DNA Vaccines

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History of Vaccine Development

The year 1996 marked the 200th anniversary of the first vaccine developed against smallpox by Edward Jenner. In the now-famous 1796 experiment, Jenner scratched the arm of eight-year-old James Phipps, infecting the boy with cowpox pus taken from a milkmaid carrying the virus. Two months later, he scratched James again, this time with small pox virus. The rest is history: James Phipps did not come down with small pox. Since then, the pioneering work of Louis Pasteur, Albert Sabin and Jonas Salk has led to the development of vaccines against diseases such as rabies, polio, etc. The last century witnessed rapid advances in vaccine development and several new or improved vaccines have been introduced. It is estimated that of the 30 years added to average human life span in the 20th century, 10-15 years have resulted from vaccination. Vaccination essentially results in the induction of an immune response capable of protecting the host against the disease when it encounters a virulent form of the specific pathogen. Put simply, a vaccine is a non-disease causing mimic of an infectious agent. Successful vaccination protects both individuals and populations. Individuals are protected against the development of disease; populations are protected against the spread of the disease-causing agent.

When Edward Jenner and Louis Pasteur demonstrated protection of individuals against smallpox and rabies respectively, they had very little knowledge of the pathogenic proteins responsible for the induction of protective immunity. However, their experiments clearly demonstrated that live, attenuated or killed, inactivated forms of pathogens can be used as vaccines. This empirical approach to vaccine development led to the development of first generation vaccines which essentially con-

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Box 1. Glossary of Terms

Antigen: A molecule which reacts with preformed antibody and the specific receptors on T and B cells.

Antibody: A molecule produced by animals in response to an antigen. It has the particular property of combining specifically with the antigen which induced its formation.

Antigen Presentation: The process by which certain cells in the body (antigen-presenting cells) express antigen on their cell surface in a form recognizable by lymphocytes.

Antigen Processing: The conversion of an antigen into a form in which it can be recognized by lymphocytes.

Cell-mediated Immunity: Immune reactions that are mediated by cells rather than by antibodies or other humoral factors.

Cytotoxic T-cells: Cells which can lyse virally infected targets expressing antigenic peptides presented by MHC class I molecules.

Major Histocompatibility Complex (MHC): A genetic region found in all mammals whose products are primarily responsible for the rapid rejection of grafts between individuals, and function in signalling between lymphocytes and cells expressing antigen.

T-cells: Lymphocytes that differentiate primarily in the thymus and are central to the control and development of immune responses. The principal subgroups are cytotoxic-T cells and T-helper cells.

Plasmids: Autonomously replicating, extrachromosomal circular DNA molecules, distinct from the normal bacterial genome and nonessential for cell survival under nonselective (normal) conditions.

sisted of attenuated, live or killed pathogens as vaccines. This mode of vaccination was primarily responsible for eradicating diseases such as polio, small pox and measles at least in the developed nations. Large-scale vaccine production involved inoculation of a large number of animals such as sheep, goats or mice with the pathogen. Rapid progress in animal cell culture technology led to development of cell culture-based vaccines, wherein the pathogenic organisms were grown in cells in culture rather than in animals. As we gained more knowledge on the pathogenic proteins, as well as structure and function of various components of the immune system, the empirical approach to vaccine development paved way for a rational approach. It became clear that inoculation of one or more proteins of the pathogen rather than the entire pathogen is sufficient to evoke a

protective immune response. This led to the development of subunit vaccines wherein specific proteins of a pathogen, instead of the entire pathogen, were used for vaccination. Further advances in immunology led to the discovery that short regions of 8-12 amino acids in a pathogenic protein are sufficient to evoke an immune response and, thus, the use of antigenic peptides as vaccines rather than entire proteins was advocated. With the advent of recombinant DNA technology, antigenic proteins of various pathogens could be made economically in large amounts in microorganisms such as bacteria or yeast, and the use of such recombinant proteins for immunization marked the advent of second generation vaccines. For example, the recombinant Hepatitis B vaccine that is currently being used for prevention of Hepatitis B is nothing but the viral envelope protein produced and purified from yeast cells. However, the modern cell culture-based vaccines as well as recombinant protein vaccines require expensive virus/protein purification techniques as well as cold storage facilities, rendering them less popular in developing countries. As a result, many vaccine-preventable diseases which are eradicated in the western hemisphere continue to remain major health hazards in developing nations. Thus, there is an urgent need to develop safe, inexpensive and room temperature-storable vaccines for the eradication of infectious diseases in the developing countries. It is in this context that the use of plasmid DNA as a vaccine assumes great significance since it can be produced at a very low cost and can be stored at room temperature.

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History of DNA Vaccination

Many vaccines began as serendipitous discoveries. Louis Pasteur discovered attenuated vaccines when old cholera cultures lost their virulence. When chickens were inoculated with aged cultures, they unexpectedly developed immunity to cholera. Similarly, DNA vaccination was discovered by chance when a group of researchers in the United States observed that mouse skeletal muscle can take up naked DNA and express proteins encoded by the DNA. Naked DNA was actually used as a control

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in their experiments whose objective was to identify lipids that enhance DNA delivery into skeletal muscle. When DNA encoding an influenza virus protein was injected into the skeletal muscle of mice, synthesis of the virus protein in the mouse muscle triggered an immune response resulting in protection of the mice from a subsequent influenza infection. These results, published in *Science* in the year 1993, marked the beginning of DNA vaccines also known as nucleic acid vaccines or genetic vaccines. Since 1993, the principle of DNA vaccination has been demonstrated for a variety of bacterial, viral and parasitic diseases (*Table* 1). Several clinical trials have been initiated in the last few years and the major challenge for this field in this century is to demonstrate the clinical utility of DNA vaccines.

What are DNA Vaccines?

By definition, DNA vaccine is a circular double stranded DNA molecule, referred to as a plasmid, containing genes encoding one or more proteins of a pathogen. Plasmids are extra-chromosomal circular DNA molecules that are often present in multiple copies in bacterial cells. The plasmid contains a bacterial origin of replication by virtue of which it can replicate autonomously inside bacterial cells but not in eukaryotic cells. Such plasmids can be isolated from bacteria using simple and inexpensive protocols. Using recombinant DNA techniques, it is possible to insert a foreign gene into the plasmid molecule to generate a recombinant plasmid. When the gene is inserted downstream of

Table 1. Diseases for which DNA vaccines have been shown to induce protective immune responses in animal models.

Viruses: Avian influenza, bovine herpes, bovine viral diarrhoea virus, dengue fever, encephalitis, feline immunodeficiency virus, hepatitis B, hepatitis C, herpes, human cytomegalovirus, herpes simplex virus, human immunodeficiency virus, influenza, measles, papilloma, pseudorabies, rabies, respiratory syncitial virus, rotavirus, simian immunodeficiency virus, simian virus, ebola.
Bacteria: *Borrelia burgdorferi* (Lyme disease), cholera, Enterotoxic *E. coli*, *Moraxella bovis*, *Mycobacterium tuberculosis*, Mycoplasma, Ricketsia, *Salmonella*, tetanus toxin.
Parasites: *Cryptosporidium parvum*, *Leishmania*, *Plasmodium falciparum* (malaria), *Schistosoma*.
Cancer-associated antigens: Carcinoembryonic antigen (CEA), melanoma-associated antigen, MHC molecule HLA-B7.

DNA sequences referred to as promoter and enhancer elements to which eukaryotic RNA polymerase II and a host of proteins known as transcription factors bind, then the resultant plasmid is referred to as an eukaryotic expression plasmid (*Figure*1). When such plasmids are introduced into eukaryotic cells, the gene of interest is transcribed by the RNA polymerase II and other accessory proteins resulting in the synthesis of messenger RNA (*m*RNA) that is translated into the corresponding protein in the cytoplasm of the host cells. Thus, by inserting the gene of your choice into the plasmid, it is possible to synthesize the protein of your choice inside eukaryotic cells.

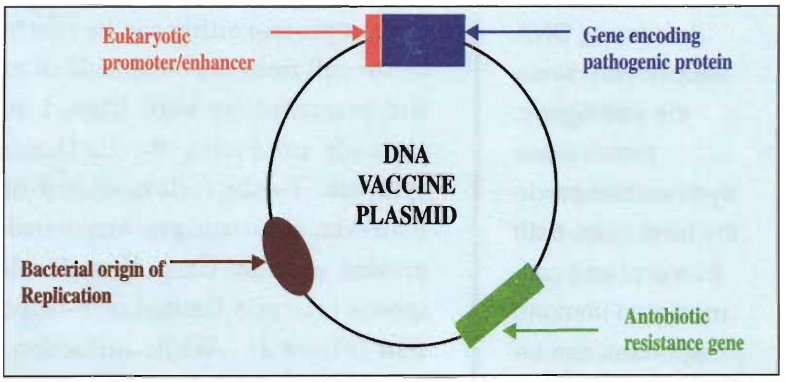

Figure 1. Key features of a DNA vaccine plasmid.

For example, for the treatment of genetic disorders such as hemophilia by gene therapy, one can insert the genes encoding proteins such as Factor VIII or Factor IX into plasmids. Such plasmids, when introduced into eukaryotic cells, start producing Factor VIII or Factor IX leading to the possibility of correction of hemophilia. In case of DNA vaccines, gene(s) encoding antigenic proteins of a pathogen are inserted into the plasmid so that synthesis of these proteins inside the host cells followed by antigen presentation results in the induction of an immune response. Antigen presentation essentially involves proteolytic degradation of the foreign proteins inside the eukaryotic cells and association of the proteolytic fragments with two different types of *major histocompatibility complex* (MHC) proteins referred to as Class I and Class II MHC proteins. Both of these present proteolytically degraded fragments of proteins to T-cells. Class I molecules present fragments of proteins to cytotoxic T-cells. Class II molecules present protein fragments to T-helper cells. In most instances, Class I molecules present foreign proteins synthesized in a cell. For presentation by Class II molecules, the

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foreign protein either can be synthesized in the cell or taken up by the cell from the outside. If an antigen is synthesized in a cell and presented by both Class I and Class II molecules, both antibody producing B-cells (humoral immune response) and cytotoxic T-cells (cell-mediated immune response) are raised. However, if an antigen originated outside of a cell and is expressed only by Class II molecules, the specific immune response is largely limited to T-helper cells and antibody production (*Figure* 2). While induction of strong humoral immune responses is required for protection against bacterial infections, both humoral and cell-mediated immune responses are required for protection against viral infections. In case of DNA vaccination, since the pathogenic proteins are synthesized inside the host cells, both humoral and cell-mediated immune responses can be induced. Thus, DNA vaccination is ideally suited for fighting not only infections caused by extracellular pathogens such as bacteria but also those caused by intracellular pathogens such as viruses and parasites.

Advantages and Limitations of DNA Vaccines

In addition to their ability to induce both humoral and cell-mediated immune responses, DNA vaccines have several other advantages over traditional vaccines. Many microbial proteins have folded structures that are altered during purification. If the shape of the recombinant protein is different from that of the native protein of the pathogen, antibodies induced by the recombinant protein will not recognize the native protein of the pathogen leading to vaccine failure. In case of DNA vaccination, since the pathogenic protein is synthesized in its native form inside the host cell, the chances of vaccine failure are minimal. In addition, DNA vaccines are non-infectious, economical to produce in large amounts, and easy to purify using simple and inexpensive procedures. Moreover, all DNA vaccines can be produced using similar fermentation, purification, and validation techniques. This ability to use generic production and verification techniques simplifies vaccine development and production. Another major advantage of DNA vaccines is

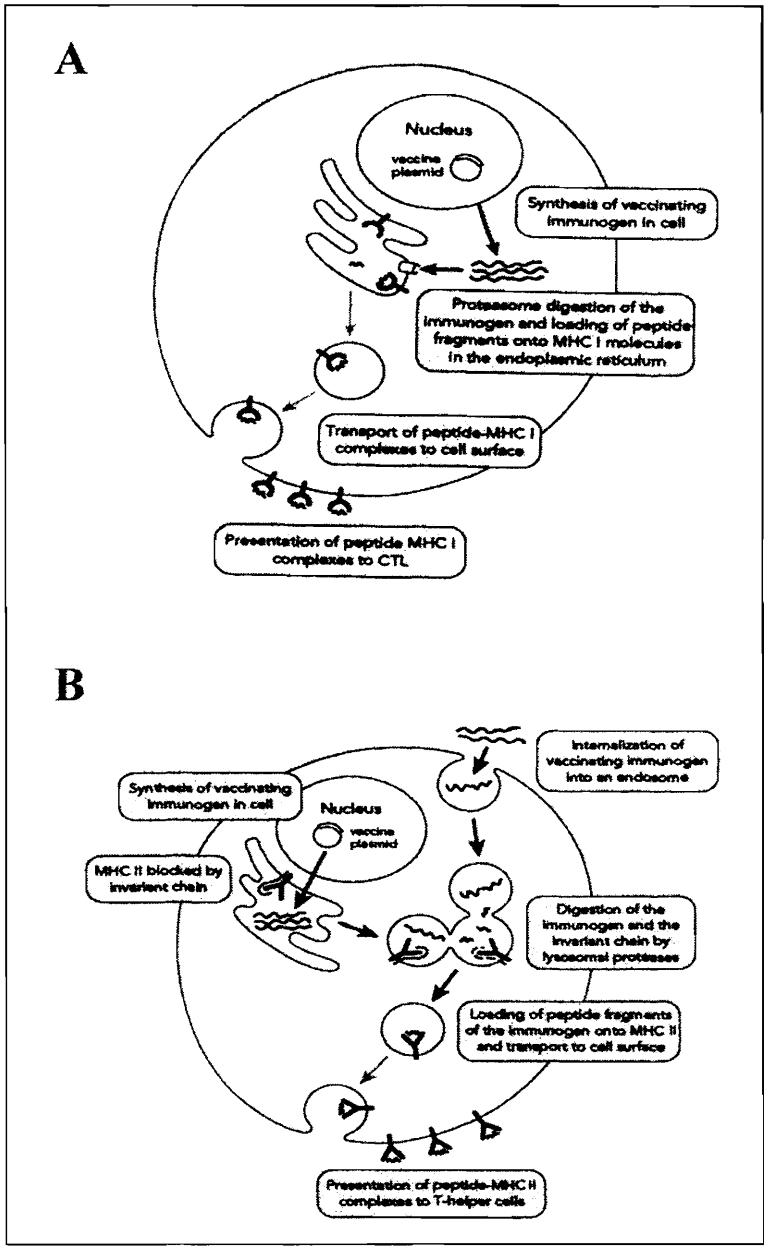

Figure 2. Synthesis of an immunogen inside an eukaryotic cell transfected by DNA vaccine plasmid and presentation of the immunogen in association with either Class I MHC (A) or Class II MHC (B) molecules.

that they do not require a cold-chain. Cold-chain refers to the series of refrigerators required to maintain the viability of a vaccine during its distribution. Currently, maintaining the cold-chain represents nearly 80% of the cost of vaccinating individuals in developing nations. Since DNA vaccines can be

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stored either dry or in an aqueous solution at room temperature, there is no need for the cold chain. DNA vaccine plasmids can be constructed using simple recombinant DNA techniques and therefore it is possible to coinoculate multiple plasmids encoding different antigens of the same pathogen or different pathogens. Such multivalent approach is especially important for diseases such as malaria, AIDS and tuberculosis, wherein a single antigen alone may not offer complete protection.

While DNA vaccination offers several advantages over conventional vaccines, there are certain drawbacks as well. This approach can be used to induce immune responses only against the protein components of the pathogen and cannot substitute for polysaccharide-based subunit vaccines for diseases caused by pathogens such as *Pneumococcus*. Although induction of protective immune responses following DNA vaccination has been demonstrated for a variety of diseases, the mechanism of DNA vaccine action is still not completely understood. For example, does DNA vaccination obey classical rules of inducing immune responses or are DNA-transfected muscle cells or skin cells, and not lymphoid cells, presenting the antigen? If lymphoid cells present the antigen, how do they obtain the antigen? Does DNA directly transfect these cells, or do they acquire protein from transfected skin or muscle cells? Since expression of the plasmid-encoded antigen appears to persist for long periods of time, there is a concern that unresponsiveness, rather than protective immunity, might result.

A major concern about DNA vaccines is whether the plasmid DNA integrates into the genome randomly, potentially leading to insertional mutagenesis. Efforts to find integrations of DNA vaccine plasmids into mouse genomic DNA have failed to detect insertions of the injected plasmid. These studies could have detected one integration event for each 150,000 nuclei, a mutation rate estimated at 1,000 times less than the spontaneous mutation rate of DNA. A careful study on the potential of genomic integration of plasmid DNA after intramuscular injection revealed that about 3-30 copies of plasmid DNA remain

associated with genomic DNA, although it was not possible to ascertain whether this was a covalent association, or the plasmid was simply associated with genomic DNA adventitiously. However, under the assumption that 30 copies of the plasmid DNA were covalently integrated with genomic DNA, and that each such integration would result in a mutational event, the calculated rate of mutation was 3000 times less than the spontaneous mutation rate for mammalian genomes. This level of integration, if it occurs, is not considered to pose a significant safety concern.

Induction of anti-DNA antibodies by DNA vaccination appears a remote possibility.

Another major concern is the induction of anti-DNA antibodies by plasmid DNA. Antibodies to DNA can cause disease and are associated with systemic lupus erythematosus. Studies on the induction of anti-DNA antibodies by DNA vaccination have led to the following observations. Purified double stranded DNA does not readily induce anti-DNA antibodies. Nonpathogenic anti-DNA antibodies generated during bacterial infections are found in most humans and these are specific for the DNA of that particular bacterial species and do not cross-react with mammalian DNA. Moreover, vaccination of lupus-prone mice with purified plasmid DNA has no effect on the levels of anti-DNA antibodies. Finally, vaccination of normal animals with DNA vaccines has not been seen to induce anti-DNA antibodies. Thus, induction of anti-DNA antibodies by DNA vaccination appears a remote possibility.

Future Prospects

As is true for any drug or vaccine, the critical issue is the risk-benefit ratio of the treatment for a given disease. While safety ranks as the most important component for prophylactic DNA vaccines, the particular disease and population under consideration would affect the estimation of the risk-benefit ratio. To this end, it is important to carefully evaluate the safety of DNA vaccines in pre-clinical and then clinical studies, while at the same time considering the benefit of a protective vaccination. Unlike other recombinant protein pharmaceuticals, in the case

Suggested Reading

[1] D B Weiner and R C Kennedy, Genetic Vaccines, *Scientific American*, July 1999.

[2] S Gurunathan, D M Klinman and R A Seder, DNA vaccines: Immunology, Application and Optimization, *Ann. Rev. Immunol.*, Vol. 18, pp. 927-974, 2000.

of DNA vaccines, the plasmid DNA itself rather than the protein produced by it is the final product. Since large-scale fermentation conditions and processes already exist for various recombinant DNA products, efficient and cost-effective production of DNA vaccines may not be a major problem. The technologies for removal of impurities such as endotoxins, genomic DNA and proteins from the plasmid DNA preparations are well worked out and pharmaceutical grade plasmid DNA for clinical trials is already being produced. Since the technology is novel and its clinical utility is not fully proven, the academic community, pharmaceutical companies and the regulatory agencies have to work together to bring out the world's first DNA vaccine into the market. For some applications the potential benefits clearly outweigh the risks, and expeditious approval of DNA vaccine clinical trials will provide important safety information for other applications where the potential risks initially appear of greater importance. The World Health Organization and the US Food and Drug Administration have already released documents that prescribe guidelines for producing and testing DNA vaccines in humans. While the potential benefits of DNA vaccines are enormous, the media should inform the public about DNA vaccine research with cautious optimism. Unrealistic expectations and hype could lead to a backlash, if hopes fail to become reality in a timely fashion. While DNA vaccine research has taken off in a big way, some researchers are already toying with the idea of using the genome sequence data of several pathogenic microbes to develop genomic vaccines. This approach, also known as expression library immunization, envisages immunization of animals with groups of plasmids encoding 100-1000 genes of a pathogen. The group that confers protection against the pathogen is split into a smaller groups of 10-100 genes and retested. Using such a reductionist approach, it is possible to identify single gene in each group responsible for protection. Let us hope that the extensive studies on animal models, and the various human clinical trials of DNA vaccines initiated in the 20th century, will be translated into a clinical reality in the 21st century.

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