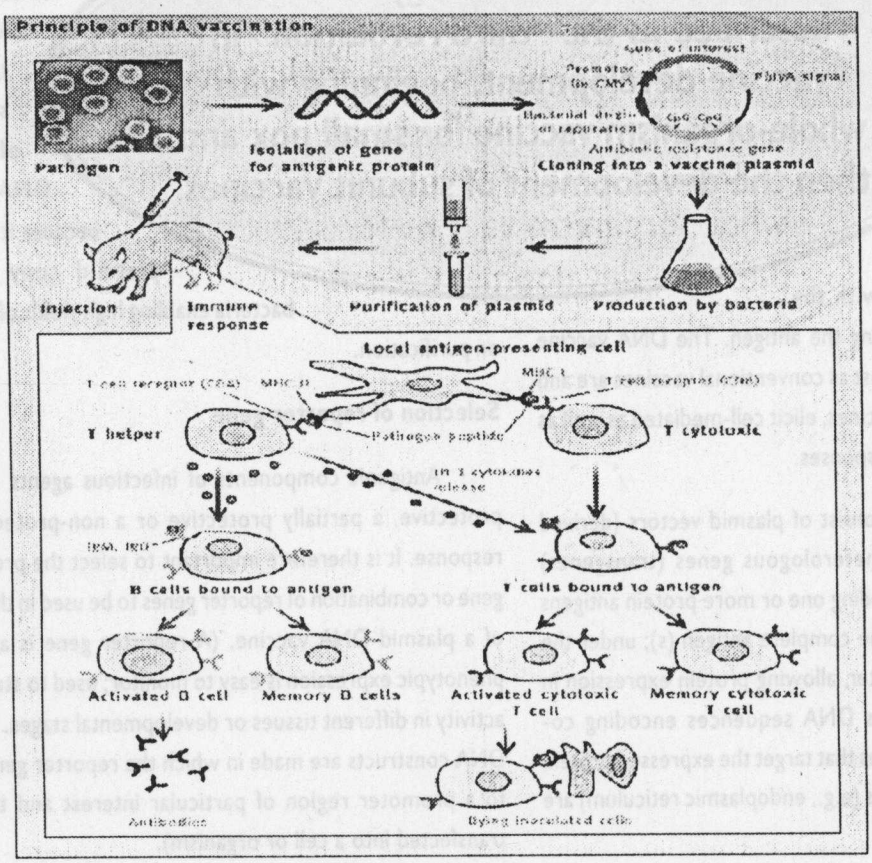
DNA Vaccines - a revolution in vaccine development

DR. SARITA G. BHAT

Over 200 years ago, in 1796 Edward Jenner showed that inoculating people with material from cowpox skin lesions (L. *vaccinus*, of cows) protected them from the highly contagious and fatal disease smallpox. The terms vaccination and vaccine is credited to him and since then the term has been retained for any preparation of dead or weakened pathogens, or their products; that when introduced into the body, stimulates the production of protective antibodies or T cells without causing the disease. Vaccination is also called active immunization because the immune system is stimulated to develop its own immunity against the pathogen. Passive immunity in contrast, results from the injection of antibodies formed by another animal (e.g., horse, human) which provide immediate, but temporary protection for the recipient.



Conventional active vaccines are made of killed or attenuated forms of the infectious agent, a modified product of the infectious agent (toxoid) or a constituent such as the capsule. Relatively high and repeated doses are administered when a non-viable (killed organism, toxoid, capsule) vaccine is used and the protective immunity obtained is not long lasting. Moreover, usually a humoral but not a cell-mediated immune response is generated. On the other hand, when low doses of a viable attenuated vaccine are used, both humoral and cell-mediated immune responses are generated and immunity is usually long lasting. But successful vaccines for many infectious agents, in particular intracellular parasites and tumours are yet to be developed.

But in 1990, Wolfe and colleagues demonstrated the direct gene transfer of plasmid DNA into mouse muscle *in vivo* without the need for a special delivery system and paved the way for the development of DNA vaccines. DNA vaccination is a relatively recent development in vaccine technology and is possibly the most hopeful and powerful alternative to traditional vaccines. This technology has been described as the "third revolution" in vaccine development, beginning with whole-organism vaccine for small pox and then the development of subunit vaccines. Here, DNA coding for the foreign antigen is directly injected into the animal so that the foreign antigen is directly expressed by the host cells and so in theory, these vaccines would be extremely safe and devoid of side effects. In addition, DNA vaccines can theoretically result in more long-term production of an antigenic protein when introduced into a relatively non-dividing tissue, such as muscle. As DNA is relatively inexpensive and easier to produce than conventional vaccines, this technology will definitely improve the availability of vaccines and the success rates of vaccination programmes in developing nations.

Considering that the emergence and outbreaks of new, highly infectious diseases like the SARS are very high and also the constant threats of bio-terrorism, the relatively short period required for the DNA vaccine development will help in the timely immunization, control and containment of disease outbreaks.

The first clinical trials using injections of DNA began for HIV in 1995. Four other clinical trials using DNA vaccines were also carried out in 1996 against influenza, herpes simplex virus, T-cell lymphoma. The technique that is being tested in humans involves the direct injection of plasmids - loops of DNA that contain genes for proteins produced by the organism being targeted for immunity. DNA vaccines for a number of infectious agents have been prepared and reported to induce both humoral and cell mediated immune responses in animal models. Moreover, DNA vaccines for some tumours and some allergens have also been prepared.

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Structure of the DNA vaccine

With DNA vaccines, the subject is not injected with the antigen but with DNA encoding the antigen. The DNA vaccine can be injected into a muscle just as conventional vaccines are and in contrast to conventional vaccines, elicit cell-mediated as well as antibody-mediated immune responses.

DNA vaccines usually consist of plasmid vectors (derived from bacteria) that contain heterologous genes (transgenes) inserted, DNA sequences encoding one or more protein antigens or, often, simply epitopes of the complete antigen (s); under the control of a eukaryotic promoter, allowing protein expression in mammalian cells. Sometimes DNA sequences encoding co-stimulatory molecules-sequences that target the expressed protein to specific intracellular locations (e.g., endoplasmic reticulum) are also present.

An important consideration when optimising the efficacy of DNA vaccines is the appropriate choice of plasmid vector. The basic requirements for the backbone of a plasmid DNA vector are

- A eukaryotic promoter,
- A cloning site,
- A polyadenylation sequence,
- A selectable marker, and a bacterial origin of replication.

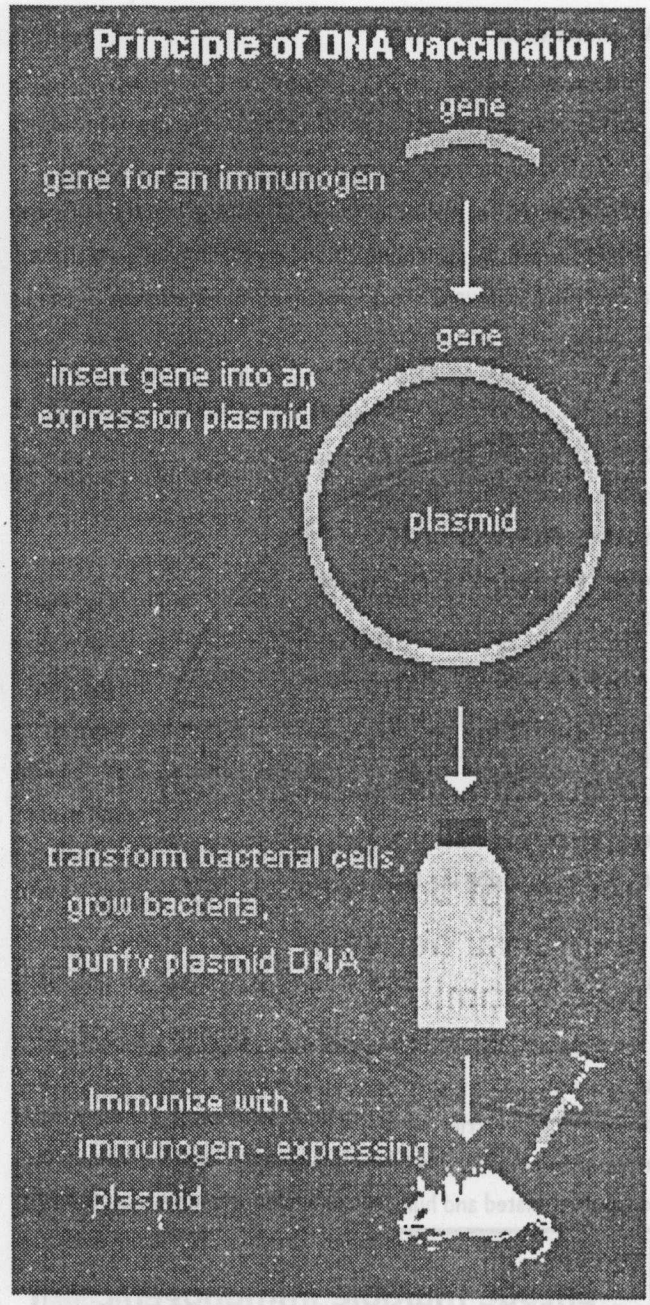
A strong promoter may be required for optimal expression in mammalian cells. For this, some promoters derived from viruses such as human cytomegalovirus (CMV) or simian virus 40 (SV40) have been used. A cloning site downstream of the promoter should be provided for insertion of heterologous genes, and inclusion of a polyadenylation (polyA) sequence such as the Bovine Growth Hormone (BGH) or SV40 polyadenylation sequence provides stabilisation of mRNA transcripts. The most commonly used selectable markers are bacterial antibiotic resistance genes, such as the ampicillin resistance gene. However, since the ampicillin resistance gene is precluded for use in humans, a kanamycin resistance gene is often used. Finally, the *Escherichia coli* ColE1 origin of replication, which is found in plasmids such as those in the pUC series, is most often used in DNA vaccines because it provides high plasmid copy numbers in bacteria enabling high yields of plasmid DNA on purification.

Selection of reporter gene

Antigenic components of infectious agents may induce a protective, a partially protective or a non-protective immune response. It is therefore important to select the proper reporter gene or combination of reporter genes to be used in the preparation of a plasmid DNA vaccine. (A reporter gene is a gene whose phenotypic expression is easy to monitor; used to study promoter activity in different tissues or developmental stages. Recombinant DNA constructs are made in which the reporter gene is attached to a promoter region of particular interest and the construct transfected into a cell or organism).

Immune Response

The antigenic portion of the pathogen or tumour cell in the DNA vaccine will be expressed *in situ*. The plasmid is taken up by an antigen-presenting cell (APC) like a dendritic cell. The gene(s) encoding the various components are transcribed and translated.



The protein products are degraded into peptides. These are exposed at the cell surface nestled in class I histocompatibility molecules where they serve as a powerful stimulant for the development of cell-mediated immunity.

In the antibody-mediated response, if the plasmid is taken up by other cells (e.g. muscle cells), the proteins synthesized are released and can be engulfed by antigen-presenting cells (including B cells). In this case, the proteins are degraded in the class II pathway and presented to helper T cells. These secrete lymphokines that aid B cells to produce antibodies.

Administration

Several routes of DNA vaccine inoculation have been undertaken in animal models. These include intra-muscular, subcutaneous, intra-peritoneal, intra-dermal, subcutaneous, intravenous, oral, rectal, intra-bursal, intra-orbital, intra-tracheal, intra-nasal, and vaginal routes. In the case of a plasmid DNA vaccine for a tumour, it can be injected directly into the tumour site. The most common routes of administration are by injecting the plasmid DNA dissolved in saline intra-muscularly or intra-dermally using a hypodermic needle or by bombarding plasmid DNA coated onto colloidal gold micro-particles in the dermis or muscle using a gene gun. The gene gun accelerates the particles into the target tissue by a controlled discharge through a shock wave created by a chemical propellant, expansion of a compressed gas or an electric spark.

Results of studies have indicated that long lasting immunity is attained when a DNA vaccine is used. The observed prolonged duration of the immune response was probably due to the persistence of the antigen produced in the host.. An immunization regimen that may result in an optimal immune response is to prime the host using the DNA vaccine and subsequently boosting with the antigen.

Advantages

Plasmid DNA is non-infectious, does not replicate and encodes only the antigen of interest, as opposed to live attenuated vaccines or viral carrier systems. It does not contain heterologous protein components to which the host may respond. It induces both cell mediated and humoral immunity, which are long lasting.

Encode multiple immunogenic epitopes

Evoke both humoral and cell mediated immune responses

Large-scale manufacturing procedures available

Longer shelf life (Thermostable)

Allows a more simplified and effective quality control process

DNA vaccines induce *in vivo* expression of immunogens thus conserving the native conformation of epitopes. They may be constructed to include more than one immunogen gene, thus potentially decreasing the number of vaccinations required in children. They offer the possibility of generating effective immune responses against diseases such as malaria and HIV where other types of vaccines have failed. Moreover, they may be safer to use than live attenuated vaccines. They are stable, do not get denatured by heat, easy to freeze dry and reconstitute, and can be manufactured inexpensively in large quantities at high levels of purity

Vaccines developed and under trial

So far, most of the work on DNA vaccines has been done in mice where they have proved able to protect them against tuberculosis, SARS, smallpox, and other intracellular pathogens. In addition, more than a dozen different DNA vaccines against HIV-1 are in clinical trials. Scientists at Rocky Mountain Laboratories in Hamilton, Mont., which is part of the National Institute of Allergy and Infectious Diseases, have now developed a DNA vaccine for rabies. The DNA encodes a glycoprotein found on the rabies virus. In a test on 12 cynomolgus monkeys, the DNA vaccine proved as effective as the commercial one. The attributes of DNA vaccines is also valuable in the fight against rotavirus. This virus causes diarrhoea that kills almost 900,000 children every year, most in developing countries. When tested in mice, the encapsulated vaccine survived a trip through the animal's stomach and unleashed its DNA in the intestines or bloodstream. While an injectable, traditional vaccine against the virus exists, the new rotavirus DNA vaccine developed, is both edible and durable, making it the first oral DNA vaccine, Animal experiments are being conducted. Clinical trials for Malaria (Naval Medical Institute), Influenza (Johns Hopkins University), HIV (University of Pennsylvania), Colon cancer (University of Alabama), Hepatitis B (University of Wisconsin), Herpes (University of Washington) are on. Plasmid vaccines haves been containing the polymorphic epithelial mucin (PEM) gene protected mice against PEM-expressing tumour cells and containing the gene that codes for the 65Kda heat shock protein of *M. tuberculosis*. The list is long. ....

DNA vaccines are now being tested in Phase I and Phase II clinical trials, yet further safety studies should be undertaken and potential dangers should not be neglected. The manner by which they are to be administered, the amount of plasmid DNA to be administered, the number of boosters to be given and time interval between boosters need to be optimized.

Potential Limitations

Enthusiasm for DNA vaccination in humans is tempered despite their multiple advantages, as DNA vaccines might not be devoid of some limitations and potential dangers. If plasmid DNA integrates into the host genome it may either activate oncogenes or suppress tumour suppressor genes, which may lead to a malignant transformation. Another potential downside is that extended immunostimulation by the foreign antigen could in theory provoke chronic inflammation or autoantibody production. It has the disadvantage of — on rare occasions — regaining full virulence and causing the disease. For this reason, the Salk vaccine has once again become the preferred vaccine in the U. S. However, there is no reason to be concerned if the vaccine is administered intradermally as the transfected epidermal cells are lost within 10-14 days because of the normal sloughing of keratinized skin tissue; or they may be administered intramuscularly in which case as the transfected muscle cells are non-dividing and random insertion is more likely to occur in replicating cells in which DNA is actively being synthesized.

Dr. Sarita G. Bhat is a Lecturer at the Department of Biotechnology, Cochin University of Science and Technology, Cochin.