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Increased immunogenicity through advancements in DNA vaccine vectors, non-mechanical delivery techniques, and molecular Adjuvants

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Abstract

The capacity to produce both humoral and cellular immune responses is a fundamental benefit of DNA vaccination. DNA vaccines are currently utilised in veterinary medicine, but due to their limited immunogenicity in early clinical research, they have not gained universal acceptability for use in humans. Recent clinical data, on the other hand, has re-established the utility of DNA vaccines, especially in priming high-level antigen-specific antibody responses. Advancements in DNA vaccine vector design, the addition of genetically modified cytokine adjuvants, and novel non-mechanical delivery methods have all been researched as ways to improve DNA vaccine efficacy. These techniques have demonstrated promise in mice and big animal models, leading in improved adaptive immune responses. Here, we look at recent developments in each of these domains that have the potential to improve the immunogenicity of DNA vaccines.

Keywords: DNA Vaccine, immunogenicity, molecular adjuvant, plasmid, vaccine delivery

Introduction

The ongoing appearance and reemergence of known and novel infections forces scientists to develop new vaccination methods that allow for the quick development of safe and effective vaccines. These issues are met by nucleic acid (DNA and RNA) vaccines, which offer qualities such as ease of production, scalability, consistency between batches, storage, and safety. Bacterial

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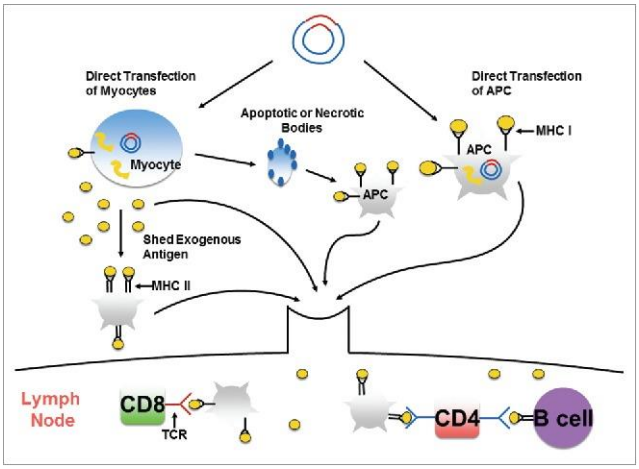
plasmids that encode the polypeptide sequence of potential antigens are commonly used in DNA vaccine development. The encoded antigen is expressed under the control of a powerful eukaryotic promoter, resulting in high transgenic expression levels. Incorporating transcriptional enhancers, such as Intron A, improves the rate of polyadenylation and messenger RNA trafficking into the nucleus (mRNA). Vaccine plasmids are typically grown in bacterial culture, purified, and then administered to the host.

In most cases, modern DNA vaccine design depends on nucleic acid synthesis and possible one-step cloning into the plasmid vector, which reduces both the cost and time to produce. Plasmid DNA is also quite stable at ambient temperature, which eliminates the requirement for a cold chain during transport. Vaccination with DNA plasmid eliminates the need for infectious pathogen protein purification, boosting safety. Furthermore, DNA vaccination has a high safety profile in the clinic, with mild irritation at the injection site being the most prevalent side effect. The in vivo generation of antigen allows for presentation on both class I and class II major histocompatibility complex (MHC) molecules, making DNA vaccines a safe, non-live vaccine way to producing balanced immune responses. Antigen-specific antibodies are elicited, as well as cytotoxic T lymphocyte responses (CTL), which are rare in non-live vaccinations. DNA vaccines have also been shown to induce follicular T helper populations, which are necessary for inducing high-quality antigen-specific B cell responses.



In various animal models, DNA vaccination has been shown to be effective in preventing or curing infectious illnesses, allergies, cancer, and autoimmune. Small animal investigations were initially successful, which led to a number of human clinical trials. When DNA vaccines were provided alone by needle delivery, however, the protective immunity seen in small animals and non-human primates was not seen in human investigations. DNA can be administered in a variety of ways,

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including intramuscular (IM), intradermal (ID), mucosal, or transdermal administration, just like traditional protein-based vaccines. Because DNA plasmids must reach host cell nuclei to be translated into mRNA, DNA vaccines' early failure to elicit robust responses in humans was largely owing to their delivery by needle injection, which deposits the DNA in intracellular spaces rather than within cells. Improved delivery technologies, such as intramuscular or intradermal electroporation, have been employed to improve DNA transport into cells, resulting in significantly improved immunogenicity in both clinical and non-clinical investigations. In one study, patients who received an HPV DNA vaccine expressing the E6 and E7 genes of HPV16 and HPV18, respectively, had more polyfunctional antigen-specific CD8+ T cells after electroporation-enhanced DNA vaccination. Following DNA delivery, the majority of DNA vaccinated patients saw complete regression of their cervical lesions as well as viral clearance. Other mechanical delivery methods, such as particle bombardment (gene cannon), use physical force to transfer DNA plasmids into specified tissues or cells, and have had some clinical success. In participants who have previously failed to respond to a licenced subunit vaccination, particle bombardment delivery of a Hepatitis B DNA vaccine resulted in maintained antibody titers. Needle-free pneumatic or jet injectors, which work by delivering a high-pressure, narrow stream of injection liquid into the epidermis or muscles of test subjects, have also showed promise in animal and human clinical studies. Several more options are being investigated to improve the immunogenicity of DNA vaccines in humans, in addition to these improved mechanical delivery methods. Non-mechanical administration, inclusion of molecular adjuvants, and advancements in DNA vaccine vectors are three of these approaches that offer promise for developing DNA vaccines.

Non-mechanical DNA vaccine delivery

The most significant hurdle to DNA vaccination, as previously stated, is low immunogenicity due to difficulty in transporting DNA plasmid into the host cell. Several barriers must be overcome in order for DNA vaccination plasmids to enter cellular nuclei. Endocytosis or pinocytosis are required for the vaccine plasmid to cross the phospholipid cellular membrane, avoid destruction in endosomes and lysosomes, survive cytosolic nucleases, and translocate over the nuclear envelope. Chemical delivery technologies, in contrast to physical delivery systems, utilise biopharmaceuticals to improve DNA vaccine transfection efficiency.

Liposomes have become a preferred carrier molecule for DNA vaccine administration since they not only improve transfection efficiency but also have an adjuvant effect. Liposomes are spherical vesicles made up of phospholipids and cholesterol organised in a lipid bilayer that allows them to fuse with cell membrane lipids. The DNA plasmid might be attached to the liposome surface or encapsulated within the liposome's hydrophobic core. This makes it easier for the DNA vaccination plasmid to get into the cells. Importantly, lipid vesicles can be unilamellar or

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multilamellar in nature. Multilamellar vesicles enable vaccine administration to be sustained over a long length of time. While using liposomes for intramuscular injection has caused some reactogenicity difficulties, liposome/DNA vaccine complexes have shown to be immunologically beneficial. When compared to vaccine alone, IM injection of a liposome/influenza nucleoprotein mixture boosted antibody titers 20-fold. Antibody titer boosts had no effect on the cytotoxic T cell response. Similarly, adding a liposome formulation to a P. falciparum vaccination increased IFN- production. A subsequent human trial using DNA plasmids containing the influenza H5 HA, nucleoprotein, and M2 genes revealed cellular immune response rates and antibody titers comparable to those seen with inactivated protein-based H5 vaccines now on the market. Liposomes have also showed potential as a delivery vehicle for DNA vaccines to mucosal tissue. A recent study found that immunisation with liposome-encapsulated influenza A virus M1 resulted in protective humoral and cellular immune responses against respiratory illness. Intranasal DNA vaccination with liposomes has also been found to be an efficient delivery strategy for providing protective immune responses against infection.

Biodegradable polymeric micro- and nanoparticles made up of amphiphilic molecules about 0.5–10 m in size can also be used to deliver DNA vaccines. Plasmid molecules can be encapsulated or adsorbed onto the surface of nanoparticles, similar to how DNA plasmids are loaded onto liposomes. These particles serve as a carrier system for the vaccination plasmid, preventing it from being degraded by extracellular deoxyribonucleases. In addition to protecting plasmid DNA from nucleases, micro- and nanoparticles increase vaccine release over time rather than the bolus delivery typical of larger submicrometer complexes. When it comes to aggregating DNA vaccination plasmid, high molecular weight cationic polymers have proven to be far more successful than cationic liposomes. Plasmid DNA trapped within biodegradable chitosan-coated polymeric microspheres (with diameters ranging from 20 to 500 micrometres) can elicit mucosal and systemic immune responses. Microspheres can be administered orally or intraperitoneally, allowing for direct dendritic cell (DC) transfection and hence increased DC activation. Microsphere formulations have been found to be effective against a variety of diseases in mice, nonhuman primates, and humans, including hepatitis B, tuberculosis, and cancer. These findings imply that microparticle-based delivery technologies can improve cellular and humoral immune responses while also increasing DNA vaccine immunogenicity.

Clinical trials have shown that using liposomes or nanoparticles is safe and well tolerated. Microparticle-based delivery technologies can boost gene expression as well as the immunogenicity of DNA vaccines. Despite the fact that many of the first carrier formulations failed to show a meaningful clinical advantage, the more recent trials discussed below have generated encouraging clinical results. Because microparticles may be made with a wide range of structural characteristics (size, surface charge, and lipid content), they provide a lot of flexibility

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in vaccine formulation. This enables for vaccine optimization based on the clinician's individual needs.

Molecular adjuvants for DNA vaccines

As 'genetic adjuvants,' many vaccination plasmid-encoded immune-stimulatory molecules, such as different cytokine genes or PRR ligands, have been investigated. Recombinant DNA technology allows these genetic or molecular adjuvants to be encoded in the same plasmid as the vaccination or a co-administered plasmid.

Table 1.

Molecular adjuvants tested *in vivo*.

Molecular Adjuvant	Molecule Type	Animal Model	Adaptive Response Effect
CD40L	Co-Stimulatory	Mice	Cellular
CD80/86	Co-Stimulatory	Mice, NHP	Cellular
GM-CSF	Cytokine	Mice	Humoral
ICAM-1	Co-Stimulatory	Mice	Cellular
IFN-γ	Cytokine	Mice, NHP	Cellular
IL-2	Cytokine	Mice	Cellular, Humoral
IL-4	Cytokine	Mice, NHP	Humoral
IL-7	Cytokine	Mice	Cellular, Humoral
IL-8	Chemokine	Mice	Cellular, Humoral
IL-10	Cytokine	Mice	Cellular
IL-12	Cytokine	Mice, NHP	Cellular
IL-15	Cytokine	Mice, NHP	Cytokine
IL-18	Cytokine	Mice, NHP	Cytokine
MCP-1	Chemokine	Mice	Humoral
M-CSF	Cytokine	Mice	Cellular
MIP-1α	Chemokine	Mice	Humoral
RANTES	Chemokine	Mice	Cellular

Ligands of pattern recognition receptors

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TLRs are a type of membrane-bound PRR that play an important role in the innate immune system. There are 13 related human TLR genes (TLR1–TLR13) that have been discovered so far. TLR3 and TLR9 ligands have been shown to act as molecular adjuvants in the recognition of dsRNA and ssDNA, respectively. Poly (I:C) is a TLR3 ligand that has been around for a long time. In mice administered a DNA cancer vaccine, a poly (I:C) adjuvant improved generating CTL immunity and reduced tumour burden. Poly (I:C) boosted responses to an HPV-16 E7 DNA vaccination in a similar way. In mice, a DNA vaccine against eastern equine encephalitis virus with a combination CpG/Poly (I:C) adjuvant improved immunogenicity. Similarly, CpG, a TLR9 ligand, has been utilised to improve the immunogenicity of DNA vaccines. Double-stranded RNA is sensed by RIG-I-like receptors, which are key intracellular proteins. eRNA41H, a RIG-I ligand, improved the humoral immune response to a flu DNA vaccination. Similarly, a RIG-I 546-nucleotide ligand produced from the Sendai virus improved the immunogenicity of an influenza DNA vaccine. Th2 cell differentiation can be triggered by TLRs, RIG-I-like receptors (RLRs), the inflammasome, and STING-dependent cytosolic DNA sensor ligands. The cytosolic DNA sensor DAI's ligands have also been found to be effective molecular adjuvants for DNA cancer vaccines.

Plasmid-encoded cytokines

Cytokines are tiny proteins that are produced naturally and are essential for immune cell signalling. Local expression of cytokines at the injection site reduces the potential toxicity of systemically injected cytokines, and cytokine-encoding plasmids can be generated with antigen-expressing plasmids. Interleukin-2 (IL-2) stimulates T and NK cell proliferation and has been intensively researched as a genetic adjuvant. In mice, a fusion construct of the carboxy terminal region of the Mycoplasma pneumoniae gene with IL-2 resulted in improved vaccination responses. Immune responses were also improved in a therapeutic vaccination for chronic myeloid leukaemia that combined BCR/ABL-pIRES and IL-2.

DCs and monocytes both release IL-12, a proinflammatory cytokine. Th1 immune responses have been demonstrated to be enhanced by IL12 expression plasmids. A bicistronic plasmid producing Yersinia pestis epitopes and IL-12 boosted mucosal IgA and serum IgG, as well as protecting mice from infection. In a clinical trial of a poorly immunogenic hepatitis B DNA vaccine, IL-12 expression plasmids were also employed. A recent study found that using an IL12 genetic adjuvant increased the immunogenicity of the hepatitis C DNA vaccine by stimulating the production of IL-4 and IFN-. In a study of a Toxoplasma gondii DNA vaccine, researchers discovered that adding an IL-12 genetic adjuvant improved immune responses and survival rates. A DNA prime/protein boost research with an IL-12-adjuvanted HIV/SIV DNA vaccine was likewise successful. The PENNVAX-B HIV1 DNA vaccine, which is made up of three expression plasmids expressing HIV-1 Clade B Env, Gag, and Pol and adjuvanted by the IL-12 DNA plasmid, was found to be

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safe. The combination of PENNVAX-B and IL-12 plus electroporation resulted in considerable dosage reduction and improved CD4+ and CD8+ T-cell immunogenicity.

APC are known to be recruited to vaccination sites by GM-CSF, which also promotes DC maturation. It's been used in DNA vaccines before, including ones using Pseudorabies virus gB-encoding, SIV encoding, and DENV serotype 2 prM/E encoding. However, a recent study found that co-administration of the GM-CSF plasmid can be harmful, causing a DNA vaccination against dengue virus types 1 and 2 to be suppressed and failing to increase the response elicited by the HCV vaccine. Furthermore, too much GM-CSF can promote the growth of myeloid suppressor cells and inhibit adaptive immune responses. GM-CSF expressed in recombinant SIV and MAV vaccines did not improve protection in preclinical macaque tests. As a result, fine-tuning of GM-CSF expression levels must be considered when utilised as a molecular adjuvant.

IL-15 is a cytokine that promotes the growth of NK and T cells. A DNA vaccination against Toxoplasma gondii infection showed a synergistic effect of IL-15 and IL-21. The IL-6, IL-7, and IL-15 genes were given in order to improve CD4+ T cell memory to a DNA vaccination against foot and mouth disease. As a result, combining several cytokines in a DNA vaccine formulation or sequential cytokine immunisation may improve vaccine efficacy.

The ease with which cytokine genes may be cloned makes them interesting candidates for use as DNA vaccine adjuvants. The modest but longer-lasting expression of plasmid-expressed cytokines at the injection site helps address the difficulty of many cytokines' short half-lives while reducing the possibility of a systemic cytokine "storm" by limiting cytokine expression to the injection site. Despite the lack of human data on the use of cytokine-encoding plasmids as vaccine adjuvants, this appears to be a viable avenue for fine-tuning immune responses to DNA vaccines.

Plasmid-encoded signalling molecules

Understanding of immunological signalling pathways has advanced significantly in the last ten years, allowing for the testing of signalling compounds such as vaccine adjuvants. TRIF and HMGB1 signalling molecules have been successfully tested as genetic adjuvants for DNA vaccinations. Similarly, HSP70 co-transfection improved CTL responses to DNA vaccinations. DNA-vaccine-induced CD8+ T cell responses against HIV were demonstrated to be enhanced by PD-1-based plasmids. In hens infected with the H5N1 influenza virus, MDA5, a RIG-I-like dsRNA receptor, improved DNA vaccination. Recent research found that a plasmid expressing interferon regulatory transcription factor (IRF) increased CTL and IFN- responses to an HIV-1 Tat vaccine, whereas IRF3 and IRF7 plasmids had no effect. The innate immune regulator NF-B is a master. Co-administration of a plasmid expressing the NF-B subunit p65/RelA improved DNA vaccine immunogenicity, according to a recent study. Tbet, a T-cell transcription factor, proved

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successful in inducing a Th1 response to a tuberculosis vaccination based on Ag85B DNA. DNA can trigger innate immune pathways when it binds to cellular receptors. According to a recent study, a short noncoding DNA fragment of 300bp increased electroporation-mediated gene transfer in vivo as well as the immunological efficacy of an HBV vaccination.

shRNA or siRNA as molecular adjuvants

The post-transcriptional gene silencing mechanism RNA interference (RNAi) is activated by double-stranded small hairpin RNA (shRNA) structures. Since its development, RNAi has mostly been utilised as a research tool for studying target gene loss of function. RNAi can be used to inhibit genes that prevent DNA vaccines from working. The use of shRNA to inhibit caspase 12, a cell death mediator elevated following DNA vaccination, boosted plasmid gene expression as well as T-cell and antibody responses. Similarly, RNAi-mediated suppression of Foxo3, a key suppressor of T cell proliferation, improved the effectiveness of a HER-2/neu cancer vaccination. The IL10 receptor was also demonstrated to improve vaccine potency when it was knocked down. In HBV transgenic mice, RNAi inhibition of the PD-1 ligand (PD-L1) increased DC-mediated T cell responses and antiviral immunity. The combination of IL-10 siRNA and CpG in a recent cancer vaccination research revealed improved protective immunity against B cell lymphoma. Another cancer therapeutic vaccine found that combining GM-CSF with furin shRNA knockdown improved vaccine effectiveness. The RNAi knockdown of APOBEC expression also improved the immunogenicity of DNA vaccines. Thus, using RNAi against target genes that limit plasmid expression could be a strong new strategy for DNA vaccine improvement, particularly for tumour vaccines, although the safety of this approach must still be thoroughly validated in animal research.

Conclusion

While DNA vaccine has significant advantages over more traditional immunisation procedures, it still has to be improved before it becomes the standard in human patients. Despite some setbacks, tremendous progress has been achieved in resolving the human immunogenicity challenge. A better understanding of the immunological mechanisms driving DNA vaccine immunogenicity has revealed many pathways that could help improve DNA vaccine efficacy even further. A huge number of cytokines, chemokines, adhesion molecules, and transcription factors are being investigated as molecular adjuvants, albeit each will need to be thoroughly evaluated for safety and tolerability. Similarly, continuing to develop vaccine delivery systems appears to be fruitful. New vaccine formulations, such as slow-releasing micropatches or multilamellar vesicles, are on the horizon. The tremendous appeal of needle-free injection and mucosal delivery, as well as the ease of design and recent clinical achievements with DNA vaccines, suggest that this technique is on the verge of reinventing vaccinology.

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