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DNA Vaccines in Immunity - A Comprehensive Review

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Abstract

Traditionally vaccines are based on immunogens delivered as attenuated live microbes, inactivated pathogens, purified proteins or virus-like particles. Newer generation vaccines are based on the delivery of genes encoding for a protein antigen that can be transcribed and translated by host cells. Despite current challenges to improve delivery and immunogenicity, DNA vaccination has several major advantages over traditional vaccines or over other types of investigational vaccine platforms. DNA vaccines do not integrate into the host genome, they are stable, can be manufactured with relative ease and efficiency, have been safe in clinical trials and do not require a preservative in final preparation. The lack of vector-specific immunity allows the potential for DNA vaccines to be used as a platform technology for emerging viral diseases by allowing the simple exchange of genes encoding vaccine antigens in a stable plasmid backbone.

Keywords: DNA Vaccine, Advantages, Types, Applications

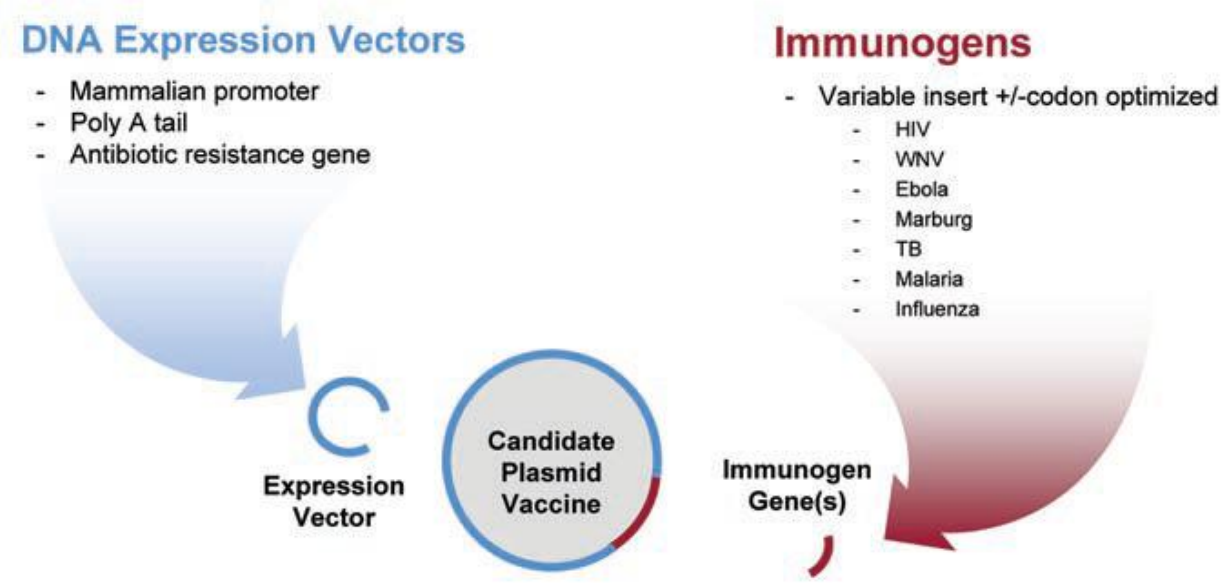
Introduction

Since Jenner's original discovery and the subsequent eradication of smallpox, vaccines have made a dramatic positive impact on public health. The field of vaccinology has evolved in direct association with scientific advances in molecular biology, immunology and infectious disease pathogenesis under pressure from emerging infectious diseases and pathogens that have eluded vaccine solutions. Traditionally vaccines have been based on immunogens delivered as attenuated live microbes, inactivated pathogens, and purified proteins or as particles simulating wild-type structures. Newer generation vaccines are based on the delivery of genes encoding for a specific protein antigen that can be transcribed and translated by host cells.1 The host cell can present authentic protein conformations as well as processed proteins in the context of class I and class II major histocompatibility complexes (MHC). The host immune system is thereby able to mount humoral and cellular immune responses to microbial protein. Once the gene is delivered and the encoded gene is transcribed and translated, the host cell is recognized and destroyed by the host immune response. Importantly, the ability to mount a gene-based vaccine-induced immune response appears to be irrespective of HLA type or other individual characteristics as shown by the high frequency of immune response among subjects in

early phase I and II clinical trials. Gene-based vaccination can be accomplished through a variety of platforms. Altered microbes designed to express genes encoding vaccine antigens are generically called vectors. Vaccine vectors can be replication-competent or replication-defective. They utilize the tools evolved by viruses, bacteria, fungi or other microbes to gain entry into host cells to deliver the genetic payload. Genebased vaccines vectors have been developed that efficiently deliver genes for vaccine antigen production in host cells. However, there are issues involving manufacturing, toxicity and vector-specific immunity that significantly alter risk: benefit and cost: benefit considerations. Therefore, gene-based vaccination using DNA plasmid technology has a number of advantages over vector-based vaccine approaches.

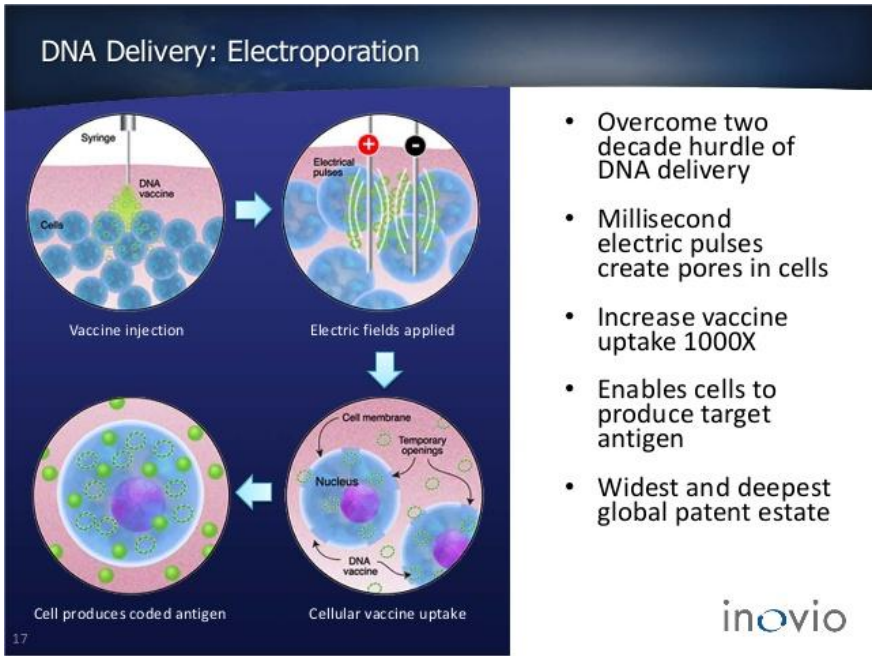
Construction of DNA Vaccines

Genes of required interest coupled with a suitable promoter are injected directly into muscle or coated into gold micro particles and "shot" into the skin by pressurized gas using a gene gun. This can induce cellular and humoral immunity in experimental animals for a longer period of time. The mechanism appears to be through uptake and expression of the DNA in antigen presenting cells (APCs).[2] The diagrammatic representation of DNA vaccines has been shown in figure 1. DNA vaccines raise both humoral and cellular immunity. The injected gene of the concerned DNA vaccine is expressed in the injected muscle cell and also in nearby APCs. The protein is made up of peptides which, after being processing as endogenous antigens through the MHC class I pathway, form the protein encoded by the concerned DNA and are expressed on the surface of both cell types. Cells that present the antigen in the context of class I MHC molecules stimulate development of cytotoxic T cells. The protein encoded by the injected DNA is also expressed as a soluble, secreted protein. This is taken up and finally processed, and presented through class II MHC molecules. This pathway provokes B-cell immunity and generates antibodies and B-cell memory against the protein. This response serves to defend the host from the concerned microorganism for which the particular DNA vaccine has been made.[11] The researchers later proposed three different mechanisms that contribute to the immunogenicity of DNA vaccines. First, the antigens encoded by the DNA are presented by somatic cells (myocytes or keratinocytes) to CD8 T cells through their MHC class I pathway. Second, the DNA immunization results in direct transfection of professional antigen presenting cells (APC) (e.g. dendritic cells). Third, cross-priming results from transfected somatic cells are phagocytosed by professional APCs which then present the antigens to T cells. As the muscle cells are not up to the mark at presenting antigens through MHC class I, the latter two mechanisms appear to be more appropriate to DNA vaccines.[2] Currently, attempts are also underway to incorporate DNA into the nasal tissue by using nasal drops. It should be noted that once inside the cells of the recipient, the plasmid does not replicate, but only expresses itself, and protein is produced. Usually, bacterial plasmids are used, and a gene encoding the antigen is inserted into the control of mammalian promoter and this chimeric plasmid is then introduced into the recipient. The recipient cell then expresses the foreign antigenic protein coded by the introduced DNA into the host. The immune system then responds to the antigen as to any other antigen entering the body.[5]



Points to keep in mind while constructing DNA Vaccine

A number of features have to be kept in mind while designing a DNA vaccine. The selection of antigens, vector, delivery route, dose, timing, adjuvants, and boosting agents will all affect the outcome of vaccination. The reason behind this is that they affect the magnitude and quality of immunity elicited. The selection of target antigens should be given the first priority while designing a DNA vaccine. An individual must select the genes from the pathogen and also the form of the gene, whether the gene is mutated or wild type, intracellular or membrane-bound or secreted. After the selection of the desired gene, one can proceed for its modification to achieve the immunogenicity of the DNA vaccine. The vectors used for expression of the antigen can also have a large impact on immunogenicity. Promoters, enhancers, and introns can affect the level of antigen expression. Most DNA vaccine studies use plasmids carrying promoters that constitutively yield high levels of protein in most mammalian tissues. Additional modifications can be made to increase protein production in transfected host cells. The most effective of these is codon optimization. In order for a DNA vaccine to work, it is essential to incorporate DNA coding an appropriate antigen, to elicit the required antibody response of the immune system. A variety of factors may affect the route of choice. DNA vaccines can be easily injected with needles. They can be easily prepared in saline. The main advantages of biolistic technology, such as Gene Gun (Bio-Rad, USA) or Biojector 2000 (Bioject Medical Technologies, USA) lie in the fact that the technology possesses high efficiency.[2]



Advantages and disadvantages of DNA Vaccines

DNA vaccines appear to have certain advantages over conventional vaccines, for example the ability to induce a wider range of types of immune response. A number of advantages and disadvantages are listed in tables I and II, respectively.

Advantages of DNA vaccines

Advantages of DNA vaccines
Inexpensive
Long-term persistence of immunogenicity
Subunit vaccination with no risk for infection
Antigen presentation by both MHC class I and class II molecules
Ability to polarize T-cell help toward type 1 or type 2
Ease of development and production
Immune response focused only on antigen of interest
Stability of vaccine for storage and shipping
In vivo expression ensures that the protein resembles the normal eukaryotic structure more closely, with accompanying post-translational modifications
DNA vaccines are safer, more stable, and easy to handle
DNA vaccines induce protective humoral and cellular immune responses
DNA vaccines are heat stable
A mixture of plasmids could be used to form a broad spectrum vaccine

Disadvantages of DNA vaccines

Disadvantages of DNA vaccines
Limited to protein immunogens (not useful for non-protein based antigens such as bacterial polysaccharides). Certain vaccines, such as those for pneumococcal and meningococcal infections, use protective polysaccharide antigens
Inducing antibody production against DNA
May induce immunologic tolerance by antigens expressed inside host body
DNA vaccines may have a relatively poor immunogenicity
Atypical processing of bacterial and parasite proteins
Insertion of foreign DNA into the host genome may cause the cell to become cancerous

Application of DNA Vaccines

Tests of DNA vaccines in animal models have shown that these vaccines are able to induce protective immunity against a number of pathogens including influenza and rabies viruses. At present, human trials are under way with several DNA vaccines, including those for malaria, AIDS, influenza, Ebola and herpesvirus.

DNA Vaccine against Cancer

DNA vaccination has become an effective strategy for the development of vaccines against cancer, including cervical carcinoma (CC). Persistent infection with human papillomaviruses (HPV) is the main etiological factor in cervical cancer, the second most common cancer in women worldwide.[2] The formation of CC is associated with HPV infection. Viral E6 and E7 oncoproteins are suitable targets for therapeutic vaccination. In this context, DNA vaccine against HPV type 16 was reported.

DNA Vaccine against Tuberculosis

Use of therapeutic DNA vaccines is a promising strategy against TB. DNA vaccine expression of IL-2 and the HSP65 fusion gene was studied. It elevated the immunogenicity and protective as well as therapeutic effects of the HSP65-DNA vaccine against TB in mice. This was achieved by improving the Th1-type response3. Addition of immunostimulatory motifs in the transcribed region of a plasmid DNA vaccine elevated Th1 immune responses and the therapeutic effect against *Mycobacterium tuberculosis* in murine models.[5] Recent studies have described the efficacy of T-bet as Th1-inducing adjuvant in the context of Ag85B DNA-based vaccination. It could also prove to be a promising candidate for DNA vaccine development against TB[6] A novel TB DNA vaccine was reported to have been synthesized. This vaccine utilizes an HIV-1 p24 protein backbone. It confers protection against *Mycobacterium tuberculosis* and simultaneously elicits humoral and cellular response to HIV-1.

DNA vaccine against HIV

Human immunodeficiency virus (HIV) causes acquired immunodeficiency syndrome (AIDS) and remains one of the most serious threats to global health. Today there are no vaccines to prevent HIV infection. As far as the knowledge of this author is concerned, all of the candidates explored so far are in the experimental stage. HIV-negative people were used to study the effect of preventive vaccine candidates to see if they can prevent infection.[1] The safety, stability, and ability for repeated homologous vaccination encourage the DNA vaccine platform as important candidate for an effective HIV-1 vaccine. The immunogenicity of DNA vaccines for HIV has been increased through improvement of the DNA vector, through the inclusion of molecular adjuvants, heterologous prime-boost strategies, and delivery with electroporation. [2]The principle behind electroporation is that it applies a small electric field across the site of injection that causes temporary membrane instability and

produces an electric gradient, which elevates the cellular uptake of DNA. It is a useful technique as it increases the transfection efficiency of DNA vaccines *in vivo*.[8] Nanoparticles as drug-delivery systems have also been explained by the Editor-in-chief of this Journal in a previous editorial.[4] The study of nanoparticles provides a strong platform to combining protein- and DNA-based vaccines/antiretrovirals which can help the production, preclinical evaluation and the clinical testing in the near future.

DNA Vaccine against Anthrax

Anthrax is an infectious zoonotic disease caused by *Bacillus anthracis*, a spore-forming encapsulated bacteria. In human beings, three forms of anthrax have been recognized. They are cutaneous, gastroenteritis and pulmonary forms.[4] This disease is not common in western countries but the countermeasures against this disease are important because the spores of *B anthracis* can be used as bio-terror weapons.[5] DNA vaccination resulted in varying degrees of protection and appears to be a promising approach in this field.[6] The immunogenicity and efficacy of an anthrax/plague DNA fusion vaccine in a murine model has been described.

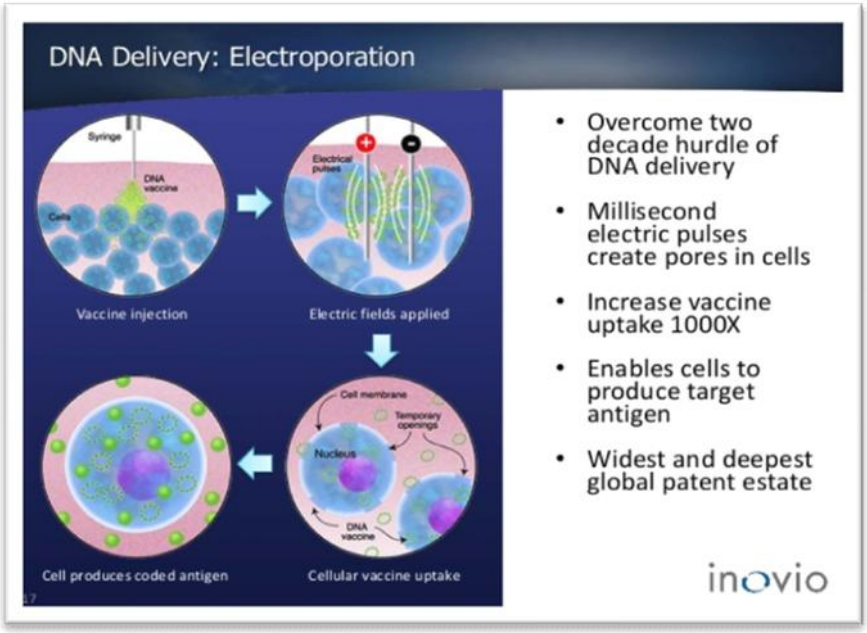
DNA Vaccine against Influenza

Each year, particularly in the months of February and September, the World Health Organization (WHO) recommends the influenza viruses to be included in influenza vaccines for the forthcoming winters in the Northern and Southern hemispheres respectively. Generally, influenza vaccines are often updated so as to be most effective against newly emerging strains of human influenza viruses that are likely to circulate in the forthcoming influenza season.[8] Influenza viruses A and B are associated with significant morbidity and mortality in humans. Influenza virions contain two major surface glycoproteins, hemagglutinin (HA) and neuraminidase (NA), and these are the predominant antigens of these viruses. Several influenza genes have been evaluated as potential DNA vaccine candidates, including HA, NA, matrix protein (M1), nucleoprotein (NP) or nonstructural protein (NS1).49 An epidermal DNA vaccine for influenza, immunogenic in humans, has been reported.9 Intramuscular influenza HA DNA vaccines have been shown to be immunogenic in preclinical models.[5] Preparation and immunological effectiveness of a swine influenza DNA vaccine encapsulated in chitosan nanoparticles has also been reported.[5] Complete protection against a H5N2 avian influenza virus by a DNA vaccine expressing a fusion protein of H1N1 HA and M2e has been described.

DNA Vaccine against Malaria

Malaria is a major cause of disease and death. Approximately half of the world's population is at risk of malaria.[3] The United States National Institute of Health is supporting about ten International Centers of Excellence for Malaria Research throughout the world.[4] In South Asia, India has more than three million square kilometers of land, and vast amounts of these lands are well suited for the breeding of mosquitoes, leading to the propagation of malaria parasites.[5] Various strategies have been developed to prevent this burden, aimed at diagnosis, treatment, and vector control. DNA vaccination is one of the novel approaches for developing new generation vaccines against malaria. Coated DNA vaccines have been shown to exhibit good immunogenicity and show protective levels of antigen-specific IgG, an elevated proportion of CD4+, CD8+ T cells, INF-γ and IL-12 levels in the serum and cultured splenocyte supernatant, as well as INF-γ-producing cells in the spleen. An effective delivery system for malaria vaccination has been described for an NP-coated, MSP-1 DNA-based vaccine which confers protection against lethal *Plasmodium yoelii* infection in mouse models across various routes of administration[5] Molecular adjuvants for malaria DNA vaccines based on the modulation of host-cell apoptosis have been described.[7] Field literature describes Vaxfectin (Vical, USA) as having the ability to elevate antibody response and T cell response to each component of a 5-gene *Plasmodium falciparum* plasmid DNA vaccine mixture.[7] It has been hypothesized by

some of the researchers that a malaria therapeutical vaccine targeting the erythrocyte stage of the parasite through erythrocyte sickling can lower the parasite density and also control the progression and severity of this disease.[8]



DNA Vaccine against dengue

Dengue is a mosquito-transmitted infectious disease. It also has an important impact on human health globally. This disease has increased dramatically in the past century throughout the globe, and is now among the most common causes of febrile illness in travelers.[9] The human immune system produces antibodies against a number of dengue proteins, namely C, prM, E, NS1, NS3, NS4B and NS5. Most of the anti-dengue neutralizing antibody epitopes have been mapped to the E protein. That is why the E gene has been chosen for constructing DNA vaccines. It has also been reported that the prM gene is essential for the proper processing and folding of the E protein and hence the prM gene has also been included. A number of DNA dengue vaccine have also been studied and presented and a West Nile virus CD4+ T cell epitope appears to improve the immunogenicity of dengue virus serotype 2 vaccines. Immunogenicity and protective efficacy of a Vaxfectin-adjuvanted tetravalent dengue DNA vaccine has also been discussed.[6]

DNA Vaccine against typhoid

Salmonella infection is a food borne infection. Typhoid fever is a prolonged febrile illness caused by bacterium *Salmonella typhi*. It has a global distribution and is a worldwide problem as described by Khan et al. Typhoid can be treated by using antibiotics.[7] Vaccination and herbal drugs also showed interesting results. A number of plants have been reviewed by this author for their medicinal assessment. Recently, a number of vaccines against *Salmonella* have been developed including live-attenuated as well as DNA vaccines and their clinical trials exhibited promising results.

DNA vaccine against other diseases

A recent study has reported the efficacy of DNA vaccine-generated duck polyclonal antibodies as post-exposure prophylaxis to prevent hantavirus pulmonary syndrome.[8] The development of DNA vaccines against foot-and-mouth disease has also been studied in detail.[3] The efficacy of *Leishmania donovani* ribosomal P1 gene as DNA vaccine in experimental visceral leishmaniasis has also been reported.[3] Development of a DNA vaccine

targeting Merkel cell polyomavirus has also been studied. A number of DNA vaccines against different antigens and the concerned system in which the vaccine was tested are listed in table III.

DNA vaccines against different antigens

Name of DNA vaccine	Antigen against which the DNA vaccine was directed	System in which the DNA vaccine was experimented
P^{CE6}	Eta6	Fish
P^{CE18}	FliC	Fish
S iniae DNA vaccine in the form of plasmid pSia10	Sia10	Fish (turbot model –*Scophthalmus maximus*)
pcDNA3-LTDNA vaccine	MCPyVlarge T antigen (LT) (aa1-258)	Mice
pIDSia10	Sia10	Fish
pIDOmpU	OmpU	Fish
pSiVa1	Sia10 and OmpU	Fish

DNA Vaccine and prophylaxis

A guideline was released in 1996 by the FDA to assist the people engaged in developing DNA vaccines. The name of the guidance document is "Points to consider on plasmid DNA vaccines for preventive infectious disease indications".[8] The document provided necessary information regarding the pre-clinical and clinical issues concerned with the development of DNA vaccines. Moreover it also raised the safety concerns to be taken into account by workers before the starting of clinical trials. Further, the guidance was revised in the year 2007 to understand more preclinical and clinical issues for DNA vaccine manufacturing. The main aim of FDA guidance is to have a full watch on the methods, processes and facilities availed to manufacture vaccines so that the vaccine is pure and potent. It also checks the safety of vaccine before it goes on to clinical trial. The guidance document framed during the year 2007 explained that there is no requirement of sponsor to perform a preclinical trail to assess the effect of a vaccine on autoimmunity. It further concluded that the established clinical monitoring procedures were enough to asses any adverse effect. Moreover the adverse effect also includes autoimmune disease. Once a claim has been made that the vaccination induces protection in adults, preclinical studies in appropriate animal models can help to study in forward direction in younger individuals.

Conclusion

The field of DNA vaccination has recorded significant progress during the past decades. Better-designed constructs and promoters, as well as novel delivery technologies have been tested in animal models and advanced in the clinic. The author explored the strategies for construction and working of DNA vaccines. The applications of DNA vaccines in different diseases were highlighted. Much stress has to be required by the researcher to develop DNA vaccines against various diseases. It is also the requirement of the present time to develop ways and means to develop the vaccine in a limited period of time, in order to help eradicate emerging infectious diseases.

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