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DNA Vaccines – An Overview

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1.1
Rationale for DNA Vaccines

Administration of genes via DNA or RNA may be considered the next-generation of scientific development following the use of recombinant proteins for prophylactic vaccines or for therapy. The use of DNA vaccines for the generation of immune responses arose from efforts to find immunogens that would be able to overcome some of the limitations of other modalities of vaccination. With the discovery of the potential widespread applications of DNA plasmids came appreciation of certain of the characteristics of DNA as a product: namely, its advantages, relative to other biologicals, for manufacturing (Chapter 3), product characterization, storage (Chapter 3), and delivery (Chapters 5–12).

From the standpoints both of therapeutics and of vaccines, the use of DNA arose from the desire to have a protein be produced *in situ*. For a variety of applications, ranging from cytokine administration to gene therapy for metabolic and inherited disorders, it was clear that administration of the gene rather than the protein could have multiple advantages: proteins synthesized *in situ* from DNA could potentially persist locally or systemically for longer periods of time without the toxicities associated with the high levels of intravenously administered proteins, certain proteins such as cytokines could be administered to the desired site (i.e., intratumorally) (Chapter 7) more readily when administered as genes, and a protein synthesized from the gene would have mammalian posttranslational modifications, thus avoiding one of the significant challenges that can arise when making recombinant proteins in nonmammalian hosts.

Although vaccines have been considered perhaps the greatest human health achievement, being successful even to the point of eliminating an entire wild-type disease from the planet (smallpox), certain diseases have remained unconquered by vaccination. Two key reasons for this are that the traditional approaches have either simply not worked, or have been considered potentially too risky for a disease such as HIV. As an example, although live attenuated virus vaccines have been extremely effective against a variety of diseases, they have at least the theoretical

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risk of reversion to wild type, which in the case of HIV would render the vaccinee infected with a virus that causes what today is still a fatal infection.

As understanding of immune responses to disease increased, it became clear that the use of vaccines that induced primarily antibody responses might not be able successfully to target diseases that required a strong CD8+ T cell responses. Proteins that enter the cellular processing pathway resulting in the generation of CD8+ T cell responses generally have to be endogenously synthesized within a cell. Means to deliver the gene for an antigen, rather than the antigen itself, directly into cells were therefore sought, as the latter would generally result in the exogenous protein being taken into the endolysosomal processing pathway, with the resultant generation of MHC Class II-restricted CD4+ T cells rather than CD8+ T cells. The observation that plasmid DNA could directly transfect cells *in vivo* [1] came as a surprise given the complexity of viral structures that are designed for infecting cells. The process of DNA transfection is very inefficient and, moreover, the best transfected cell type is the muscle cell. Myocytes lack the immune accessory surface molecules needed to activate immune-responding cells appropriately, so it was a surprise to find that direct transfection of myocytes by immunization with unformulated plasmid DNA could indeed result in the generation of CD8+ T cells and protection against a lethal viral challenge [2].

DNA vaccines had further appeal as a product, in additional to their immunologic rationale. The manufacturing process promised to be fairly generic in comparison with those for other biologicals. Traditional live virus vaccines require years of challenging work to attenuate the pathogen properly and to design a cellular production system. Even recombinant proteins can be challenging, because of the need to find the correct producer cell able to make the antigen in the correct form (such as with the correct folding or posttranslational modifications). Because DNA vaccines are bacterial plasmids, the production is quite similar for different vaccines because they differ only in the gene sequence encoding the antigen. The majority of the plasmid, such as the backbone, can be identical or similar. Moreover, DNA vaccines at their simplest, being just plasmids, are potentially more stable (Chapter 3) than live viruses, an attribute that should facilitate their use in resource-poor settings.

1.2 Preclinical Proof of Concept

The initial demonstration that direct immunization with a simple plasmid of DNA encoding a protein from a pathogen could not only result in the generation of both arms of the immune response (cytotoxic T lymphocytes as well as antibodies), but could also protect from an otherwise lethal challenge [2] opened up the field of DNA vaccines. The ability to protect animals from a strain of virus different from the strain from which the gene was cloned generated considerable interest because it offered a potential means to make vaccines for diseases that have multiple strains, such as influenza or HIV. The influenza vaccine, for example, has to contain antigens

for three strains and needs to be reformulated each year as new strains arise. Not only is this a cumbersome process making the adequate yearly supply of vaccines problematic, but such a vaccine does not protect against the epidemic strains differing from the strain in the vaccine that occasionally arise mid-season. Of even more concern is the fact that such a vaccine will not protect against novel pandemic strains of influenza that periodically may arise, most notably in the 1919 Spanish influenza that killed millions of people worldwide. The demonstration that a DNA vaccine made from the genetic sequence of one strain was able to protect against challenge not just with a slightly different drifted strain, but against a different subtype, raised hopes for the ability of DNA vaccines to be effective against a variety of diseases.

From those initial studies, the scientific literature rapidly grew to thousands of publications demonstrating the ability of DNA vaccines to induce immune responses and protective and therapeutic benefits in a variety of preclinical disease models. These models not only included various infectious diseases, including those caused by viruses, bacteria, and parasites, but also encompassed other types of disease, such as cancer, allergy, and autoimmunity (reviewed in [3, 4]). Additional applications for autoimmune diseases and allergies are based upon the ability of the DNA to alter the type of generated T cell help specifically for the particular protein antigen. Autoimmune responses are thought to be due to the inappropriate overproduction of either T helper 1- or T helper 2-type responses. In animal models, DNA vaccines have been shown to be able to alter the form of T cell help, and DNA vaccines have thus been able to prevent or ameliorate the disease in preclinical models of asthma [5] and diabetes [6].

It soon became evident, however, that DNA vaccines, while robust in small animal models, were less immunogenic in nonhuman primates and humans (reviewed in [3, 4]). This has given rise to a variety of approaches for making DNA vaccines of increased potency, as is explored below.

1.3 Clinical Trials

Clinical trials have been performed for DNA vaccines encoding antigens from pathogens and tumors. In addition, however, trials have been performed with DNA encoding therapeutic proteins where not an immune response, but rather expression of the therapeutic protein, is desired. Such studies have included the therapeutic administration of a gene encoding a normal growth factor such as Fibroblastic Growth Factor, or other growth factors, the intent being not to replace a defective or missing protein, but rather to administer a supraphysiologic amount of the growth factor to a local site for a period of time more prolonged than would be achievable by administration of the recombinant protein [7, 8]. The factor then induces the growth of new blood vessels to ameliorate the ischemic condition of the limb or myocardium. DNA has also been used for what is more traditionally considered to be the purview of gene therapy: DNA encoding a form of the muscle

protein dystrophin, for example, has been administered to patients with forms of muscular dystrophy who are lacking in the production of any (or any normal) dystrophin ([9], Chapter 11). In both of these types of clinical applications, the hope is that no immune responses against the therapeutic protein will be generated. In the case in which the DNA is intended to provide additional amounts of a therapeutic protein locally, the individual is already tolerized to the protein, so the administration of the gene through the use of a plasmid should not break the tolerance. The use of a DNA plasmid is thought to be potentially less immunogenic for these purposes than the use of viral vectors, another widely studied approach.

Of course, the most important observation in all the vaccine and therapeutic clinical trials has been that the vaccines have been safe to administer. Secondly, antibody and cellular immune responses, albeit generally low, have been observed in the patients in clinical trials. Interestingly, in HIV patients with long exposure to high levels of viral antigens (due to their high viral loads), new antibody but particularly T helper and cytolytic T cell responses were seen after DNA immunization [10, 11], the DNA somehow eliciting immune responses that the virus could not. This represents the important observation that different methods of producing an antigen *in vivo*, or the effects of different vectors, may result in different immune responses, an observation consistent with the results of preclinical prime-boost studies (see below).

1.4 Second-Generation Vaccines

Perhaps the simplest approach to increasing the potency of DNA vaccines has been to design the plasmids to produce more protein antigen [12] and/or to increase the doses used in clinical trials, even up to milligram doses per vaccine [13, 14]. Another approach, described more fully in this book, is to formulate the DNA in such a way as to facilitate its uptake into cells, or to protect it from degradation. Alternative delivery modalities, such as combining injection (Chapters 6, 7 and 10) with *in vivo* electroporation (Chapters 11 and 12) to increase the amount of transfection, are also being explored.

The coding sequences of DNA vaccines have also been modified to include genes encoding cytokines or other molecules that may enhance immune responses. Because the bacterial DNA in DNA vaccines has sequences that activate Toll-like receptors, the DNA is not simply an inert carrier of the genes, but itself also activates the innate immune system, which may in turn augment the cognate immune responses (reviewed in [15]). Efforts to increase this innate immune stimulation by increasing the number of CpG motifs in the plasmid have met with limited success, but the principal of harnessing the innate immune response to aid in the antigen-specific response is the focus of considerable attention.

DNA vaccines have also been delivered by a variety of routes, variously to increase potency, to generate specific forms of immunity (e.g., mucosal), or to facilitate delivery. The earliest demonstration of the ability of DNA plasmids to generate

antibody responses utilized a 'gene gun' to propel DNA-coated gold beads into the cells of the skin (Chapter 10) [16]. This approach has successfully resulted in the generation of antibodies against hepatitis B surface antigen in clinical studies [17]. In these studies, the titers were lower and required more immunizations that with the licensed protein vaccine, but nevertheless demonstrated the desired immune response in humans. Importantly, though, even patients who had not responded well to the traditional recombinant protein vaccine responded to the DNA vaccine [18]. Additional means of delivery have included the production of biodegradable to which the DNA is adhered (reviewed in [19]) or particles containing the DNA for oral delivery [20] (Chapters 5 and 8). Additional devices that propel the free DNA directly into the skin [21] or mucosa [22] have been developed. *In vivo* electroporation to increase the number of cells that are transfected is also being developed [23] (Chapters 11 and 12).

One of the most promising approaches has been the combination of DNA vaccines with viral vectors or recombinant protein [24, 25] (reviewed in [4]). In this approach a DNA plasmid encoding a given antigen is injected, and the subsequent immunizations then utilize a heterologous delivery system such as a viral vector encoding the same antigen, or a different form of the antigen (e.g., a recombinant protein). This has been referred to as the 'prime-boost' approach. While the mechanism for its efficacy has not been completely determined, a variety of different viral vectors, including adenoviruses and pox vectors, have been utilized. Interestingly, it appears that the approach is most effective when the DNA vaccine is given first, rather than the other way around.

1.5 Conclusions

Although the second generation of DNA vaccines includes more complex formulations and devices, the inherent simplicity of the core of the vaccine (i.e., the plasmid DNA) nevertheless remains an attraction. For scenarios in which the formulation of final product may be more complex (such as the inclusion of two different vectors), it is felt that if that is what is required to overcome the challenges of making a vaccine for HIV, this will nevertheless be a critical part of the medical armamentarium. The potential for developing a somewhat generic, even if complex, approach to a variety of diseases, including diseases that have hitherto been resistant to prevention or therapy, makes these studies of continued high interest.

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