

Western Maryland Health System
Infection Prevention and Control Department

DNA Vaccines—A viable alternative?

The recent rapid progression of the H1N1 2009 influenza pandemic again brought to the fore the delay in making an influenza vaccine to meet the need for an evolving outbreak. One can think of the process as a multistep endeavor that includes:

1. Identification and growth of the virus (or other etiologic agent in the case of other diseases);
2. Propagation of the virus in large quantities;
3. Purification of the virus and admixture PRN;
4. Safety testing;
5. Distribution;
6. Administration.

Some of these steps cannot be changed or expedited readily owing to their nature. Etiologic agent identification, safety testing and administration will, at least for the foreseeable future, retain their *status quo*. However, the use of DNA vaccines may accelerate or facilitate some of the other aspects of vaccine production and distribution.

DNA immunization is a novel technique used to efficiently stimulate humoral and cellular immune responses to protein antigens. The direct injection of genetic material into a living host causes a small amount of its cells to produce the introduced gene products. This “foreign” gene expression within the host results in the specific immune activation of the host against the gene-delivered antigens.

Experiments outlining the transfer of DNA into cells of living animals were reported as early as 1950. The use of purified genetic material confirmed that the injection of genes in the absence of viral vectors results in the expression of the inoculated genes in the host. Additional experiments demonstrated that recombinant DNA molecules could be delivered directly into a host and proteins would be produced.

Since these early trials DNA-based immunization has become a novel approach to vaccine development. Direct injection of naked plasmid DNA induces strong immune responses to the antigen encoded by the gene-containing vaccine. Once the plasmid DNA is injected the host cells take up the foreign DNA, expressing the viral gene and producing the corresponding viral protein inside the cell. This form of antigen presentation and processing induces both major histocompatibility complexes (MHC class I and class II), as well as restricted cellular and humoral immune responses.

DNA vaccines are composed of bacterial plasmids. Plasmids used in DNA-based vaccination normally contain two units: the antigen coding unit composed of the information needed to have the host cells readily able to accept the nucleic acid, and the production unit composed of bacterial sequences necessary for plasmid amplification. The construction of bacterial plasmids with vaccine inserts is accomplished using recombinant DNA technology. Once constructed, the vaccine plasmid is transformed into bacteria, where bacterial growth produces multiple plasmid copies. The plasmid 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.

Several possible routes of plasmid delivery have been described. Successful immunization has been demonstrated after delivery of plasmids through intramuscular, intradermal and intravenous injection. The skin and mucous membranes are considered the best sites for immunization due to the high concentrations of dendritic cells, macrophages and lymphocytes. Intradermal injection of DNA-coated gold particles with a “gene gun” has also been used.

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 host. After uptake of the plasmid, the protein is produced endogenously. It is then processed intracellularly into small antigenic peptides by the host proteases. The peptides then enter the lumen of the endoplasmic reticulum (ER) by membrane-associated transporters. In the ER, peptides bind to MHC class I molecules. These peptides are presented on the cell surface in the context of the MHC class I. Subsequent CD8+ cytotoxic T cells (CTL) are stimulated and they evoke cell-mediated immunity.

The foreign protein can also be presented by the MHC class II pathway by antigen-presenting cells (APCs) which elicit helper T cell (CD4+) responses. These CD4+ cells are able to recognize the peptides formed from exogenous proteins that were endocytosed or phagocytosed by APCs, then degraded 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. This is the same manner in which traditional vaccines work.

DNA immunization offers many advantages over the traditional forms of vaccination. It is able to induce the expression of antigens that resemble native viral epitopes more closely than standard vaccines do since live attenuated and killed vaccines are often altered in their protein structure and antigenicity. Plasmid vectors can be constructed and produced quickly and the coding sequence can be manipulated in many ways. DNA vaccines encoding several antigens or proteins can be delivered to the host in a single dose. The cost of rapid and large-scale production is lower than traditional vaccines, and they are also very temperature stable making storage and transport much easier. Another important advantage of genetic vaccines is their therapeutic potential for ongoing chronic viral infections. The continuous expression of the viral antigen caused by gene vaccination in an environment containing many APCs may promote a successful therapeutic immune response which cannot be obtained by other traditional vaccines.

Although DNA can be used to raise immune responses against pathogenic proteins, certain microbes have outer capsids that are made up of polysaccharides. This limits the extent of the usage of DNA vaccines because they cannot substitute for polysaccharide-based subunit vaccines. Conversely, DNA vaccines may be effective in antibody production of “foreign” cells such as some cancers, thereby increasing the possibility of better cancer immunotherapeutics.

It should be kept in mind that DNA vaccine studies have been ongoing for at least two decades and we have yet to see an available commercial version for human use. The closest we have come so far may be the hepatitis B vaccines that are composed of microbially generated viral proteins. How long we will have to wait until these are available remains to be seen.

A “Ponderment” Moment

Formal taxonomic systems first identified species based on visual traits such as fins or fur. Later, the species concept changed, specifying that two organisms should be capable of breeding. Today biological diversity can be ascertained by sampling DNA and tracking how a species descended from a common ancestor. Is this the end of the story? (to be continued).