Potential Advantages of DNA Immunization for Influenza Epidemic and Pandemic Planning

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Immunization with purified DNA is a powerful technique for inducing immune responses. The concept of DNA immunization involves insertion of the gene encoding the antigen of choice into a bacterial plasmid and injection of the plasmid into the host where the antigen is expressed and where it induces humoral and cellular immunity. The most effective routes and methods for DNA immunization are bombardment with particles coated with DNA ("gene gun" technique), followed by the intramuscular and intradermal routes. DNA immunization technology has the potential to induce immunity to all antigens that can be completely encoded in DNA, which therefore include all protein, but not carbohydrate, antigens. DNA immunization results in presentation of antigens to the host's immune system in a natural form, like that achieved with live-attenuated vaccines. The DNA immunization strategy has the potential to rapidly provide a new vaccine in the face of an emerging influenza pandemic.

Each February, the World Health Organization collaborating laboratories meet to review information obtained from >110 laboratories worldwide and decide which strains best represent those that are likely to be dominant in the next year. Experience at reviewing the data and some good guesswork have resulted in surprisingly good matches with the influenza virus that has become dominant in recent years. After the decision on vaccine strains is made, preparation of high-growth reassortants and of the vaccine takes up to 6 months, and since the viruses continue to evolve in nature, the vaccine strain used can be as much as 1 year out of date by the time the vaccine is administered to humans. Therefore, although the development of a "new" vaccine each year is truly remarkable, in terms of virus variability, the time required is too long. This lag could pose serious problems when preparing for an influenza pandemic, especially in the worst-case scenario of reappearance of a highly pathogenic "Spanish-type" influenza. DNA immunization may offer certain advantages over conventional vaccination strategies involving inactivated intact virus or subunit vaccines.

The Concept and the Construct Used for DNA Immunization

The concept of DNA immunization is illustrated in figure 1. A DNA copy of the RNA segment of influenza virus encoding, for example, the hemagglutinin (HA) protein is inserted into a bacterial plasmid. The circular plasmid, which can replicate in bacteria, contains control elements including a strong eukaryotic promoter of cytomegalovirus (cytomegalovirus early promoter), intron A, and sequences encoding a tissue plasminogen activator, which enables expression of the HA protein in the B cells of a mammalian host. Other characteristics that are desirable in expression vectors for DNA immunization include a high copy number and lack of replication or integration into the host chromosome. The plasmid DNA for vaccination must be free of any contamination, particularly toxic or inherently antigenic substances such as endotoxins and antibiotics. The aim is to express (in mammalian cells) high levels of a gene product defining antigenic domains that will stimulate the desired immune response.

Administration of DNA Vaccines

The first report of protective immunity induced by DNA of an infectious agent (in this case, influenza) was published in 1993 [1, 2], and this technology was quickly applied to a wide range of disease agents, including viruses, bacteria, parasites, and cancer-associated pathogens [3]. The routes of inoculation used for DNA immunization include intramuscular, intradermal, and gene-gun delivery to the skin [4–6]. One of the most effective methods for potentiating the efficiency of transfection is the use of a gene-gun to shoot DNA-coated gold beads directly into cells [7]. The most dramatic effect of particle bombardment with DNA was found on induction of immunity after DNA transfer to the epidermis [6]: in mice, as little as 0.04 μg of a plasmid containing the HA of influenza A/PR/8/
the birds for a pronounced secondary response. Subsequent studies demonstrate that the immune response induced by H7-DNA is subtype-specific, and bursectomy experiments demonstrate that immunity is antibody-mediated (author’s unpublished data).

(2) Induction of cross-reactive (heterotypic) immunity to influenza viruses. Influenza viruses show continuous antigenic drift, which makes it necessary to change human vaccine strains almost every year to keep abreast of antigenic changes in the HA and neuraminidase (NA). The nucleoprotein (NP) of influenza virus, one of the antigenically conserved internal proteins, is important in the induction of cross-reactive cytotoxic T lymphocytes (CTLs) and recovery from infection, but it does not provide significant protection from initial infection.

The demonstration that DNA immunization with the NP of influenza A provides heterotypic immunity between different subtypes of influenza A in mice [2, 8] is important if this phenomenon is shown to extend to animals naturally infected with influenza, including humans, pigs, horses, and chickens. Studies on the preclinical efficacy of contemporary human vaccine strains in ferrets and nonhuman primates suggest that DNA expression vectors encoding HA and internal proteins were more efficient than were conventional inactivated or subvirion vaccines [9]. Preliminary studies with DNA vaccine to the NP of A/PR/8/34 (H1N1) in mice failed to provide protection from homologous challenge [10], indicating some controversy about the claims of cross-protection between subtypes and a need for more studies to determine if heterotypic immunity to influenza induced by NP applies across all subtypes and in naturally infected hosts.

(3) Induction of long-term B-cell immunity to influenza in mice. Comparative studies of conventional and DNA vaccines to influenza virus have established that DNA vaccination is equivalent to live vaccination in protecting mice from challenge [11, 12]. Two doses of intradermal gene-gun-administered DNA vaccine provided 100% protection, as did a live-virus vaccine, whereas a subunit vaccine provided only 70% protection. The antibody-producing cells localized in the spleen and bone marrow after vaccination; after challenge these cells were found in the lymph nodes draining the lungs. CD4+ cells were vital for induction of antibody-forming cells because treatment with anti-CD4+ antibody completely abolished antibody-forming cells from all compartments. The immunity induced by DNA immunization was maintained in the spleen and bone marrow for at least 1 year [11]. Studies in ferrets and nonhuman primates [9] confirm that DNA immunization is more effective than use of conventional influenza virus subunit vaccines.

(4) Broadening of the antibody response to the HA of influenza virus. Preliminary evidence suggests that DNA immunization may more effectively induce cross-reactive antibody than vaccination with live influenza virus. Immunization of ferrets with DNA encoding the HA of A/PR/8/34 (H1N1) induced antibodies that cross-reacted with A/swine/Shope/30 (H1N1) [13]. This cross-reaction was not found after natural
infection of ferrets. These studies indicate the potential for inducing high-affinity antibodies. In addition, these preliminary results warrant further study, for if cross-reactive antibodies are induced, it may be possible to reduce the frequency at which influenza vaccine strains are changed.

(5) Modulation of the antibody isotype by DNA immunization. There is increasing evidence that DNA immunization permits the investigator to modulate the immune response by targeting a T-helper (TH)-1 or TH-2 response [14]. Intramuscular or intradermal DNA vaccination of mice with the HA of influenza virus induces primarily IgG2a antibodies, whereas gene-gun immunization produces mainly IgG1 antibodies. Therefore, different methods of delivering DNA vaccines can influence the isotype of the antibodies produced [15]. Intramuscular immunization may result in TH-1-like responses, due to the predominance of IgG2a antibodies, CTL activity, and IFN-γ production and to the lack of IL-4 release. In contrast, gene-gun immunization appears to elicit a TH-2 response [16].

(6) Manipulation of the immune response with cytokine genes. In addition to altering the character of the immune response, the cytokines induced by the plasmids used for DNA immunization can alter the intensity of the response [14]. Cytokines play a central role in the immune response by promoting the activation of specific and nonspecific effector mechanisms. Inoculation of mice with vectors that expressed granulocyte-macrophage colony-stimulating factor and the rabies glycoprotein enhanced the B and TH cell activity against rabies virus, whereas concurrent inoculation with an additional plasmid expressing IFN-γ resulted in a decreased immune response [17].

Coexpression of other cytokines, including IL-6 [18], has also been shown to augment the immune response to DNA vaccines encoding the HA of influenza viruses. Therefore, inoculation of mixtures of plasmids that encode antigen or cytokines or of polycistronic vectors that express both may improve the efficacy of DNA immunization. Most importantly, DNA immunization has the potential for yielding further insight into the interplay of antigen and cytokine in the modulation of immune responses.

Although this article concentrates on the immune response induced by DNA immunization for influenza virus, the available evidence indicates that it is equally applicable to other respiratory pathogens, including respiratory syncytial virus, pneumococcus, and all protein-based antigens.

Safety Considerations

The introduction of genetic information into mammalian hosts raises several safety considerations. These include (1) the formation of antibodies to DNA, (2) the unexpected and untoward consequences of persistent expression of a foreign antigen, and (3) the potential for causing transformation [19].

(1) Antibodies to DNA. Studies to date indicate that there is no evidence of antibody induction against the DNA of mice or humans, even after repeated injections [20, 21]. To provide rigorous testing of the immunogenicity of plasmid DNA, mice prone to autoimmunity (B/W) and control mice (Balb/c) were immunized with a plasmid encoding the glycoprotein of Plasmodium yoelii [22]. The patterns of the immune responses in both strains were similar, and there was no evidence that the B/W mice produced antibodies to DNA that interfered with vaccine effectiveness. Therefore, the formation of antibodies to DNA and the induction of autoimmune disease are considered to be unlikely consequences of the administration of plasmid DNA.

(2) Altered immune state. Events that may lead to an altered immune state include the induction of tolerance, autoimmunity, anaphylaxis, hyperimmunity, and autoaggression. Studies in neonatal mice with a vector that expressed the rabies virus glycoprotein induced B and T cell responses, with no evidence of induction of tolerance [23]. At the present time, all of these consequences remain theoretical considerations. Thus far, no evidence suggests that these possible scenarios are practical concerns [19].

(3) Transformation. Theoretically, the introduction of extraneous DNA could lead to the formation of tumor cells through the insertion into or deactivation of a suppressor gene [24]. PCR analysis was used to look for sequences from an influenza NP-DNA vaccine plasmid and demonstrated that no integration could be detected [25]. Although direct inoculation of oncogene DNA can induce tumors, the risk of induction of tumors by plasmids specifically designed for DNA immunization is several orders of magnitude below that associated with the spontaneous mutation rate of human DNA. Additional testing for integration is needed before DNA vaccines will be generally accepted for use in humans.

Measures of Induction of Immunity to DNA Immunization

In part, live attenuated vaccines are so effective because they activate CD8+ and CD4+ precursor cells that give rise to the major antiviral effector cells: the major histocompatibility complex (MHC) class I–restricted CTLs and the class II–restricted TH cells [26]. The reason for this resides in the cell biology of the synthesis, assembly, and transport of the MHC class I molecule. The effective specific immune response to viral and parasitic diseases that is induced by DNA immunization involves both cell-mediated and antibody-mediated immunity. The available information supports the idea that like live-attenuated vaccines, DNA immunization results in presentation of antigens in a natural form to the host immune system (figure 2). One model for the priming of humoral and cellular immune responses by DNA vaccines reflects the distinct pathways of MHC class I and class II antigen processing in cell culture. Immune responses are thought to occur when antigen that is encoded by the plasmid-containing myocyte or keratinocyte is released by secretion or cell death. The antigens are then taken.
up by macrophages and B cells, thereby initiating a TH-dependent antibody response.

**Potential Advantages of DNA Immunization and Future Directions**

The potential advantages of DNA immunization are that it (1) may increase the rapidity with which new vaccines with genetic identity are generated; (2) induces long-lasting protective immunity; (3) mimics immunization with live-attenuated vaccine while eliminating the possibility of contamination with undesirable adventitious agents from the culture system; (4) provides correct MHC class I presentation of antigen; (5) allows concurrent administration of multiple DNA-encoded antigens and/or cytokines; (6) provides genetic stability of the immunizing plasmid; (7) obviates the need for a “cold chain” for vaccines in developing countries; (8) may permit modulation of the immune response; and (9) may allow creation of vaccines for agents that cannot be grown in culture.

We are at the very dawn of the DNA vaccine era. The optimal plasmid construct for DNA immunization of humans probably has not yet been designed, and the role of immunostimulatory sequences in the antigenic DNA and the influence of its methylation status have not been established. One question that remains to be answered for influenza viruses is how long-lasting immunity to DNA vaccines will influence the immune response to subsequent vaccination (or infection) with variants of that subtype. Will an “original sin” response be detrimental? This potential disadvantage of DNA immunization needs to be addressed in future studies.

Of immediate practical importance is the need to rapidly manufacture vaccines in response to the next influenza pandemic, and distributing and administering vaccines to an increasing world population will continue to be an enormous logistic problem. Our as-yet limited knowledge of DNA vaccination technology already suggests that DNA vaccines may greatly alleviate these concerns. Understanding the mechanisms by which plasmid DNA is taken up and expressed and the means to manipulate the multiple parameters involved in B and T cell responses may lead us to discover strategies for directing the immune response to conserved but currently nonantigenic regions of the influenza HA and NA. Providing that DNA vaccines meet the safety requirements discussed above, this method of immunization will likely play an important role in future strategies to control influenza and many other diseases.

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This stamp, with others, was issued in 1964 for the 16th anniversary
of the Proclamation of the State of Israel to publicize Israel’s contributions
to science. It depicts the DNA helix and macromolecules of the
living cell. (From the medical philately collection of Dr. J. N. Shan-
berge, University of Michigan.)