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COMMENTARY

Plasmid DNA: A New Era in Vaccinology

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ABSTRACT. DNA vaccination is a novel approach for inducing an immune response. Purified plasmid DNA containing an antigen's coding sequences and the necessary regulatory elements to express them is introduced into the tissue via intramuscular injection or particle bombardment. Once the DNA reaches the tissue, the antigen is expressed in enough quantity to induce a potent and specific immune response and to confer protection against further infections. The effectiveness of DNA vaccines against viruses, parasites, and cancer cells has been demonstrated in numerous animal models. This new approach comes as an aid for the prevention of infectious diseases for which the conventional vaccines have failed. Research on DNA vaccines is providing new insights into some of the basic immunological mechanisms of vaccination such as antigen presentation, the role of effector cells, and immunoregulatory factors. In addition, DNA vaccines may enable us to manipulate the immune system in situations where the response to agents is inappropriate or ineffective. The study of the potential deleterious effects of DNA vaccines is furthering our knowledge regarding the relationship between bacterial DNA and the immune system, as well as its potential application for the study of neonatal tolerance and autoimmunity. BIOCHEM PHARMACOL **55**;8:1151–1153, 1998. © 1998 Elsevier Science Inc.

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Two hundred years after Jenner's introduction of the concept of vaccines, a new revolutionary technology, "DNA Vaccination," has been conveyed into the world of medicine.

DNA vaccines are based on the direct inoculation of purified DNA in order to raise immune responses. Administration of a plasmid encoding the gene or genes for an antigenic portion of a virus results in the *in situ* expression of the antigen and leads to the induction of antigen-specific immunity. A report of the first demonstration of the protective efficacy of a DNA vaccination in an animal model was published in *Science*, as recently as 4 years ago. In their studies, Margaret Liu and her colleagues at the Merck Research Laboratories reported that direct injection of a gene from the influenza A virus triggered a full spectrum of immune responses, including antibody production, recruitment of cytotoxic T lymphocytes and T-helper cells, and the induction of immunologic memory, in mice. When subsequently challenged with the influenza virus, the vaccinated mice were able to resist infection [1]. Since then, the number of publications on the subject of plasmid DNA has grown significantly (see review [2]), and considerable public interest has been aroused. There is even an Internet site dedicated exclusively to DNA vaccination [3]. Although DNA vaccines are relatively new, some observers already regard the new technology as "the third revolution in vaccine development," on a par with Pasteur's groundbreaking studies with whole organisms and the development of subunit vaccines.

HOW DOES THE DNA VACCINE WORK?

DNA vaccines contain the gene or genes coding for an antigenic portion of a virus (the viral core or envelope proteins), parasite, or cancer. It has been proposed that following intramuscular injection, plasmid DNA is endocytosed by the myocytes located at the injection site [4]. These host cells are then thought to take up the foreign DNA, express the viral gene, and make the corresponding viral protein [5]. An important advantage of this system is that the foreign protein enters the cell's MHC† class I pathway (only proteins originating inside a cell are processed in this manner). MHC class I molecules then carry the peptide fragments of the foreign protein to the cell surface, where they evoke cell-mediated immunity by stimulating $CD8^+$ cytotoxic T cells. This is in contrast to standard vaccine antigens, which are taken up into cells via phagocytosis or endocytosis and are processed through the MHC class II system pathway, thereby primarily stimulating antibody responses [6].

Recently, the efficacy of myocytes in the role of antigen presenting cells has been challenged and the question of how DNA vaccines work has been reconsidered. Although myocytes are known to express MHC class I molecules and to present endogenously synthesized viral peptides to the $CD8^+$ cells, they lack the costimulatory molecule B7.1,

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† *Abbreviations:* MHC, major histocompatibility complex; IL, interleukin; and IFN-γ, interferon-γ.

which is important for antigen presentation. Thus, they are not considered professional antigen presenting cells in the classic sense. In our studies, the initial immune response following injection of plasmid DNA was localized to the draining lymph nodes, as opposed to the injection site muscle [7]. It is likely, then, that following vaccination migratory antigen presenting cells directly take up the plasmid DNA at the injection site and transport it to the lymph nodes or spleen where it is finally expressed and presented to $CD8^+$ cells [5, 7].

Plasmid DNA vaccination is characterized by low antigen levels and long-lasting antigen expression. Only a few hundred cells are believed to engage in antigen production and, although protein levels have been difficult to measure, the expressed antigen concentration is estimated to be in the nanogram range. Both the presence of plasmid and antigen expression have been detected intramuscularly 13 months after vaccination [8, 9].

DNA DELIVERY METHODS

DNA immunization can be accomplished via intramuscular saline injection or particle bombardment. Intramuscular injection is the most commonly used form of plasmid DNA delivery. Using either chloramphenicol, acetyl transferase, luciferase, or β-galactosidase, Wolff *et al.* [10] demonstrated for the first time that injection of purified DNA results in the expression of the protein within the muscular cell. Particle bombardment, in turn, involves coating of the plasmid DNA with gold microparticles. Delivery of the DNA into the epidermis is achieved using a device called a *Gene Gun*, which creates a shock wave capable of accelerating the DNA-coated gold particles into the target tissue via a controlled discharge. In this way, both the depth of gold particle penetration and the amount of DNA delivered per cell can be regulated [11]. This method appears to be a highly efficient way to achieve immunization, since a protective immune response can be elicited with considerably less DNA (as little as 40 ng) than what is typically used for intramuscular injection (10–400 mg). The direct intracellular delivery achieved via particle bombardment is largely responsible for the increased efficiency of this method [12, 13].

ADVANTAGES OF DNA VACCINATION

Vaccination is an invaluable medical aid for the prevention of human morbidity and mortality and represents the most commonly employed immunological intervention in medicine. Through vaccination we have been able not only to prevent the devastation caused by active infection and its sequelae, but also to eradicate numerous diseases, most notably chicken pox. Traditionally, attenuated or inactivated pathogens have been used for vaccination. More recently, new advances in molecular biology have resulted in the development of experimental subunit vaccines based on single antigens or even antigen fragments.

In spite of great success and ongoing advances, current vaccines target only a minuscule fraction of the spectrum of infectious diseases. In addition, most current vaccines induce primarily antibody responses, resulting in weak or absent cellular responses, and, therefore, have limited efficacy. Malaria, where a humoral response has been shown to be of little protective value against infection, exemplifies this last caveat. More importantly, vaccination for the more common and fatal infections afflicting the Third World is limited by the high cost and inefficient distribution of vaccines.

The advantages of DNA vaccines originate in the chemistry and molecular biology of DNA. The plasmid encoding the sequence for the antigenic protein also serves as the physical vector for the genes. In this way and mimicking viral infections, DNA-based vaccines are able to stimulate the intracellular synthesis of foreign proteins. Another advantage of DNA vaccination is that it results in the *in vivo* production of the purified and structurally intact antigen. This is important since the interaction between molecules and the cells of the immune system is highly dependent on their three-dimensional shape. Hence, the paradox—in order to be recognized by the immune system, the components of a vaccine must retain their native conformation, yet purification procedures often result in the denaturation and aggregation of the single antigens or antigen fragments of subunit vaccines.

The possibility of DNA serving not only as a vector but also as an adjuvant in the process of DNA-mediated immunization is even more remarkable. The plasmid vectors are propagated in bacteria and bear both characteristic bacterial sequences and patterns of base methylation. In a series of elegant experiments, Klinman and colleagues [14] have shown that these patterns of base or CpG motifs induce lymphocytes to secrete inflammatory cytokines, such as IL-6, IL-12 and IFN-γ. Even in the presence of small antigen levels, this intrinsic effect of the DNA vector would result in an optimal cytokine environment for the induction of an active and potent immune response.

Finally, from a public health point of view, DNA is very stable and resists extreme temperatures, thus facilitating the storage, transport, and distribution of vaccines. This is of critical importance for countries lacking the infrastructure to provide and to guarantee the proper storage and efficient distribution of vaccines.

DANGERS OF DNA VACCINATION

Although the immunogenicity of DNA vaccines is well established, concerns have been raised regarding their safety, more specifically their potential to induce deleterious immune responses, such as autoimmunity, and the development of tolerance in immunized individuals.

The potential of DNA vaccines to result in the formation of anti-DNA antibodies in healthy persons, as well as in individuals with autoimmune diseases [such as systemic lupus erythematosus (SLE)], is of special concern. An

additional safety concern associated with the use of DNA vaccines is that myocytes could potentially become targets for antigen-specific T-cells after taking up the injected plasmid and expressing the encoded antigen. Such a process could lead to the development of autoimmune myositis. Our studies in normal BAL/c mice have demonstrated that DNA vaccination may result in the production of low levels of IgG anti-DNA, but we were not able to detect anti-muscle cell antibody production. DNA vaccination of (NZB X NZW)F1 mice, an animal model for the study of SLE, did not induce or accelerate the development of systemic or muscle cell specific autoimmune disease [15].

Of concern, as well, is the possibility that vaccinated newborns may develop tolerance rather than immunity because of the immaturity of their immune system. This is especially relevant since most vaccines intended for human use are administered to infants and children and since numerous studies have demonstrated that in the early stages of postnatal development the immune system is unresponsive to antigenic challenge [16]. Since the protein encoded by a DNA vaccine is produced endogenously and is expressed in the context of self MHC, it is possible for the neonatal immune system to recognize it as self, leading to the development of tolerance. We have shown that a plasmid vaccine encoding the circumsporozoite protein of the malaria parasite, *Plasmodium yoelii*, induces tolerance rather than immunity when administered to 2- to 5-day-old mice [15]. Animals in which neonatal tolerance was induced were unable to develop cytokine or cytotoxic responses *in vivo* or *in vitro* when rechallenged with the DNA vaccine. This study demonstrated the existence of an epitope-specific induction of tolerance dependent on the manner in which the protein is presented to the immune system cells and the age of the individual at the time of immunization. Tolerance was never achieved by exogenous administration of the protein or when the plasmid DNA was administrated to mice >2 weeks of age. These results pose serious challenges to DNA vaccine developers, as they highlight the risks of inducing tolerance by giving vaccines to newborns with immature immune systems. On the other hand, the tolerance-inducing effect of DNA vaccine could potentially be used for the treatment of autoimmune diseases in which the immune system destroys its own tissues.

CONCLUSION

From both a practical and scientific standpoint, DNA vaccines have expanded the horizon of gene therapy and immunoprotection, and continue to provide new insights into essential microbiologic and immunologic processes.

In spite of being a relatively new technology, plasmid DNA vaccination has created high expectations for its use and future applications. At the present time, several clinical trials are underway with promising results, especially those studies related to the development of a vaccine for HIV and melanoma.

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