Advances in DNA vaccines for Cancer and many other Diseases

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Abstract
DNA vaccination, a recent smart approach that deals with the quality and stable vaccine platform for immunotherapy to manage the severity of the fatal diseases as well as to prevent epidemic diversity of the diseases even cancer. It can be a choice of great interest for its simplicity, safety, stability and potentiality. However it lacks a major disadvantage of delivering genetic material to the immune cells, which must be targeted. Because somatic cells are almost avoid of MHC Class receptor to present antigenic peptides and also the efficiency of exocytosis of antigenic peptides as well as the proper target sequence of the peptides, which were originated from proteasomes. Here recent advances of DNA vaccine were discussed in order to orchestrate its future promising perspectives.

Key words: Acute Poisoning, Epidemiology, Ethiopia, Global.

Introduction
DNA vaccines are going to a broadly useful vaccine and immunotherapy platform with applications for human and animal health. DNA vaccines for cancer immunotherapy are designed to deliver one or several genes encoding tumor antigens, thereby eliciting or augmenting anti-gen-specific immune responses against antigens that play a central role in tumor initiation, progression and metastasis. Vaccine efficacy can be significantly improved by implementing strategies for enhancing antigen presentation and immunogenicity, such as new delivery systems, addition of molecular adjuvants and immune-stimulatory signals, optimized prime-boost strategies or blockade of immune check points.

DNA technology enables vaccination with versatile combinations of antigens that can simply be substituted. 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. Over the past decade of clinical research and trials, several possible routes 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 pro-injection or co-delivery with various drugs.

How does a DNA vaccine work?
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 the host. After uptake of the plasmid,
the protein is produced endogenously and intracellularly processed into small anti-genic 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. Unfortunately most of the somatic cells don’t present antigen to the T-Cell. In this aspect, antigen presenting cells APC like dendritic cells, B cells and macrophage, should be targeted to deliver genetic materials. These peptides are presented on the cell sur-face in the context of the MHC class I. Subsequent CD8+ cytotoxic T/cells (CTL) are stimu-lated and they evoke cell-mediated immunity. CTLs inhibit viruses through both cytolysis of infected cells and noncytolysis mechanisms such as cytokine production (Encke et al, 1999). 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.

**Diversity of a DNA vaccine**

A DNA vaccine career usually consists of a plasmid vector, which contains epitopic gene of interest for antigenic protein. Codon optimization of genes, improved delivery, and DNA vector improvements have enhanced the immunogenicity of DNA vaccines, and a number of DNA vaccine candidates have been successful in both animal and human studies. A DNA prime inactivated vaccine boost is highly effective in eliciting higher protective immune response than using either a DNA vaccine or protein vaccine alone. Therefore, it is highly advisable to use DNA vaccine as the first dose of immunization that can be given either long before the pandemic (prepandemic vaccination) or shortly after the outbreak. Furthermore DNA vaccine can be stockpiled for a long period of time, which makes the whole system even more attractive.

There have been dozens of human clinical trials of DNA vaccines against infectious and non-infectious diseases such as influenza, hepatitis B, HIV, malaria and cancer; however, with dis-appointing outcomes, suffering from lower immunogenicity than that had been ob-served in other mammals. Safety issues such as integration of plasmid DNA into genomic DNA, the risk of autoimmunity or antigen tolerance were successfully addressed in those studies. It was concluded that despite its low immunogenicity, DNA vaccination is a safe form of immunization.

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 recent decade.

**DNA vaccines to attack cancer**

DNA vaccines are simple vehicles for in vivo transfection and antigen production.

Epitope-specific DNA vaccination leads to powerful antitumor attack and can activate immunity.

Broad spectrum of cytotoxic T-lymphocytes outcome is an outpouring of cytokines including IL-6, IL-12, IFN-γ and IFN-α, TNF-α and TNF-β. Cytotoxic T-Lymphocytes (CTL) release the cytotoxins proteins like Perforin, Granzymes, granulysin and others (Fas ligand). Interleukin-12 (IL-12) is a very exciting cytokine. It is a heterodimeric protein that promotes T cell and NK activity and is a growth factor for B cells. It is involved in the stimulation and maintenance of Th1 cellular immune responses, including the normal host defense against various intracellular pathogens, such as Leishmania, Toxoplasma, Measles virus, and Human immunodeficiency virus 1 (HIV).

IL-6 is a multifunctional cytokine that is involved in the regulation of the acute-phase immune system response to infection and injury. It stimulates B-cell to release antibody.

**DNA Vaccines for prostate cancer**

Prostate cancer (PCa) is the second most common cause of male cancer death in the UK and USA. The prostate is a part of the male reproductive system that helps make and store seminal fluid. It is located in the pelvis, under the urinary bladder and in front of the rectum.

Prostate cancer is classified as an adenocarcinoma, or glandular cancer, that begins when normal semen-secreting prostate gland cells mutate into cancer cells.

One research group showed that DNA fusion gene vaccines induce cytotoxic T-cell attack on naturally processed peptides of human prostate-specific membrane antigen.

In patients with prostate cancer, induction of cytolytic T cells is desirable. Several lineage-specific target proteins are known and algorithms have identified candidate MHC class I-binding peptides, particularly for HLA-A*0201. Figure 2 showed DNA fusion vaccines incorporating a domain of tetanus toxin fused to candidate tumor-derived peptide sequences.
Figure 1. Schematic diagram of a DNA vaccine how it activates host immune system against cancer and other fatal immune disorders.

Figure 2. DNA fusion vaccines, containing prostate specific antigens.
Three separate peptide sequences from prostate-specific membrane antigen (PSMA) (peptides PSMA(277), PSMA(663), and PSMA(711)), induced high levels of CD8+ T cells against each peptide in a HLA-A(*) 0201 preclinical model. In contrast, the full-length PSMA sequence containing all three epitopes was poorly immunogenic.

Plasmid DNA vaccine encoding prostatic acid phosphatase is effective in eliciting autologous antigen-specific CD8+ T cells

In figure 3, recombinant vaccinia virus was constructed by first cloning the cDNA encoding the entire length of human PAP and this vector contains a multiple cloning site downstream of the p7.5 viral promoter. Multiple immunizations with a DNA vaccine encoding the rat PAP homologue (pTVG-RP) could overcome peripheral self-tolerance against rPAP and generate a Th1-biased antigen-specific CD4+ and CD8+ T cell response. The pTVG-HP and pTVG-RP constructs were derived from the pTVG4 immunization vector and this construct was cloned the cDNA encoding the entire length of hPAP (pTVG-HP) or rPAP (pTVG-RP) protein.

**DNA Vaccines for Malignant Myeloma**

Multiple myeloma (MM) is a plasma-cell malignancy that remains incurable in the vast majority of patients. Patients with MM are diagnosed around 15,000 people each year in the United States alone. A type of white blood cell normally responsible for the production of antibodies. After treatment with high-dose chemotherapy and autologous stem cell support, complete remission rates 50%. However, most patients relapse.

Active Vaccination with Dickkopf-1 Induces Protective and Therapeutic Antitumor Immunity in Murine Multiple Myeloma

Dickkopf-1 (DKK1), broadly expressed in myeloma cells but highly restricted in normal Tissues. DKK1 (peptide)-specific cytotoxic T lymphocytes can effectively lyse primary myeloma cells in vitro. Figure 5 showed various tumor sizes against DKK1 peptides and/ also with other adjuvants.

**DNA Vaccines for Influenza virus**

Millions of people worldwide are infected with influenza virus every year. Although most yearly outbreaks are characterized by fewer than 40,000 deaths in the USA. Highly virulent strains can evolve that cause worldwide pandemics, resulting in a dramatically increased incidence of death. Ribavirin and oseltavir can be used to combat infection, there has been recent emergence of strains resistant to these drugs. Two glycoproteins, hemagglutinin (HA) and neuraminidase (NA), contribute to the considerable antigenic variation of influenza virus because they have 16 and 9 subtypes: H1N1, H2N3, H5N1, H9N2, and H7N7

The worst influenza pandemic to date are H1N1 and H5 N1.

A Human Multi-Epitope Recombinant Vaccinia Virus as a Universal T Cell Vaccine Candidate against Influenza Virus

One research group designed a novel influenza virus immunogen based on the NP backbone containing human T cell epitopes for M1, NS1, NP, PB1 and PA proteins (referred as NPmix) as well as a construct containing the conserved regions of influenza virus neuraminidase (N-terminal) and hemagglutinin (C-terminal) (referred as NA-HA).

In figure 7, viral genes were codon optimized and contain either an
Figure 4. CD4+ and CD8+ T-Cell populations against respective antigenic plasmids

Figure 5: Tumor sizes with days after treatment with antigenic plasmids and adjuvants. DKK1 cDNA fragment (726 bp) was amplified by reverse transcription polymerase chain reaction (RT-PCR), from DKK1-expressing mouse cells with primers 5’-actgcagtcgacaccttgaactcagttctcatcaattc-3’ and 5’- ttagactgtcggtttagtgtctctggc-3’. The cDNA was then sub-cloned into pCMVE-Chemokine-sFv vector. 50μg of DKK1 plasmid was intramuscularly injected followed by adjuvant CpG (ODN1826; 50μg/mouse TCCATGACGTTCCTGACGTT

Figure 6: DKK1 vaccine elicited a strong DKK1- and tumor-specific CD4+ and CD8+ immune responses
N-terminal FLAG tag (NPmix) or a C-terminal His tag (NA-HA). The genes were individually cloned into pCIneo for mammalian expression, and also cloned into the TK locus of vaccinia virus using the transfer vector pCyA. In this vector NPmix was inserted alone, or in front of NA-HA, both of which were driven by the vaccinia virus early/late promoter (pE/L). They observed an increase in the number of influenza virus-specific IFNγ-secreting splenocytes, composed of populations marked by CD4+ and CD8+ T cells producing IFNγ or TNFα.

**DNA Vaccines for Head and Neck cancer**

Head and neck cancer refers to a group of biologically similar cancers that start in the upper aero-digestive tract, including the lip, oral cavity (mouth), nasal cavity (inside the nose), paranasal sinuses, pharynx, and larynx. 90% of head and neck cancers are squamous cell carcinomas (SCCHN), originating from the mucosal lining (epithelium) of these regions.

Head and neck cancer is strongly associated with certain environmental and lifestyle risk factors, including tobacco smoking, alcohol consumption, UV light, particular chemicals used in certain workplaces, and certain strains of viruses, such as human papillomavirus.

Innovative DNA vaccine for human papillomavirus (HPV)-associated head and neck cancer.

The viral-encoded oncogenic proteins E6 and E7 represent ideal targets for immunotherapy against HPV-associated head and neck cancers. DNA vaccine encoding an invariant chain (Ii), in which the class II-associated Ii peptide (CLIP) region has been replaced by a Pan-DR-epitope (PADRE) sequence to form Ii-PADRE, is capable of generating PADRE-specific CD4+ T cells in vaccinated mice.

DNA vaccine encoding Ii-PADRE linked to E6 (Ii-PADRE-E6)
will further enhance E6-specific CD8+ T cell immune responses through PADRE-specific CD4+ T helper cells.

They found that mice vaccinated with Ii-PADRE-E6 DNA generated comparable levels of PADRE-specific CD4+ T cell immune responses as well as significantly stronger E6-specific CD8+ T cell immune

**DNA Vaccines for HIV**

Recent advances in increasing the potency of DNA vaccines via novel adjuvants, formulations, and delivery systems, along with prime/boost strategies and nonhuman primate studies, were reviewed in terms of their potential for developing an HIV/AIDS vaccine for clinical usage.

DNA vaccine encoding multiple HIV CD4 epitopes elicits vigorous polyfunctional, long-lived CD4+ and CD8+ T cell responses.

Safety and Comparative Immunogenicity of an HIV-1 DNA Vaccine in Combination with Plasmid Interleukin 12 and Impact of Intramuscular Electroporation for Delivery

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**Figure 9.** CD4 Cell populations with the connection of IFN-gamma secretion

**Figure 10.** CD8 Cell populations with the connection of IFN-gamma secretion
A DNA vaccine encoding 18 conserved multiple HLA-DR binding HIV-1 CD4 epitopes (HIVBr18), capable of eliciting broad CD4+ T cell responses in multiple HLA class II transgenic mice. The artificial gene (EZBiolab) was cloned into the HindIII/XhoI restriction site of the pVAX1 vector (Invitrogen) to generate the HIVBr18 plasmid.

The percent of proliferating CD4+ and CD8+ CFSE low cells were determined in the CD3+ cell population. HIVBr18 immunization was able to induce polyfunctional CD4+ and CD8+ T cells that proliferate and produce any two cytokines (IFNγ/TNFα, IFNγ/IL-2 or TNFα/IL-2) simultaneously in response to HIV-1 peptides.

**DNA Vaccine for Tuberculosis**

Tuberculosis (TB) is a re-emerging disease, that is a major human priority as well as an important disease of livestock. TB is due to infection with mycobacteria of the Mycobacterium tuberculosis complex, and is responsible for over two million human deaths annually.

An approach of DNA vaccine to live mycobacterial vaccines for protective immunization against tuberculosis, was recently exercised.

*Immunogenicity and protective efficacy of mycobacterial DNA vaccines incorporating plasmid-encoded cytokines against Mycobacterium*[^10] [^31]

In Figure 12, Mice that received immunization with DNA constructs encoding M. bovis antigen 85A (Ag85–A) and arget (ESAT-6) produced measurable interferon-gamma (IFN-γ) responses to CD4+ T-cell epitope-peptide recall antigens in vitro. The magnitude of these responses was enhanced by co-delivery of a construct encoding murine cytokines (macrophage inhibitory protein (MIP)-1α or interleukin (IL)-7).

Another group developed a DNA vaccine combination expressing mycobacterial heat shock protein 65 (HSP 65) and interleukin-12 (IL-12) by using the hemagglutinating virus of Japan (HVJ)-liposome (HSP 65 + IL-12/HVJ).

Hsp65+mIL-12/HVJ vaccination resulted in a greater degree of protection than that evoked by BCG. This protective efficacy was associated with the emergence of IFN-gamma-secreting T cells and activation of proliferative T cells and cytokines (IFN-gamma and IL-2) production upon stimulation with Hsp65 and antigens from M. tuberculosis.

**DNA Vaccines for Alzheimer’s disease**

The most common form of dementia, There is no cure for the disease, which worsens as it progresses, and eventually leads to death. The most common symptom in early stage is difficulty in remembering recent events. Amyloid beta (Aβ) deposits

They developed a DNA vaccine, p(Aβ(3-10))(10)-IL-4, encoding ten tandem repeats of Aβ(3-10) fused with mouse cytokine interleukin-4 (IL-4) as a molecular adjuvant. Mice were injected intramuscularly with p(Aβ(3-10))(10)-IL-4 followed by in vivo

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[^10]: Reference 10
[^31]: Reference 31
Figure 12: INF gamma and IL-2 production after immunization with DNA vaccine.

Figure 13. Comparison of a normal aged brain (left) and the brain of a person with Alzheimer’s (right).

Immunization with a new DNA vaccine for Alzheimer’s disease

Figure 14. A, B and C, Cloning of plasmid vector, which elicited Th2 immune response in BALB/c mice by in vivo electroporation.
Figure 15. A, B and C, DNA vaccination and anti-Aβ antibodies as well as IgG1/IgG2a, INFγ, IL4.

Figure 16. IL-4 and INF gamma production after DNA vaccination
electroporation. Ex vivo cultured splenocytes isolated from mice immunized with p(AB(3-10))(10)-IL-4 exhibited a low IFN-γ response and a high IL-4 response compared with the control group.

**Future perspectives of DNA vaccines**

Both CD8+ T and CD4+ T Combined administration of the vaccine concept may provide sustained help for CD8+ T cells and antibody responses in future perspective of DNA vaccines

It might be a potential application if cytokine motif gene could be inserted with leader peptide motif for a promising DNA vaccine ahead. Miura, Shaheen, Akita et al. reported on an artificial nanoparticle that can achieve these; a multifunctional envelope-type nanodevice modified with KALA, a peptide that forms α-helical structure at physiological pH (KALA-MEND). KALA modification and the removal of the CpG-motifs from the pDNA synergistically boosted transfection efficacy. In parallel, transfection with the KALA-MEND enhances the production of multiple cytokines and chemokines and co-stimulatory molecules via the Toll-like receptor 9-independent manner. Endosome-fusogenic lipid envelops and a long length of pDNA are essential for this immune stimulation.

DNA manipulation, xenogeneic antigen use, immune stimulatory molecule and immune response regulator application, DNA prime-boost immunization strategy use and different DNA delivery methods are of great interest in the future perspective of a successful DNA vaccine. However, cancer cells, immune cells are heterogenous and likely to be resistant to target as well as exocytosis of intracellular antigenic peptides from myocytes, which might restrict a DNA Vaccine.

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