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# DNA Vaccine

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**Abstract:** ***In the two decades since their initial discovery, DNA vaccines technologies have come a long way. Unfortunately, when applied to human subjects inadequate immunogenicity is still the biggest challenge for practical DNA vaccine use. Many different strategies have been tested in preclinical models to address this problem, including novel plasmid vectors and codon optimization to enhance antigen expression, new gene transfect ion systems or electroporation to increase delivery efficiency, protein or live virus vector boosting regimens to maximise immune stimulation, and formulation of DNA vaccines with traditional or molecular adjuvants. Better understanding of the mechanisms of action of DNA vaccines has also enabled better use of the intrinsic host response to DNA to improve vaccine immunogenicity. This review summarizes recent advances in DNA vaccine technologies and related intracellular events and how these might impact on future directions of DNA vaccine development.***

**Keywords:** DNA vaccine, preparation of vaccine, vector design, delivery method, mechanism, advantages and disadvantage and future of DNA vaccine

## 1. Introduction

DNA vaccine is DNA sequence used as a vaccine. DNA vaccination is a technique for protecting an organism against disease by injecting it with genetically engineered DNA to produce an immunological response. The direct injection of genetic material into a living host causes a small amount of its cells to produce the introduced gene products.

This inappropriate gene expression within the host has important immunological consequences, resulting in the specific immune activation of the host against the gene delivered antigen. In this way, DNAvaccine provides immunity against that pathogen. Nucleic acid vaccines are: Experimentally provedapplied to a number of viral, bacterial and parasitic diseases experimentally proved.DNA vaccines have a number of advantages over conventional vaccines, including the ability to induce a wider range of immune response types.

## 2. History

The story of vaccines did not begin with the first vaccine– Edward Jenner's use of material from cowpox pustules to provide protection against smallpox. Rather, it begins with the long history of infectious disease in humans, and in particular, with early uses of smallpox material to provide immunity to that disease.

Edward Jenner's innovations, begun with his successful 1796 use of cowpox material to create immunity to smallpox, quickly made the practice widespread. His method underwent medical and technological changes over the next 200 years, and eventually resulted in the eradication of smallpox.

Louis Pasteur's 1885 rabies vaccine was the next to make an impact on human disease. And then, at the dawn of bacteriology, developments rapidly followed. Antitoxins and vaccines against diphtheria, tetanus, anthrax, cholera, plague, typhoid, tuberculosis, and more were developed through the 1930s.

The middle of the 20th century was an active time for vaccine research and development. Methods for growing viruses in the laboratory led to rapid discoveries and innovations, including the creation of vaccines for polio. Researchers targeted other common childhood diseases such as measles, mumps, and rubella, and vaccines for these diseases reduced the disease burden greatly.

More than the science behind vaccines, these timelines cover cultural aspects of vaccination as well, from the early harassment of smallpox variolators (see the intimidation of a prominent minister described in the 1721 Boston Smallpox Epidemic entry) to the establishment of vaccination mandates, to the effect of war and social unrest on vaccine-preventable diseases. Edward Jenner, Louis Pasteur, and Maurice Hilleman, pioneers in vaccine development receive particular attention as well.

## 3. Types of Vaccines

### 3.1 First Generation Vaccines

Are whole-organism vaccines – either live and weakened, or killed forms. Live, attenuated vaccines, such as smallpox and polio vaccines, are able to induce killer T-cell (TC or CTL) responses, helper T-cell (TH) responses and antibody immunity. However, there is a small risk that attenuated forms of a pathogen can revert to a dangerous form, and may still be able to cause disease in immunocompromised people (such as those with AIDS).

While killed vaccines; do not have this risk, theycannot generate specific killer T cell responses, and may not work at all for some diseases. These were the earliest vaccines.Attenuated (live, attenuated microorganisms eg.Yersiniapestis EV is used for plague immunization) Toxoid ( inactivated toxic compounds that cause illness rather than the micro-organism. Eg.tetanus and diphtheria)

### 3.2. Second Generation Vaccines

Second generation vaccines were developed to minimize the risks of the live attenuated vaccines. Protein antigens (such as tetanus or diphtheria toxoid) or recombinant protein components (such as the hepatitis B surface antigen).These, too, are able to generate TH and antibody responses, but not killer T cell responses.

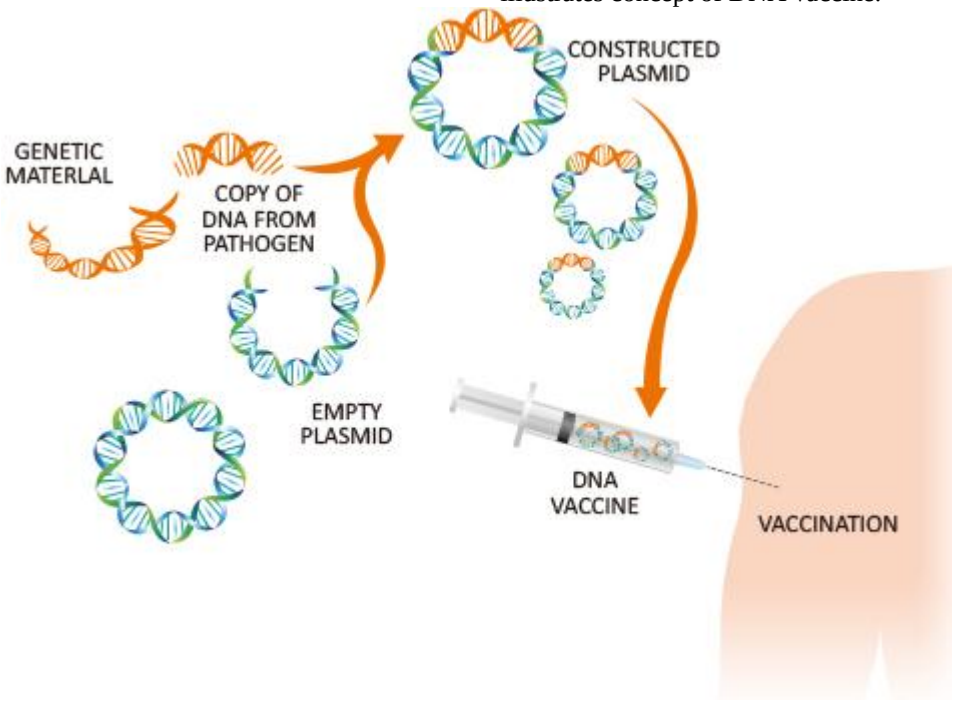
### 3.3. Third Generation Vaccines

DNA vaccines are third generation vaccines, and are made up of a small, circular piece of bacterial DNA (called a plasmid).The vaccine DNA is injected into the cells of the body, whereThe "inner machinery" of the host cells "reads" the DNA andConverts it into pathogenic proteins.

Because these proteins are recognized as foreign, when they are processed by the host cells anddisplayed on their surface, implies; the immune system is alerted, which then triggers a range of immune responses

## 4. DNA Vaccine

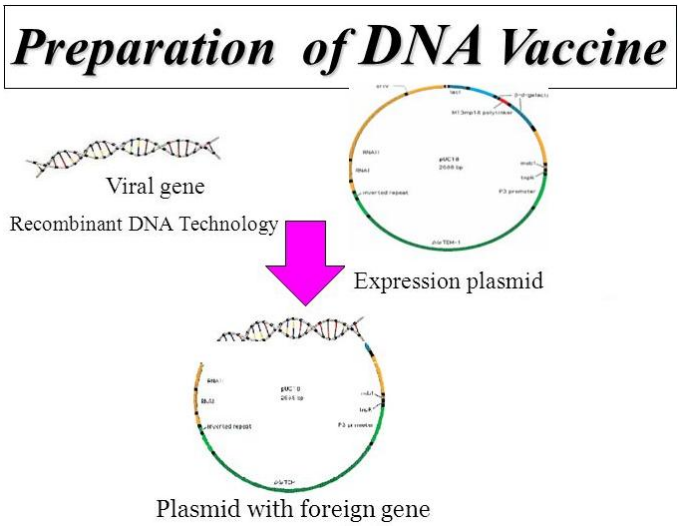
These vaccines usually consist of synthetic DNA containing the gene that encodes the disease-agent protein. Usually, the plasmid DNA used as vaccine is propagated in bacteria such as E. coli and they are isolated and purified for injection. This "naked" DNA is usually injected intramuscularly or intradermally. The principle behind a DNA vaccine is that the antigen can be expressed directly by host cells in a way that simulates viral infection and invokes an immune response from the host. This is similar to GenScript's DNA Immunization Technology which is a powerful tool that aids in custom antibody production against membrane proteins, other problematic antigens, as well as for early DNA vaccine development studies. DNA immunization technique allows antigen production to occur in vivo, bypassing the need to produce and purify protein antigen in vitro. Schematic below illustrates concept of DNA vaccine.




## 5. How DNA Vaccines are made?

Plasmid vectors for use in vaccination:

DNA vaccines are composed of bacterial plasmids. Expression plasmids used in DNA-based vaccination normally contain two units:The antigen expression unit composed of promoter/enhancer sequences, followed by antigen-encoding and polyadenylation sequences and the production unit composed of bacterial sequences necessary for plasmid amplification and selection. The construction of bacterial plasmids with vaccine inserts is accomplished using recombinant DNA technology. Once constructed, the vaccine plasmid is transformed intoϖ bacteria, where bacterial growth produces multiple plasmid copies.Theplasmid DNA is then purified from the bacteria, by separating the circular plasmid from the much larger bacterial DNA and other bacterial impurities.This purified DNA acts as the vaccine.




### 5.1 Vector Design

DNA vaccines elicit the best immune response when highly active expression vectors are used. These are plasmids which usually consist of a strong viralϖ promoter to drive

the in vivo transcription and translation of the gene (or complementary DNA) of interestBecause the plasmid is the "vehicle" from which the immunogen is expressed, optimizing vector design for maximal protein expression is essential. Ways of enhancing protein expression is by

1) Optimizing the codon usage of pathogenic mRNAs for eukaryotic cells.
2) Altering the gene sequence of the immunogen to reflect the codons.

**5.2 Steps for DNA Vaccine Preparation:**

**Step 1:** The vectors and copied genes have been treated with restriction enzymes, which are agents that cut DNA sequences at known locations.

**Step 2:** The enzymes have cut open the round vectors and trimmed the ends of the copied genes.

**Step 3:** Add bacteria to the vectors to allow the altered vectors to replicate.

**Step 4:** The ends of the vectors have again come together, but now with a gene spliced into the loop. Many copies of the vector/gene loop for genetic vaccine are needed.

**Step 5:** These copies can be produced with the help of bacteria. Vectors are capable of self-replicating when within a bacterial host, as long as that host is in an environment conducive to growing.

**Step 6:** Combine the vectors and bacteria, the vectors will be shocked into the bacteria

**Step 7:** Use the purifier to separate the altered vectors from the bacteria.

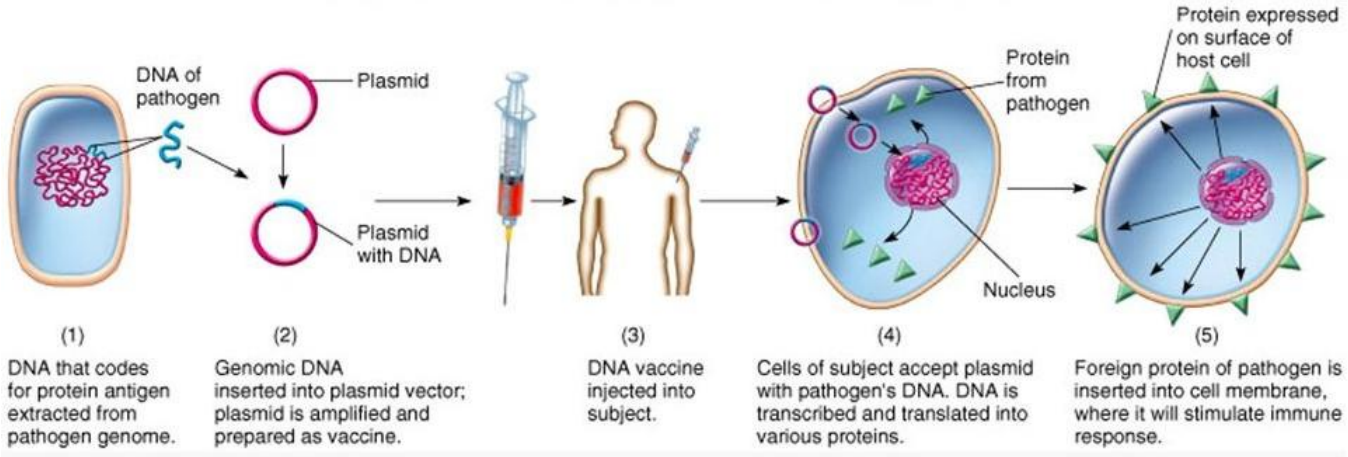
**Step 8:** The final vaccine should include only the vectors, and then separate those from the bacteria after enough copies have been produced.

**Step 9:** This can be done with a detergent, which ruptures the cell walls of the bacteria and frees the DNA within.

**Step 10:** The relatively large bacterial DNA can then be separated from the smaller DNA loop that makes up the vector.

**Step 11:** Fill the syringe with the altered vectors Upon inoculation, billions of copies of the altered vector will enter the body. Of these, only 1 percent will work their way into the nuclei of body cells. But that's enough.

The body's immune system responds to these proteins once they leave the cell. But more importantly, it also reacts to proteins that are incorporated into the cells' walls.




## 6. Delivery Methods

DNA vaccines have been introduced into animal tissues by a number of different methods. The two most popular approaches are:

**Injection in saline**

It is normally conducted intramuscularly (IM) in skeletal muscle or intradermally (ID), with DNA being delivered to the extracellular spaces. This can be assisted by electroporation; by temporarily damaging muscle fibers with myotoxins such as bupivacaine; or using hypertonic solutions of saline or sucrose. Immune responses to this method of delivery canϖ be affected by many factors including; needle type, needle alignment, speed of injection, Volume of injection, muscle type, and age, Sex and physiological condition of the animal being injected.

**Gene gun delivery**

The other commonly used method of delivery, ballistically accelerates plasmid DNA (pDNA) that has been adsorbed onto gold or tungsten micro particles into the target cells,using compressed helium as an accelerant. The method of delivery determines the dose of DNA required to raise an effective immune response.

**Mechanisms of Action**

A plasmid vector that expresses the protein of interest (e.g. viral protein) under the control of an appropriate promoter is injected into the skin or muscle of the the host. After uptake of the plasmid,protein is produced endogenously (Antigenic Protein is presented by cell in which it is produced) andintracellular processed into small antigenic peptides by the host proteases.

The peptides then enter in to lumen of the endoplasmic reticulum (E.R.) by transporters in the E.R., peptides bind to

MHC class I molecules. Subsequent CD8+ cytotoxic T cells (CTL) are stimulated andevoke cell-mediated immunity.

CTLs inhibit viruses through both cytolysis of infected cells and non cytolysis mechanisms such as cytokine production.

The foreign protein can also be presented by MHC class II pathway by APCs

These CD4+ cells are able to recognize the peptides formed from exogenous proteinsdegraded to peptide fragments and loaded onto MHC class II molecules.

Depending on the type of CD4+ cell that binds to the complex, B cells are stimulated and antibody production is stimulated.

## 7. Advantages and Disadvantages OF DNA Vaccines

| Advantages | Disadvantages |
|---|---|
| 1. Subunit vaccination with no risk for infection | 1. Limited to protein immunogens (not useful for non-protein based antigens such as bacterial polysaccharides) |
| 2.Antigen presentation by both MHC class I and class II molecules | 2. Risk of affecting genes controlling cell growth |
| 3. Able to polarise T-cell help toward type 1 or type 2 | 3. Possibility of inducing antibody production against DNA |
| 4. Immune response focused only on antigen of interest | 4. Possibility of tolerance to the antigen (protein) produced |
| 5. Ease of development and production | 5. Potential for atypical processing of bacterial and parasite proteins (Ulmer et al. 2008) |
| 6. Stability of vaccine for storage and shipping | |
| 7. Cost-effectiveness | |

## 8. Future of DNA Vaccines

It has recently been discovered that the transfect ion of myocytes can be amplified by pre-treatment with local anesthetics or with cardiotoxin, which induce local tissue damage and initiate myoblast regeneration.

Gaining a full understanding of this mechanism of DNA uptake could prove helpful in improving applications for gene therapy and gene vaccination.

Both improved expression and better engineering of the DNA plasmid may enhance antibody response to the gene products and expand the applications of the gene vaccines.

## References

[1] Rajcani J, Mosko T, Rezuchova I. Current developments in viral DNA vaccines: shall they solve the unsolved? Rev Med Virol.
[2] Abdulhaqq SA, Weiner DB. DNA vaccines: developing new strategies to enhance immune responses.
[3] Liu MA. DNA vaccines: an historical perspective and view to the future. Immunological reviews. 2011
[4] Kutzler MA, Weiner DB. DNA vaccines: ready for prime time? Nat Rev Genet. 2008
[5] Wolff JA, Malone RW, Williams P, et al. Direct gene transfer into mouse muscle in vivo. Science. 1990
[6] Tang DC, DeVit M, Johnston SA. Genetic immunization is a simple method for eliciting an immune response. Nature. 1992
[7] Ulmer JB, Donnelly JJ, Parker SE, et al. Heterologous protection against influenza by injection of DNA encoding a viral protein. Early pioneer study of DNA vaccine showed original ideas and immunogenicity of DNA vaccines in animal models.
[8] Robinson HL, Hunt LA, Webster RG. Protection against a lethal influenza virus challenge by immunization with a haemagglutinin-expressing plasmid DNA. Vaccine. 1993
[9] Faurez F, Dory D, Le Moigne V, Gravier R, Jestin A. Biosafety of DNA vaccines: New generation of DNA vectors and current knowledge on the fate of plasmids after injection. Vaccine. 2010
[10] Hemmi H, Takeuchi O, Kawai T, et al. A Toll-like receptor recognizes bacterial DNA. Nature. 2000