, K.D., Jahrling, P.B., Ulrich, R.G. and Schmaljohn, C.S. Comparison of the protective efficacy of DNA and baculovirus-derived protein vaccines for EBOLA virus in guinea pigs. Virus Res 2003; 92(2):187–193.

Miyashita, M., Joh, T., Watanabe, K., Todoroki, I., Seno, K., Ohara, H., Nomura, T., Miyata, M., Kasugai, K., Tochikubo, K., Itoh, M. and Nitta, M. Immune responses in mice to intranasal and intracranial administration of a DNA vaccine encoding Helicobacter pylori-catalase. Vaccine 2002; 20(17-18):2336–2342.

Mohamed, N., Caggett, M., Li, J., Jones, S., Pincus, S., D’Alia, G., Nardone, L., Babin, M., Spitalny, G. and Casey, L. A high-affinity monoclonal antibody to anthrax protective antigen passively protects rabbits before and after aerosolized Bacillus anthracis spore challenge. Infect. Immun. 2005; 73(2):795–802.

Moorthy, V.S., McConkey, S., Roberts, M., Gothard, P., Arulanantham, N., Degano, P., Schneider, J., Hannan, C., Roy, M., Gilbert, S.C., Peto, T.E. and Hill, A.V. Safety of DNA and modified vaccinia virus Ankara vaccines against liver-stage P. falciparum malaria in non-immune volunteers. Vaccine 2003; 21(17-18):1995–2002.

Morris, S., Kelley, C., Howard, A., Li, Z. and Collins, F. The immunogenicity of single and combination DNA vaccines against tuberculosis. Vaccine 2000; 18(20):2155–2163.

Mota, J., Acosta, M., Argotte, R., Figuera, R., MÃºndez, A. and Ramos, C. Induction of protective antibodies against dengue virus by tetravalent DNA immunization of mice with domain III of the envelope protein. Vaccine 2005; 23(26):3469–3476.

Mwau, M., Cebere, L., Sutton, J., Chiroti, P., Winstone, N., Wee, E.G., Beattie, T., Chen, Y.H., Dorrell, L., McShane, H., Schmidt, C., Brooks, M., Patel, S., Roberts, J., Conlon, C., Rowland-Jones, S.L., Bwabo, J.J., McMichael, A.J. and Hanke, T. A human immunodeficiency virus 1 (HIV-1) clade A vaccine in clinical trials: stimulation of HIV-specific T-cell responses by DNA and recombinant modified vaccinia virus Ankara (MVA) vaccines in humans. J. Gen. Virol. 2004; 85(Pt 1):911–919.

Nagata, L., Hu, W.-G., Masri, S., Rayner, G., Schmalts, F., Das, D., Wu, J., Long, M., Chan, C., Proll, D., Jager, S., Jebrailey, L., Suresh, M. and Wong, J. Efficacy of DNA vaccination against western equine encephalitis virus infection. Vaccine 2005; 23(17-18):2280–2283.

NIH. Vaccine to Prevent West Nile Virus Disease. 2005, 02/24/2007. Retrieved 11/5/2007, 2007, from http://clinicaltrials.gov/show/NCT000106769.

NIH. West Nile Virus Vaccine Development. 2007, 07/31/2007. Retrieved 11/5/2007, 2007, from http://www.nich.gov/scientificupdates_westnile.htm.

O’Hagan, D., Singh, M., Ugozzoli, M., Wild, C., Barnett, S., Chen, M., Schaefer, M., Doe, B., Otten, G.R. and Ulmer, J.B. Induction of potent immune responses by cationic microparticles with...
adsorbed human immunodeficiency virus DNA vaccines. J. Virol. 2001; 75(19):9307–9403.

Okuda, K., Ihata, A., Watabe, S., Okada, E., Yamakawa, T., Hamajima, K., Yang, J., Ishii, N., Nakazawa, M., Okuda, K., Ohnari, K., Nakajima, K. and Xin, K.Q. Protective immunity against influenza A virus induced by immunization with DNA plasmid containing influenza M gene. Vaccine 2001; 19(27):3681–3691.

Otero, M., Calarota, S., Dai, A., De Groot, A., Boyer, J. and Weiner, D. Efficacy of novel plasmid DNA encoding vaccinia antigens in improving current smallpox vaccination strategy. Vaccine 2006; 24(21):4461–4470.

Otten, G.R., Schaefer, M., Doe, B., Liu, H., Srivastava, I., Megede, J., Kazaz, J., Lian, Y., Singh, M., Ugozzoli, M., Montefiori, D., Lewis, M., Driver, D.A., Dubensky, T., Polo, J.M., Donnelly, J., O’Hagan, D.T., Barnett, S. and Ulmer, J.B. Enhanced potency of plasmid DNA microparticle human immunodeficiency virus vaccines in rhesus macaques by using a priming-boosting regimen with recombinant proteins. J. Virol. 2005; 79(13):8189–8200.

Pal, R., Wang, S., Kalyanaraman, V.S., Nair, B.C., Whitney, S., Keen, T., Hocker, L., Hudacik, L., Rose, N., Cristillo, A., Mbudjeka, I., Shen, S., Wu-Chou, T.H., Montefiori, D., Mascola, J., Lu, S. and Markham, P. Polyvalent DNA prime and envelope protein boost HIV-1 vaccine elicits humoral and cellular responses and controls plasma viremia in rhesus macaques following rectal challenge with an R5 SHIV isolate. J. Med. Primatol. 2005; 34(6):226–236.

Pal, R., Wang, S., Kalyanaraman, V.S., Nair, B.C., Whitney, S., Keen, T., Hocker, L., Hudacik, L., Rose, N., Mbudjeka, I., Shen, S., Wu-Chou, T.H., Montefiori, D., Mascola, J., Markham, P. and Lu, S. Immunization of rhesus macaques with a polyvalent DNA prime/protein boost human immunodeficiency virus type 1 vaccine elicits protective antibody response against simian human immunodeficiency virus of R5 phenotype. Virology 2006; 348(2):341–353.

Pancholi, P., Liu, Q., Tricoche, N., Zhang, P., Perkus, M.E. and Prince, A.M. DNA prime-canarypox boost with polyclonotypic hepatitis C virus (HCV) genes generates potent immune responses to HCV structural and nonstructural proteins. J. Infect. Dis. 2000; 182(1):18–27.

Perkins, S., O’Brien, L. and Phillipotts, R. Boosting with an adenovirus-based vaccine improves protective efficacy against Venezuelan equine encephalitis virus following DNA vaccination. Vaccine 2006; 24(17):3440–3445.

Pitsutthithum, P., Gilbert, P., Gurwitz, M., Heyward, W., Martin, M., van Griensven, F., Hu, D., Tapper, J.W. and Choopanya, K. Randomized, double-blind, placebo-controlled efficacy trial of a bivalent recombinant glycoprotein 120 HIV-1 vaccine among injection drug users in Bangkok, Thailand. J. Infect. Dis. 2006; 194(12):1661–1671.

Pulford, D.J., Gates, A., Bridge, S.H., Robinson, J.H. and Ulateo, D. Differential efficacy of vaccinia virus envelope proteins administered by DNA immunisation in protection of BALB/c mice from a lethal intranasal poxvirus challenge. Vaccine 2004; 22(25-26):3358–3366.

Putnak, R., Fuller, J., Vanderzanden, L., Innis, B. and Vaughn, D. Vaccination of rhesus macaques against dengue-2 virus with a plasmid DNA vaccine encoding the viral pre-membrane and envelope genes. Am. J. Trop. Med. Hyg. 2003; 68(4):469–476.

Qiu, M., Fang, F., Chen, Y., Wang, H., Chen, Q., Chang, H., Wang, F., Wang, H., Zhang, R. and Chen, Z. Protection against avian influenza H9N2 virus challenge by immunization with hemagglutinin- or neuraminidase-expressing DNA in BALB/c mice. Biochem. Biophys. Res. Commun. 2006; 343(4):1124–1131.

Ramshaw, I.A. and Ramsay, A.J. The prime-boost strategy: exciting prospects for improved vaccination. Immun. Today 2000; 21(4):163–165.

Raviprakash, K., Apte, D., Brinkman, A., Skinner, C., Yang, S., Dawes, G., Ewing, D., Wu, S.-J., Bass, S., Punnonen, J. and Porter, K. A chimeric tetravalent dengue DNA vaccine elicits neutralizing antibody to all four virus serotypes in rhesus macaques. Virology 2006; 353(1):166–173.

Raviprakash, K., Kochel, T.J., Ewing, D., Simmons, M., Phillips, I., Hayes, C.G. and Porter, K.R. Immunogenicity of dengue virus type 1 DNA vaccines expressing truncated and full length envelope protein. Vaccine 2000a; 18(22):2426–2434.

Raviprakash, K., Porter, K.R., Kochel, T.J., Ewing, D., Simmons, M., Phillips, I., Murphy, G.S., Weiss, W.R. and Hayes, C.G. Dengue virus type 1 DNA vaccine induces protective immune responses in rhesus macaques. J. Gen. Virol. 2000b; 81(P7):1659–1667.

Repique, C.J., Li, A., Collins, F.M. and Morris, S.L. DNA immunization in a mouse model of latent tuberculosis: effect of DNA vaccination on reactivation of disease and on reinfection with a secondary challenge. Infect. Immun. 2002; 70(7):3318–3323.

Richmond, J.F., Lu, S., Santoro, J.C., Weng, J., Hu, S.L., Montefiori, D.C. and Robinson, H.L. Studies of the neutralizing activity and avidity of anti-human immunodeficiency virus type 1 Env antibody elicited by DNA priming and protein boosting. J. Virol. 1998; 72(11):9092–9100.

Riemenschneider, J., Garrison, A., Giesbert, J., Jahrling, P., Hevey, M., Negley, D., Schmaljohn, A., Lee, J., Hart, M., Vanderzanden, L., Custer, D., Bray, M., Ruff, A., Ivins, B., Bassett, A., Rossi, C. and Schmaljohn, C. Comparison of individual and combination DNA vaccines for B. anthracis, Ebola virus, Marburg virus and Venezuelan equine encephalitis virus. Vaccine 2003; 21(25-26):4071–4080.

Robinson, H.L., Boyle, C.A., Felquetcq, D.M., Morin, M.J., Santoro, J.C. and Webster, R.G. DNA immunization for influenza virus: studies using hemagglutinin- and nucleoprotein-expressing DNAJs. Infect. Dis. 1997; 176(Suppl 1):50–55.

Robinson, H.L., Hunt, L.A. and Webster, R.G. Protection against a lethal influenza virus challenge by immunization with a haemagglutinin-expressing plasmid DNA. Vaccine 1993; 11(9):957–960.

Rodriguez-Carreno, M., Nelson, M., Botten, J., Smith-Nixon, K., Buchmeier, M. and Whitton, J. Evaluating the immunogenicity and protective efficacy of a DNA vaccine encoding Lassa virus nucleoprotein. Virology 2005; 335(1):87–98.

Rottthausing, S.T., Poland, G.A., Jacobson, R.M., Barr, L.J. and Roy, M.J. Hepatitis B DNA vaccine induces protective antibody responses in human non-responders to conventional vaccination. Vaccine 2003; 21(31):4604–4608.

Roy, M.J., Wu, M.S., Barr, L.J., Fuller, J.T., Tussey, L.G., Speller, S., Culp, J., Burkholder, J.K., Swain, W.F., Dixon, R.M., Widera, G., Vessey, R., King, A., Ogg, C., Gallimore, A., Haynes, J.R. and Fuller, D. Induction of antigen-specific CD8+ T cells, T helper cells, and protective levels of antibody in humans by particle-mediated administration of a hepatitis B virus DNA vaccine. Vaccine 2000; 19(7-8):764–778.

Sakhatksky, P., Wang, S., Chou, T.H. and Lu, S. Immunogenicity and protection efficacy of monovalent and polyvalent poxvirus vaccines that include the D8 antigen. Virology 2006; 355(2):164–174.

Sakhatksky, P., Wang, S., Chou, T.H. and Lu, S. Immunogenicity and protection efficacy of subunit-based smallpox vaccines using Variola Major antigens. Virology 2008; 371(1):98–107.

Schmaljohn, C., Custer, D., Vanderzanden, L., Spik, K., Rossi, C. and Bray, M. Evaluation of tick-borne encephalitis DNA vaccines in monkeys. Virology 1999; 263(1):166–174.

II. FUNDAMENTAL ASPECTS OF VACCINOLOGY
CONCLUSIONS

Ulmer, J.B., Liu, M.A., Montgomery, D.L., Yawman, A.M., Deck, R.R., DeWitt, C.M., Content, J. and Huygen, K. Expression and immunogenicity of Mycobacterium tuberculosis antigen 85 by DNA vaccination. Vaccine 1997; 15(8):792–794.

Ulmer, J.B., Fu, T.M., Deck, R.R., Friedman, A., Guan, L., DeWitt, C., Liu, X., Wang, S., Liu, M.A., Donnelly, J.J. and Caulfield, M.J. Protective CD4+ and CD8+ T cells against influenza virus induced by vaccination with nucleoprotein DNA. J. Virol. 1998; 72(7):5648–5653.

Vanderzanden, L., Bray, M., Fuller, D., Roberts, T., Custer, D., Spik, K., Kahrling, P., Huggins, J., Schmaljohn, A. and Schmaljohn, C. DNA vaccines expressing either the GP or NP genes of Ebola virus protect mice from lethal challenge. Virology 1998; 246(1):134–144.

Vuola, J.M., Keating, S., Webster, D.P., Berthoud, T., Dunachie, S., Gilbert, S.C. and Hill, A.V. Differential immunogenicity of various heterologous prime-boost vaccine regimens using DNA and viral vectors in healthy volunteers. J. Immunol. 2003; 174(1):449–455.

Wang, B., Ugen, K.E., Srikantan, V., Agadjanian, M.G., Dang, K., Rafaelli, Y., Sato, A.I., Boyer, J., Williams, W.V. and Weiner, D.B. Gene inoculation generates immune responses against human immunodeficiency virus type 1. Proc. Natl. Acad. Sci. USA 1993; 90(9):4156–4160.

Wang, R., Doolan, D.L., Le, T.P., Hedstrom, R.C., Coonan, K.M., Charoenvit, Y., Jones, T.R., Hobart, P., Margalith, M., Ng, J., Weiss, W.R., Sedegah, M., De Taisne, C., Norman, J.A. and Hoffman, S.L. Induction of antigen-specific cytotoxic T lymphocytes in humans by a malaria DNA vaccine. Science 1998; 282(5388):476–480.

Wang, R., Epstein, J., Baraceros, F.M., Gorak, E.J., Charoenvit, Y., Carucci, D.J., Hedstrom, R.C., Rahardjo, N., Gay, T., Hobart, P., Stout, R., Jones, T.R., Richie, T.L., Parker, S.E., Doolan, D.L., Norman, J. and Hoffman, S.L. Induction of CD8+ T cell-dependent CD8+ type 1 responses in humans by a malaria DNA vaccine. Proc. Natl. Acad. Sci. USA 2001; 98(19):10817–10822.

Wang, R., Epstein, J., Carucci, Y., Baraceros, F.M., Rahardjo, N., Gay, T., Bananaia, J.G., Chattopadhyay, R., dela Vega, P., Richie, T.L., Tornieporth, N., Doolan, D.L., Kester, K.E., Heppner, D.G., Norman, J., Carucci, D.J., Cohen, J.D. and Hoffman, S.L. Induction in humans of CD8+ and CD4+ T cell and antibody responses by sequential immunization with malaria DNA and recombinant protein. J. Immunol. 2004a; 172(9):5561–5569.

Wang, S., Chou, T.H., Sakkatskyy, P., Huang, S., Lawrence, J., Cao, H., Huang, X. and Lu, S. Identification of two neutralizing regions on SARS-CoV spike glycoprotein produced from mammalian expression system. J. Virol. 2005b; 79(12):7933–7937.

Wang, S., Chou, T.H., Sakkatskyy, P., Huang, S., Lawrence, J., Cao, H., Huang, X. and Lu, S. Identification of two neutralizing regions on SARS-CoV spike glycoprotein produced from mammalian expression system. J. Virol. 2005b; 79(3):1906–1910.

Wang, S., Farfan-Arribas, D.J., Shen, S., Chou, T.H., Hirsch, A., He, F. and Lu, S. Relative contributions of codon usage, promoter efficiency and leader sequence to the antigen expression and immunogenicity of HIV-1 Env DNA vaccine. Vaccine 2006a; 24(21):4531–4540.

Wang, S., Heilmann, D., Liu, F., Giehl, T., Joshi, S., Huang, X., Chou, T.H., Goguen, J. and Lu, S. A DNA vaccine producing LcrV antigen in oligomers is effective in protecting mice from lethal mucosal challenge of plague. Vaccine 2004b; 22(25–26):3348–3357.

II. FUNDAMENTAL ASPECTS OF VACCINOLOGY

Schmaljohn, C., Vanderzanden, L., Bray, M., Custer, D., Meyer, B., Li, D., Rossi, C., Fuller, D., Fuller, J., Haynes, J. and Huggins, J. Naked DNA vaccines expressing the prM and E genes of Russian spring summer encephalitis virus and Central European encephalitis virus protect mice from homologous and heterologous challenge. J. Virol. 1997; 71(12):9563–9569.

Schneider, J., Langermans, J.A., Gilbert, S.C., Blanchard, T.J., Twigg, S., Naitza, S., Hannan, C.M., Audoo, M., Crisanti, A., Robson, K.J., Smith, G.L., Hill, A.V. and Thomas, A.W. A prime-boost immunisation regimen using DNA followed by recombinant modified vaccinia virus Ankara induces strong cellular immune responses against the Plasmodium falciparum TRAP antigen in chimpanzees. Vaccine 2001; 19(32):4595–4602.

Seaman, M.S., Xu, L., Beaudry, K., Martin, K.L., Bedall, M.H., Miura, A., Sambor, A., Chakrabarti, B.K., Huang, Y., Bailer, R., Koup, R.A., Mascola, J.R., Nabel, G.J. and Letvin, N.L. Multiclade human immunodeficiency virus type 1 envelope immunogens elicit broad cellular and humoral immunity in rhesus monkeys. J. Virol. 2005; 79(5):2956–2963.

Stopeck, A.T., Hersh, E.M., Brailey, J.L., Clark, P.R., Norman, J. and Parker, S.E. Transfection of primary tumor cells and tumor cell lines with plasmid DNA/lipid complexes. Cancer Gene Ther. 1998; 5(2):119–126.

Sugawara, I., Yamada, H., Udagawa, T. and Huygen, K. Vaccination of guinea pigs with DNA encoding Ag85A by gene gun bombardment. Tuberculosis (Edinb.) 2003; 83(6365):331–337.

Sullivan, N.J., Sanchez, A., Rollin, P.E., Yang, Z.Y. and Nabel, G.J. Development of a preventive vaccine for Ebola virus infection in primates. Nature 2000; 408(6812):605–609.

Tacket, C.O., Roy, M.J., Widera, G., Swain, W.F., Broome, S. and Edelman, R. Phase 1 safety and immune response studies of a DNA vaccine encoding hepatitis B surface antigen delivered by a gene delivery device. Vaccine 1999; 17(22):2826–2829.

Tanabayashi, K., Mukai, R., Yamada, A., Takasaki, T., Kurane, I., Yamaoka, M., Terazawa, A. and Konishi, E. Immunogenicity of a Japanese encephalitis DNA vaccine candidate in cynomolgus monkeys. Vaccine 2003; 21(19–20):2338–2345.

Tang, D.C., Devit, M. and Johnston, S.A. Genetic immunization is a simple method for eliciting an immune response. Nature 1992; 356(6365):152–154.

Tanghe, A., D’Souza, S., Rosseels, V., Denis, O., Ottenhoff, T.H., Daemen, W., Wheeler, C. and Huygen, K. Improved immunogenicity and protective efficacy of a tuberculosis DNA vaccine encoding Ag85 by protein boosting. Infect. Immun. 2001; 69(5):3041–3047.

Tascon, R.E., Colston, M.J., Ragni, S., Stavropoulos, E., Gregory, D. and Lowrie, D.B. Vaccination against tuberculosis by DNA injection. Nat. Med. 1996; 2(8):888–892.

Tavel, J.A., Martin, J.E., Kelly, G.G., Enama, M.E., Shen, J.M., Gomez, P.L., Andrews, C.A., Koup, R.A., Bailar, R.T., Stein, J.A., Roederer, M., Nabel, G.J. and Graham, B.S. Safety and Immunogenicity of a Gag-Pol Candidate HIV-1 DNA Vaccine Administered by a Needle-Free Device in HIV-1-Seronegative Subjects. J. Acquir. Immune Defic. Syndr. 2007; 44(5):601–605.

Todoroki, I., Joh, T., Watanabe, K., Miyashita, M., Seno, K., Nomura, T., Ohara, H., Yokoyama, Y., Tochikubo, K. and Itoh, M. Suppressive effects of DNA vaccines encoding heat shock protein on Helicobacter pylori-induced gastritis in mice. Biochem. Biophys. Res. Commun. 2000; 277(1):159–163.

Ulmer, J.B., Donnelly, J.J., Parker, S.E., Rhodes, G.H., Felgner, P.L., Dwarki, V.J., Gromkowski, S.H., Deck, R.R., Devitt, C.M. and Friedman, A. Heterologous protection against influenza by injection of DNA encoding a viral protein. Science 1993; 259(5102):1745–1749.
II. FUNDAMENTAL ASPECTS OF VACCINOLOGY

Wang, S., Kennedy, J., West, K., Montefiori, D.C., Bansal, A., Goepfert, P., Coley, S., Lawrence, J., Shen, S., Green, S., Rothman, A., Ennis, F., Pal, R., Markham, P. and Lu, S. Balanced cellular and antibody responses induced by the polyvalent DNA prime-protein boost HIV-1 vaccine formulation DP6-001 in healthy human volunteers. Vaccine 2008a; 26(31):3947–3957.

Wang, S., Joshi, S., Mboudjeka, I., Liu, F., Ling, T., Goguen, J.D. and Lu, S. Relative immunogenicity and protection potential of candidate Yersinia Pestis antigens against lethal mucosal plague challenge in Balb/C mice. Vaccine 2008b; 26(13):1664–1674.

Wang, S., Pal, R., Mascola, J.R., Chou, T.H., Mboudjeka, I., Shen, S., Liu, Q., Whitney, S., Keen, T., Nair, B.C., Kalyanaraman, V.S., Markham, P. and Lu, S. Polyvalent HIV-1 Env vaccine formulations delivered by the DNA priming plus protein boosting approach are effective in generating neutralizing antibodies against primary human immunodeficiency virus type 1 isolates from subtypes A, B, C, D and E. Virolology 2008a; 350(1):34–47.

Wang, S., Taaffe, J., Parker, C., Soñoranzo, A., Cao, H., García-Sastre, A. and Lu, S. Hemagglutinin (HA) proteins from H1 and H3 subtypes of influenza A viruses require different antigen designs for the induction of optimal protective antibody responses as studied by codon-optimized HA DNA vaccines. J. Virol. 2008c; 82(25):11628–11637.

Wheeler, C.J., Felgner, P.L., Tsai, Y.J., Marshall, J., Sukhu, L., Doh, S.G., Hartikka, J., Nietupski, J., Manthorpe, M., Nichols, M., Plewe, M., Liang, X., Norman, J., Smith, A. and Cheng, S.H. A novel cationic lipid greatly enhances plasmid DNA delivery and expression in mouse lung. Proc. Natl. Acad. Sci. USA 1996; 93(21):11454–11459.

WHO. The global eradication of smallpox. Final report of the global commission for the certification of smallpox eradication. In: History of International Public Health. Geneva, World Health Organization. 1980; 4.

Wiker, H.G. and Harboe, M. The antigen 85 complex: a major secretion product of Mycobacterium tuberculosis. Microbiol. Rev. 1992; 56(4):648–661.

Williamson, E.D., Bennett, A.M., Perkins, S.D., Beecham, R.J., Miller, J. and Baillie, L.W. Co-immunisation with a plasmid DNA cocktail primes mice against anthrax and plague. Vaccine 2002; 20(23–24):2933–2941.

Williamson, E.D., Eley, S.M., Stagg, A.J., Green, M., Russell, P. and Tittball, R.W. A sub-unit vaccine elicits IgG in serum, spleen cell cultures and bronchial washings and protects immunized animals against pneumonic plague. Vaccine 1997; 15(10):1079–1084.

Williamson, E.D., Eley, S.M., Stagg, A.J., Green, M., Russell, P. and Tittball, R.W. A single dose sub-unit vaccine protects against pneumonic plague. Vaccine 2000; 19(4–5):566–571.

Woo, P.C.Y., Lau, S.K.P., Tsui, H.W., Chen, Z.-W., Wong, B.H.L., Zhang, L., Chan, J.K.H., Wong, L.-P., He, W., Ma, C., Chan, K.-H., Ho, D.D. and Yuen, K.-Y. SARS coronavirus spike polypeptide DNA vaccine priming with recombinant spike polypeptide from Escherichia coli as booster induces high titer of neutralizing antibody against SARS coronavirus. Vaccine 2005; 23(42):4959–4968.

Woodland, D.L. Jump-starting the immune system: prime-boosting comes of age. Trends Immunol. 2004; 25(2):98–104.

Wu, C.-J., Huang, H.-W. and Tao, M.-H. Induction of cross-protection against two wild-type Taiwanese isolates of Japanese encephalitis virus using Beijing-1 strain DNA vaccine. Vaccine 2003; 21(25–26):3939–3945.

Wu, L., Kong, W.P. and Nabel, G.J. Enhanced breadth of CD4 T-cell immunity by DNA prime and adenovirus boost immunization to human immunodeficiency virus Env and Gag immunogens. J. Virol. 2005; 79(13):8024–8031.

Wu, S.F., Liao, C.L., Lin, Y.L., Yeh, C.T., Chen, L.K., Huang, Y.F., Chou, H.Y., Huang, J.L., Shiao, M.F. and Sytwu, H.K. Evaluation of protective efficacy and immune mechanisms of using a non-structural protein NS1 in DNA vaccine against dengue 2 virus in mice. Vaccine 2003; 21(25–26):3919–3929.

Xiang, Z.Q., Spitalnik, S., Tran, M., Wunner, W.H., Cheng, J. and Ertl, H.C.J. Vaccination with a Plasmid Vector Carrying the Rabies Virus Glycoprotein Gene Induces Protective Immunity against Rabies Virus. Virology 1994; 199(1):132–140.

Xiang, Z.Q., Spitalnik, S.L., Cheng, J., Erikson, J., Wojczyk, B. and Ertl, H.C.J. Immune Responses to Nucleic Acid Vaccines to Rabies Virus. Virology 1995; 209(2):569–579.

Xiao, Y., Aldaz-Carroll, L., Ortiz, A.M., Whitbeck, J.C., Alexander, E., Lou, H., Davis, H.L., Braciale, T.J., Eisenberg, R.J., Cohen, G.H. and Isaacs, S.N. A protein-based smallpox vaccine protects mice from vaccinia and ectromelia virus challenges when given as a prime and single boost. Vaccine 2007; 25(7):1214–1224.

Xu, L., Sanchez, A., Yang, Z., Zaki, S.R., Nabel, E.G., Nichol, S.T. and Nabel, G.J. Immunization for Ebola virus infection. Nat. Med. 1998; 4(1):37–42.

Yang, J.S., Kim, J.J., Hwang, D., Choo, A.Y., Dang, K., Maguire, H., Kudchodkar, S., Ramanathan, M.P. and Weiner, D.B. Induction of potent Th1-type immune responses from a novel DNA vaccine for West Nile virus New York isolate (WNV-NY1999). J. Infect. Dis. 2001; 184(7):809–816.

Yang, Z.Y., Kong, W.P., Huang, Y., Roberts, A., Murphy, B.R., Subbarao, K. and Nabel, G.J. A DNA vaccine induces SARS coronavirus neutralization and protective immunity in mice. Nature 2004; 438(9092):561–564.

Yuan, L., Azvedo, M.S.P., Gonzalez, A.M., Jeong, K.-i., Van Nguyen, T., Lewis, P., Iosef, C., Herrmann, J.E. and Saif, I.J. Mucosal and systemic antibody responses and protection induced by a prime/boost rotavirus-DNA vaccine in a gnotobiotic pig model. Vaccine 2005; 23(30):3925–3936.

Zakhartchouk, A., Liu, Q., Petric, M. and Babiuk, L. Augmentation of immune responses to SARS coronavirus by a combination of DNA and whole killed virus vaccines. Vaccine 2005; 23(35):4385–4391.

Zarozinski, C.C., Fynn, E.F., Selin, L.K., Robinson, H.L. and Welsh, R.M. Protective CTL-dependent immunity and enhanced immunopathology in mice immunized by particle bombardment with DNA encoding an internal virion protein. J. Immunol. 1995; 154(8):4010–4017.

Zhao, B., Jin, N.-Y., Wang, R.-L., Zhang, L.-S. and Zhang, Y.-J. Immunization of mice with a DNA vaccine based on severe acute respiratory syndrome coronavirus spike protein fragment I. Virology 2008; 383(3):518–524.

Zhao, P., Cao, J., Zhao, L.-J., Qin, Z.-L., Ke, J.-S., Pan, W., Ren, H., Yu, J.-G. and Qi, Z.-T. Immune responses against SARS-coronavirus nucleocapsid protein induced by DNA vaccine. Virology 2005; 331(1):128–135.

Zhu, M.-S., Pan, Y., Chen, H.-Q., Shen, Y., Wang, X.-C., Sun, Y.-J. and Tao, K.-H. Induction of SARS-nucleoprotein-specific immune response by use of DNA vaccine. Immunology letters 2004; 92(3):237–243.