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Seven Hills College of Pharmacy, Tirupati, Andhra Pradesh.
In association with Sri Padmavathi Medical College for Women, Alipiri Road, Tirupati, Chittoor (Dist.,), Andhra Pradesh, India.
www.shcptirupati.com
shcpdic333@gmail.com

Phone: 7730084513, 7702484513

VISION

To emerge as one of the premier pharmacy colleges in the country and produce pharmacy professional of global standards.

MISSION

- To deliver quality academic programs in Pharmacy and empower the students to meet industrial standards.
- To build student community with high ethical standards to undertake R&D in thrust areas of national and international standards.
- To extend viable outreach programs for the health care need of the society.
- To develop industry institute interaction and foster entrepreneurial spirit among the graduates

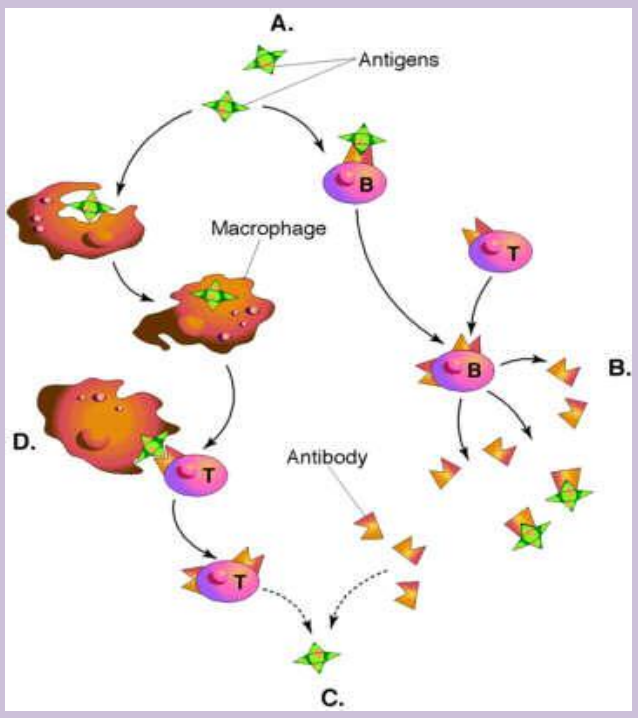
DNA VACCINES – The Facts to Know

Dr S Sirisha

Introduction:

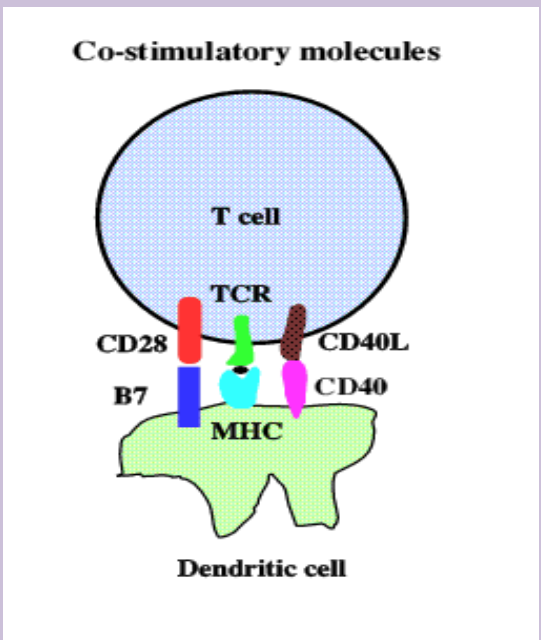
Genetic/ DNA immunization is a novel technique used to efficiently stimulate humoral and cellular immune responses to protein antigens. The direct injection of genetic material into a living host causes a small amount of its cells to produce the introduced gene products. This inappropriate gene expression within the host has important immunological consequences, resulting in the specific immune activation of the host against the gene delivered antigen.

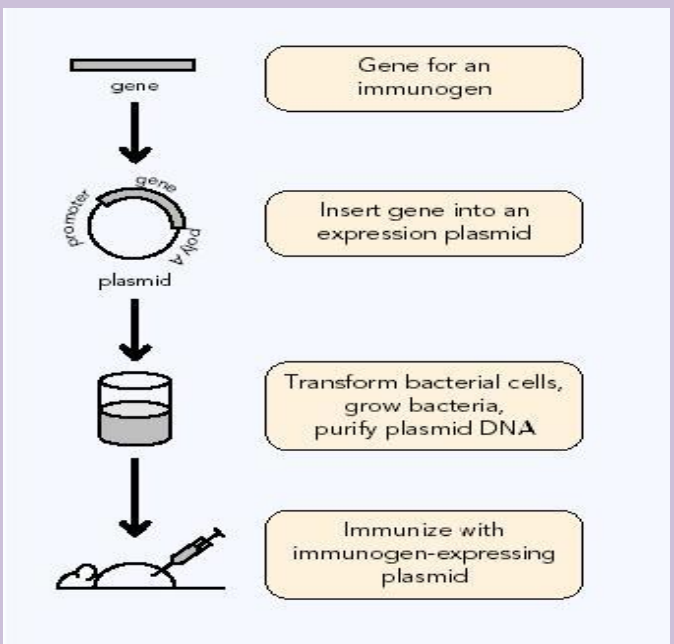
Traditional Vaccines: The development of vaccination against harmful pathogenic microorganisms represents an important advancement in the history of modern medicine. In the past, traditional vaccination has relied on two specific types of microbiological preparations to produce material for immunization and generation of a protective immune response. These two categories involve either living infectious material that has been manufactured in a weaker state and therefore inhibits the vaccine from causing disease, or inert, inactivated, or subunit preparations.



Live attenuated vaccines stimulate protective immune responses when they replicate in the host. The viral proteins produced within the host are released into the extracellular space surrounding the infected cells and are then acquired, internalized and digested by scavenger cells that circulate the body. These cells are called antigen presenting cells (APCs) and include macrophages, dendritic cells, and B cells, which work together to expand immune response. The APCs recirculate fragments of the digested the antigen to their surface, attached to MHC class II antigens. This complex of foreign peptide antigen plus host MHC class II antigens forms part of the specific signal with which APCs along with the MHC peptide complex, trigger the action of immune cells, the T helper lymphocytes. The second part of the activation signal comes from the APCs themselves, which display on their cell surface costimulatory molecules along with MHC-antigen complexes. Both drive T call expansion and activation through interaction with their respective ligands, the T cell receptor complex (TCR) and the costimulatory receptors CD28/CTLA4, present on the T cell surface. Activated T cells secrete molecules that act as powerful activates of immune cells. Also as viral proteins are produced within the host cells, small parts of these proteins surface, chaperoned by host cell MHC class I antigens. These complexes together are recognized by a second class of T cells, killer or cytotoxic cells. This recognition, along with other stimulation by APCs and production of cytokine by stimulated T cells, is responsible for the developments of mature cytotoxic T cells (CTL) capable of destroying infected cells. In most instances live infection induces lifelong immunity. Although live attenuated preparations are the vaccines of choice they do pose the risk of reversion to their pathogenic form, causing infection.

In contrast, when inoculated nonlive vaccines composed of whole or even partial viruses are not produced within the host cells, they mainly end up in the extracellular space. They provide protection by directly generating T helper and humoral immune responses against the pathogenic immunogen. In the absence of the cellular production of the foreign antigen, these vaccines are usually devoid of the ability to induce significant T cytotoxic responses. In addition, these vaccines are not actually produced in the host, and therefore, they are not customized by the host. . The immunity induced by their vaccines frequently decreases during the life of the host and may require additional boosters to achieve lifelong immunity.

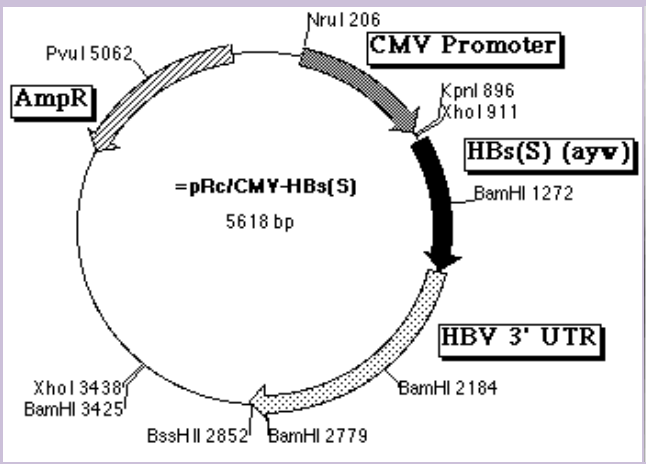


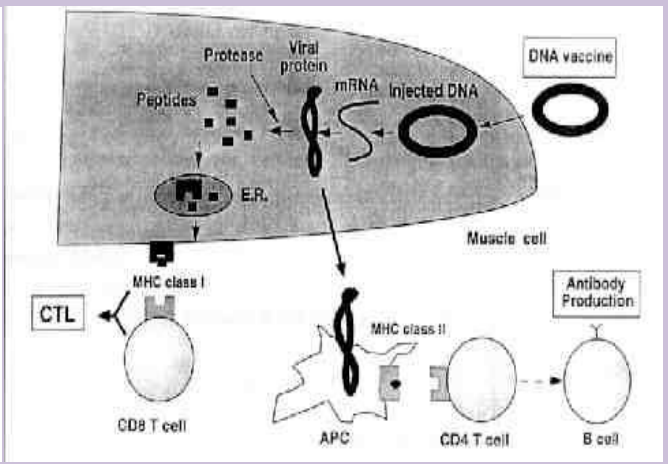


Genetic Immunization: Since its early applications in the 1950's, DNA-based immunization has become a novel approach to vaccine development. Direct injection of naked plasmid DNA induces strong immune responses to the antigen encoded by the gene vaccine. Once the plasmid DNA construct is injected the 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 induced both MHC and class I and class II restricted cellular and humoral immune responses.

History: The use of genetic material to deliver genes for therapeutic purposes has been practiced for many years. Experiments outlining the transfer of DNA into cells of living animals were reported as early as 1950. Later experiments using purified genetic material only further confirmed that the direct DNA gene injection in the absence of viral vectors results in the expression of the inoculated genes in the host. There have been additional experiments that extend these findings to recombinant DNA molecules, further illustrating the idea that purified nucleic acids could be directly delivered into a host and proteins would be produced. In 1992, scientists Tang and Johnson reported that the delivery of human growth hormone in a expression cassette *in vivo* resulted in production of detectable levels of the growth hormone in host mice. They also found that these inoculated mice developed antibodies against the human growth hormone; they termed this immunization procedure "genetic immunization", which describes the ability of inoculated genes to be individual immunogens.

Construction: DNA vaccines are composed of a bacterial plasmids. Expression plasmids used in DNA-based vaccination normally contain two unites: 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 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.





Administration- Over the past decade of clinical research and trials, several possible routs of plasmid delivery have been found. Successful immunization has been demonstrated after delivery of plasmids through intramuscular, intradermal and intravenous injection. The skin and mucous membranes being considered the best site for immunization due to the high concentrations of dendritic cells (DC), macrophages and lymphocytes. Intradermal injection of DNA-coated gold particles with a gene gun have been used. The plasmid DNA can be diluted in distilled water, saline or sucrose. There has also been positive demonstration of proinjection or codelivery with various drugs.

Mechanisms: 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 and intra-cellularly processed into small antigenic peptides by the host proteases. The peptides then enter the lumen of the endoplasmic reticulum (E.R.) by membrane-associated transporters. In the E.R., 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. 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 the MHC class II pathway by APCs which elicit helper T cells (CD4+) responses. These CD4+ cells are able to recognize the peptides formed from exogenous proteins that were endocytosed or phagocytosed by APC, 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.

Advantages: 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, only requiring a microgram of plasmids to induce immune responses. Rapid and large-scale production are available at costs considerably 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. DNA vaccination may provide an important tool for stimulating an immune response in HBV, HCV and HIV patients. The continuous expression of the viral antigen caused by gene vaccination in an environment containing many APCs may promote successful therapeutic immune response which cannot be obtained by other traditional vaccines. This is a subject that has generated a lot of interest in the last five years.

Limitations: 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.