DNA VACCINES

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General overview

Vaccines have proven to be the most successful medical intervention of human morbidity and mortality. Bacterial vaccines have resulted in the marked decrease in the incidence of human infections such as tetanus, diphteria and whooping cough. Viral vaccines have not only reduced the incidence of paedeatric diseases such as measles and poliomyletis but have resulted in the complete eradication of small pox.

Current vaccines that are available can be broadly categorised into two groups: live and dead. Live vaccines encompass attenuated microbes which are viral or bacterial that were selected for their reduced pathogenecity but with maintained immunogenicity, and recombinant vaccines which are foreign antigens expressed in a bacterial or viral vector.

Dead vaccines, on the other hand, include killed, whole pathogens, as well as soluble pathogen proteins and protein subunits.

The nature of a vaccine determines the type of immune response induced. Dead vaccines cannot efficiently enter the MHC I pathway, and may be less effective in inducing the cell-mediated immune response that is critical in protection against many diseases caused by intracellularly replicating organisms. Live vaccines on the other hand may be dangerous to immunocompromised hosts as they can revert to pathogenecity within the vaccinated person. They could also be contaminated by potentially harmful chemicals during production.

The ideal vaccine should be safe, cheap, heat stable, containing protective immunogenic sequences from multiple pathogens, and administered preferable as a single dose. To date, no such vaccine for human use meets all these requirements.

There is, however, a novel approach to the control of infectious agents in the form of DNA vaccines which could prove to be the answer to the ideal vaccine.

DNA vaccines, which are currently under development, utilize genes encoding the proteins of pathogens or tumour, rather than the pathogen or their subunits as in the more conventional approaches.

The principles of DNA vaccines is outlined in Figure 1 (1). Briefly, recombinant DNA technology is used to clone in the genes which encode one or more microbial antigens of interest (potential immunogen) into an eukaryotic expression vector. The constructed plasmid is transformed into a bacterial host (*E. coli*), grown up in large quantities and purified from bacterial contaminants. The purified plasmid construct is then directly inoculated into the host via intramuscular or intradermic injection. The DNA enters some cells where RNA transcription and protein translation of the genes encoding the bacterial antigens occurs. The expressed antigen is taken up by specialized cells of the immune system and transported to draining lymph nodes where an immune response to the disease is then elicited.

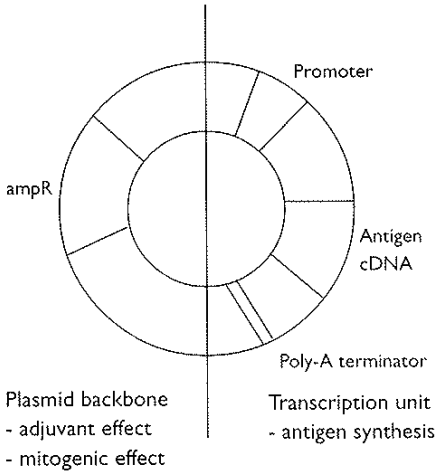

Figure 1. Principle of DNA Vaccines (adapted from Lambert and Siegrist (1997)(1)

Advantages of DNA vaccines

The advantages of DNA vaccines over conventional vaccines are numerous.

Firstly, they are easy to manufacture and much cheaper. They provide prolonged antigen expression that continuously stimulates the immune system (2). DNA vaccines also elicit qualitatively different immune responses which include induction of MHC-class I-restricted CTL and Th 1-biased immune responses (3). The technology with which they are constructed enables manipulation of the antigenicity of the protein at DNA level without the need for protein production and purification. Genes inserted into a plasmid can be modified readily, allowing the removal or insertion of carbohydrate side chains or other residues that could

affect the processing of the protein. The sequence could also be modified by site-directed mutagenesis resulting in single-amino acid changes that could enhance the antigenecity of the protein. Parts of the gene sequence could be deleted that encode for epitopes that trigger unwanted immune responses. When co-delivered with plasmid DNA-encoded cytokines or co-stimulatory molecules, a DNA vaccine offers the possibility for enhancement or modulation of the subsequent response to the DNA-encoded antigen (4). Resistance to heat would enable the use of DNA vaccines in countries where "cold chains" are difficult to maintain. DNA vaccines lack a replicating agent therefore are safer to be administered to pregnant women or immunocompromised patients. They have the capacity to induce, in murine models, adult-like antibody, Th1 and CTL responses in early life when the immune system is still immature (5).

DNA vectors

Expression of the protein of interest encoded in the vector of the DNA vaccine is influenced by several factors, one of the most important being the plasmid used. Basically, the plasmid used for DNA vaccination (see figure 2) comprises of 2 major units:

1. plasmid backbone that delivers adjuvant and mitogenic activity via immunostimulatory sequences, and
2. transcription unit comprising a promoter, antigen cDNA and polyadenylation (A) addition sequence, which together direct protein synthesis.

Most of the commercially available mammalian expression vectors carry a promoter from the human cytomegalovirus (HCMV) which has been shown to induce high level expression in many cell types (6), although alternative promoters are being studied namely plasmids containing control sequences from human papilloma viruses which result in longer term gene expression (7) Furthermore, the addition of introns and efficient transcription termination/processing units have been shown to increase gene expression in the mamalian host (8,9).

Route of administration

Protective immune responses can be generated by skin, muscle and intravenous innoculations of DNA (10). DNA immunization can been carried out by a number of methods which include direct injection of the naked DNA in saline (11), of DNA complexed with lipids (12), and by impelling DNA either by an aerosol or using a gene-gun to propel DNA-coated gold beads into cells (13).

The most widely used methods for immunization has been the direct injection of the "naked" plasmid DNA

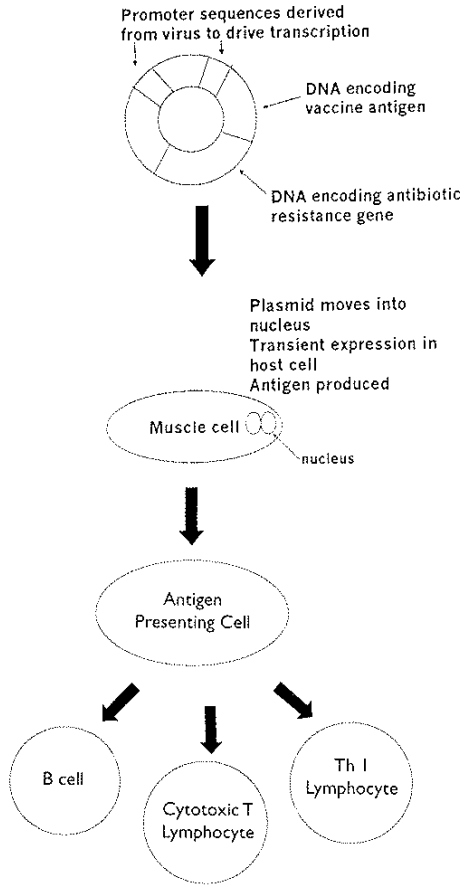

Figure 2. Schematic representation of plasmid DNA used in gene vaccination (adapted from Tighe *et al.*, 1998 (3)

into skeletal muscle and the gene-gun administration to the skin. When compared with intra-muscular immunization (i.m.), the gene-gun method requires almost 100-fold less DNA and the injections are more reproducible (14). However i.m. is easier to carry out and is more cost-effective.

Site of gene expression differs with mode of administration. Following muscle innoculations, most of the antigen expression occurs in the skeletal muscle (15) whereas following skin inoculations, expression is mostly in keratinocytes (2,16). The success of these DNA immunizations gave rise to the suggestion that the skeletal muscle and keratinocytes might be presenting the antigen to the immnune system as opposed to the conventional immunizations where the immune response is initiated by bone marrow-derived antigen presenting cells (17).

A recent study by Grillot-Courvalin *et al.* (18) has shown that a better, safer method of delivery is by using bacteria that are non-pathogenic that have been genetically modified to enter cells and release their plasmid DNA, for example E. coli bearing a deficiency in cell wall

biosynthesis and transformed with the gene encoding the protein invasin from Yersinia pseudotuberculosis, that die after entry into mamalian cells thereby releasing their contents which include plasmid DNA.

Immune response

DNA immunization results in the uptake of the DNA into cells close to the injection site. The DNA that remains episomally is subsequently transcribed and translated causing expression of the vector-encoded protein (15,19). The protein is processed like a virus-encoded antigen, resulting in presentation of antigenic fragments in association with MHC Class I molecules which result in the activation of cytolytic T cells (11). The immune response, as studied in mice, is weaker than that of conventional vaccines but has proven to be exceptionally long lasting and requires only a single dose (13,17,20). Presentation of the antigen is thought to be by the muscle cells but transfection of the plasmids into antigen-presenting cells (such as dendritic cells) residing in the muscle tissue presumably occurs (1). Activated dendritic cells up-regulate MHC and co-stimulatory molecules, secrete cytokines, and migrate to the lymph nodes where they initiate an immune response (21). One activation signal of dendritic cells is the cytokine granulocyte-macrophage colony-stimulating factor (GM-CSF) (22). In addition, stimulation of T helper cells and B cells occur (23,24) resulting in protection to the challenge.

Safety

Questions that must be addressed regarding the safety of DNA vaccines include: Does integration of the plasmid lead to insertional mutagenesis of the host genome? Are anti-DNA antibodies induced? Does immunological tolerance against the antigen occur if produced over a lengthy period? (25). Studies have shown that the integration of plasmid after intra-muscular injection is very low (26) and that seems to be no induction to tolerance whatsoever (27). Furthermore, there seems to be no significant increase in the levels of pathogenic anti-DNA antibodies (28). However, more studies are being carried out to determine the safety of these viruses vaccines.

Conclusion

DNA vaccines seem to have all the potential qualities of an ideal vaccine. However the safety, feasibility and immunogenecity of these vaccines in humans are currently under investigation. To date, many experimental trials have been successfully conducted in a variety of disease models including HIV (29), malaria (23), Hepatitis B(30) rabies (24) and cancer, specifically B-cell lymphoma (31). If DNA vaccines can be established in regard to their safety and efficacy, this may eventually lead to the replacement of existing conventional vaccines and allow the prevention of diseases that were previously unable to benefit from vaccine intervention.

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