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MOLECULAR ADJUVANTS TOWARDS IMPROVING THE EFFICACY OF DNA VACCINE

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Abstract

The capacity to quickly manufacture, adapt to newly developing infections, and maintain high stability at ambient temperatures are all advantages of DNA vaccine against infectious diseases over more traditional immunisation approaches. Furthermore, antigen is created by the vaccinated individual's cells after DNA immunisation, resulting in the activation of both cellular and humoral immune responses due to antigen presentation via MHC I and MHC II molecules. However, so far, DNA vaccines have shown the most effective immunogenicity in tiny rodent models, with larger species, including humans, still needing to be improved. This is primarily owing to inadequate DNA plasmid transport into cells and nuclei. The technologies utilised to solve this challenge are discussed here, including physical methods such as in vivo electroporation and adjuvant co-administration. Several of these techniques have already been tested in humans.
Keywords: DNA vaccines, Gene transfer techniques, Immunologic adjuvants, Infection

Introduction

The discovery that injecting bare plasmid DNA containing eukaryotic genes into mammalian muscle causes endogenous production of and a particular immune response to the encoded protein was published 25 years ago, laying the groundwork for the development of DNA vaccines. These experiments have led to the development of DNA-based immunisation as a viable weapon for combating a variety of critical challenges to human and animal health, such as infectious illnesses, cancer, and allergies. The advantages of this technology over existing methods include safety (the plasmids used are non-replicating in eukaryotic cells), the ability to stimulate potent cellular immune responses (due to MHC I-mediated presentation of the antigen produced by the transfected cells), rapid adaptation to antigenic variants (by simple cloning techniques), simple production systems (amplification and purification in Escherichia coli is uncomplicated and relatively cheap), and rapid adaptation to antigenic variant (due to the high stability of DNA).

DNA vaccines are typically made up of DNA plasmids that express antigens after being transferred into a vaccinee. They facilitate the endogenous production of a foreign protein, including its natural structure and post-translational modifications. Endogenous expression appears to be advantageous for the generation of neutralising antibodies and a balanced cellular immune response, therefore this is critical. In this context, DNA immunisation has been demonstrated to generate robust Th1-mediated cellular immune responses, as opposed to conventional approaches like inactivated pathogens or recombinant subunit vaccination [5]. Such benefits are widely acknowledged, as evidenced by the fact that some DNA vaccines have already been approved for use in the veterinary sector. DNA immunisation has been demonstrated to generate powerful priming immune

responses in combination with other vaccine approaches as booster vaccinations, such as viral vectors, recombinant proteins, or virus-like particles, in addition to serving as a vaccination platform on its own.

Until date, DNA vaccines had only been approved for use in the veterinary industry, with one application as an immune therapy for melanoma in dogs (Oncept), a vaccine for rhabdovirus disease prevention in fish (Apex-IHN), and a West Nile virus (WNV) vaccine for horses (West-Nile-Innovator). The fourth DNA plasmid licenced is not a vaccination; instead, it encodes the growth hormone releasing factor for breeding sows and is licenced for the food production business, resulting in more alive piglets in their litters and higher piglet weight. Mice, birds, and horses have all been given the WNV-DNA vaccine. The vaccine generated significant protective immunological responses in mice evaluated by intraperitoneal and mosquito exposure after a single application of 100 g or even 0.1 g DNA using a DNA electroporation (EP) equipment from Genetronic Inc. (now: Inovio Inc.). Horses, on the other hand, were only inoculated by injecting 1 mg DNA in 1 mL phosphate buffered saline without EP, as horses appear to be intolerant of electric pulses. The lack of an uptake boost could be the cause of the horses' poor immune responses after DNA immunisation. Different formulations and delivery methods were used to test the same WNV DNA vaccine in a range of bird species. However, the first genetic DNA vaccine on the market was for the rainbow trout's infectious hematopoietic necrosis virus (IHNV). Sockeye salmon with a mean weight of 150 g were injected with 25 g of naked DNA in a later DNA vaccination research, resulting in high neutralising antibody titers. Rainbow fish with a mean weight of 2 g were inoculated intramuscularly with 1, 5, or 10 g DNA vaccines, resulting in nearly full survival after IHNV challenge in all vaccinated groups. In 2010, a canine immunotherapeutic DNA vaccine was approved for the treatment of malignant melanoma. Antibody responses were effective, and the administration resulted in longer survival times. The DNA was delivered intramuscularly via needle-free injection (Biojector 2000) over the course of four vaccinations spaced two weeks apart, with doses ranging from 100 to 1,500 g per dosage.

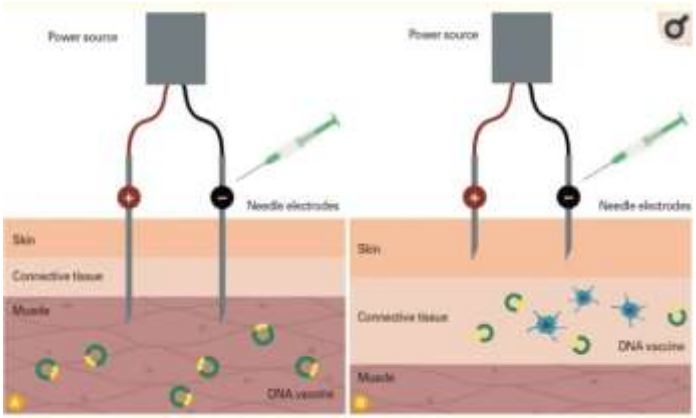
Despite these approved veterinary applications, DNA vaccine still has immunogenicity issues that have prohibited it from being used on a global scale, particularly in humans. Small rodent models yielded promising results, but not in larger species like nonhuman primates or humans. We will address approaches to overcome these limitations by enhancing the immunogenicity of DNA vaccines against infectious diseases in the following sections. Several of these approaches are currently being tested in human clinical trials to generate the first DNA vaccines.

Improving Immunogenicity of DNA Vaccines

Several research on DNA sensing by cytosolic proteins have been published in recent years, and our understanding of the innate immunity pathways induced by DNA recognition is improving. The activation of two primary types of proinflammatory pathways by the inflammatory signal triggered by cytosolic DNA recognition adjuvants the DNA vaccination itself. AIM2, IFI16, DDX41, and cGAS are some of the cytosolic DNA sensor molecules that have been discovered thus far. The interaction of these recognition molecules is managed by a critical important molecule called the stimulator of interferon gene, which transmits the signal to the innate immune response (STING). Understanding the mechanisms of DNA identification by sensor molecules and signal delivery to STING, which triggers an interferon (IFN) response, would almost certainly improve the use of DNA as a vaccine in the future.

However, in order to effectively trigger such innate immune responses while also ensuring optimal antigen expression, DNA must be properly transported into cells and deposited into nuclei. As a result, ineffective plasmid DNA transfer into mammalian cells and nuclei in vivo remains one of the key challenges in DNA

vaccinology. When small rodent models were compared to larger animal species, particularly non-human primates, a notable difference in immunogenicity after naked DNA transfer was discovered. Because numerous DNA research in non-human primates and the first human clinical trials were conducted without or with very minimal activation of immune response, this discrepancy was dubbed the "simian barrier." The reasons for the lack of repeatability of many mouse outcomes following their application in larger animals are still unknown. Variances in the ratio of applied DNA to body weight or differences in target cell DNA uptake could be possible explanations. To address these issues, a variety of solutions are being used (reviewed in Kutzler and Weiner). Gold particle bombardment was utilised in early research to improve plasmid DNA delivery efficiency. Since then, advanced physical DNA delivery technologies have become a hot topic in vaccine development. In vivo EP combines DNA injection with electric pulses into the injection site (Fig. 1), and various in vivo EP technologies have been investigated in recent years. In clinical trials, intramuscular and intradermal EP are the most used methods for delivering DNA vaccines. The in vivo uptake efficiency was studied using a variety of cell types. The majority of them only express the plasmids for a few days. Mature muscle cells, on the other hand, continue to express the plasmid-encoded protein for months. As a result, intramuscular DNA EP has shown to be the most effective delivery strategy to date. Intradermal DNA vaccination, on the other hand, results in immunogenicity, most likely due to the large abundance of antigen-presenting cells in the skin. Langerhans cells in the epidermis and dendritic cells in the dermis are examples of these cells.



The aforementioned bombardment via gold particles (gene gun), jet stream DNA injection (Biojector 2000), intradermal EP utilising various devices, plate applicator for transcutaneous EP, and DNA tattooing are all examples of physical DNA delivery systems. As a result, a range of different techniques for delivering DNA vaccines have been developed up till now. However, no side-by-side trials comparing the immunogenicity and efficacy of these various technologies have been conducted; in fact, most vaccine studies only compare one newly established device to a control group and not to other devices. As a result, we tested the immunogenicity of a DNA encoding the respiratory syncytial virus fusion protein administered by intramuscular EP, intradermal EP, DNA tattooing, or intramuscular injection without adjuvants in nonhuman primates. In this work, we found that vaccination via intramuscular and intradermal EP, as well as DNA tattooing, increased the humoral immune response to high levels. Only the intramuscular EP, on the other hand, elicited convincing systemic cellular responses to the vaccination antigen. Furthermore, the activation of mucosal T-cell responses was only polyfunctional in another group that received an adenoviral boost expressing the same antigen.

Mechanism of action of DNA vaccines

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DNA vaccine safety

In appropriate animal models, preclinical vaccine safety evaluation involves assessments of local reactogenicity and systemic toxicity, as well as histopathology. One of the first concerns about DNA vaccines was the risk of insertion mutagenesis integrating partial or whole plasmid sequences into the host genome, potentially inactivating tumour suppressor genes, activating oncogenes, or creating chromosomal instability (breaks and rearrangements). Fortunately, experimental results reveal that the rate of plasmid integration is minimal and lower than the spontaneous rate of mutation in mammalian genomes, putting these fears to rest. The long-term persistence of plasmid in tissues distal from the site of vaccine administration is uncommon after DNA injection. However, plasmids with modified backbones to improve gene expression or modified by a new delivery technique may increase the danger of integration and should be tested for plasmid DNA persistence before being used in clinical trials. Other concerns include the likelihood that DNA vaccinations may encourage the creation of anti-DNA antibodies, which have been linked to autoimmune diseases including systemic lupus erythematosus. DNA vaccinations increased the formation of anti-DNA autoantibodies in lupus-prone animals but did not cause autoimmunity in healthy animals, according to animal studies. Injecting mice with a plasmid expressing hepatitis B surface antigen (HBsAg) caused liver and kidney damage, which was explained by the creation of immunological complexes caused by the prolonged expression of HBsAg. Parker et al. found no evidence of pathogenic alterations in mice or rabbits following repeated DNA injections. The 2007 US FDA advice on DNA vaccines stated that sponsors are not needed to conduct preclinical studies to examine the effect on autoimmunity. Because their immune systems are still developing, babies who are exposed to foreign antigens may develop tolerance rather than protection. When DNA vaccinations were given to youngsters, however, immunity rather than tolerance developed. Due to the uniqueness of this method, despite initial concerns, significant recent evidence, including several clinical trials, supports the safety of DNA vaccines for routine human preventive and therapeutic usage.

Traditional adjuvants for DNA vaccines

Adjuvants have been used to boost the immunogenicity of conventional vaccinations for nearly a century. These adjuvants work by activating the innate immune system, forming antigen depots, inducing chemotaxis, increasing antigen absorption and presentation by professional APC, and upregulating co-stimulatory surface molecules on immune cells, among other things. Alum is the most extensively used vaccination adjuvant, and it works by causing cell death and subsequent release of host cell DNA, which acts as an endogenous innate immune signal (Marichal et al., 2011). In mice, guinea pigs, and nonhuman primates, adding alum to DNA vaccinations has been found to boost antibody responses (Ulmer et al., 1999). When a DNA vaccination against Toxoplasma gondii was combined with alum, it resulted in enhanced survival (Khosroshahi et al., 2012). Alum, on the other hand, activates the inflammasome and favours Th2-type immune responses (Awate et al., 2013), hence it may not be appropriate for DNA vaccines that require a cellular immune response. Plants and microorganisms, such as fungi and bacteria, produce polymeric carbohydrate molecules called polysaccharides. The delta-inulin polysaccharide adjuvant (AdvaxTM, Vaxine Pty Ltd, Adelaide, Australia) has shown promise as an adjuvant in traditional protein vaccines (Bielefeldt-Ohmann et al., 2014; Gordon et al., 2014; Honda-Okubo et al., 2014) (Petrovsky, 2011), and when given with an intramuscular or intranasal gp120 protein boost (Cristillo et al., 2011). Zymosan has also been utilised as a DNA vaccine adjuvant with effectiveness (Ara et al., 2001). Oil emulsions, such as MF59, are another typical adjuvant class. Oil emulsion adjuvants are hypothesised to work by triggering local inflammation and forming a tissue antigen store. When coupled with plasmids, injection of MF59 emulsion stimulates monocytes, neutrophils, and eosinophils, improving the immunogenicity of an HIV-1 DNA vaccine (O'Hagan et al., 2012). As a result, typical adjuvants may be useful in improving the efficiency of DNA vaccines that are otherwise not very immunogenic.

Liposomal and nanoparticle adjuvants

Liposomes are spherical vesicles made comprised of a lipid bilayer of phospholipids and cholesterol that can be utilised to transport antigens encoded by plasmids or classical antigens. By piercing the lipid bilayer of the cell membrane, liposomes entrap or bind plasmid DNA and enable DNA entry into cells (Karkada et al., 2010). DNA is also protected by liposomes against destruction by serum and cytosolic enzymes (Nakanishi and Noguchi, 2001). The incorporation of plasmids into liposomes has been proven to boost cellular and humoral immunity (Schwendener et al., 2010; Wang et al., 2007). This can be improved even further by employing scavenger or other receptors to deliver liposomes directly to APCs (Foged et al., 2004; van Broekhoven et al., 2004). Liposomes have the disadvantage of making intramuscular DNA injections more reactogenic, but they are particularly promising for mucosal immunisation. Mice inoculated orally with cationic liposome-encapsulated influenza vaccine demonstrated improved humoral and cellular immunity as well as influenza protection in a recent study (Liu et al., 2014b). Intranasal DNA vaccinations work similarly well with liposomes (Xu et al., 2014).

For vaccine distribution and adjuvants, nanoparticles composed of biodegradable and biocompatible synthetic polymers such as polyvinylpyridine, polylactide-co-glycolides (PLG), and polylactide-co-glycolide acid (PLGA) have been widely employed. Nanoparticles, like liposomes, shield plasmids against destruction and

improve cellular absorption (Xiang et al., 2010). A Treponema pallidum DNA vaccine made with chitosan nanoparticles, for example, demonstrated increased immune responses and protective effectiveness in a rabbit research (Zhao et al., 2011). In a mouse tumour model, a peptide-based gene delivery method termed MPG was found to increase Th1 cellular immune responses by forming stable non-covalent nanoparticles with DNA (Saleh et al., 2015). A multifunctional envelope-type nanoparticle modified with KALA, a peptide that forms a -helical structure at physiological pH, also produced strong cytotoxic T lymphocyte activity (Miura et al., 2015). As a result, liposomes and other polymer nanoparticles have a lot of potential as DNA vaccine adjuvants.

Conclusion

The low degree of antigen expression in DNA vaccines limits their immunogenicity in humans when compared to protein vaccinations. Various physical or molecular adjuvants can be used into DNA vaccine design to circumvent this. The number of immune genes that could be exploited as possible genetic adjuvants has expanded as a result of advances in related study areas such as genomics and systems biology. Additional tactics include optimising DNA construct design for maximum protein expression, directing expressed antigens to professional APC for efficient MHC-I and MHCII compartment loading, electroporation or other transfection tools, and DNA prime/protein or vector boost procedures. A successful human DNA vaccination is most likely to be part of a DNA prime/protein boost strategy, in which the DNA prime is used to assure efficient CD8 and CD4 T-cell priming and the protein boost is utilised to optimise antibody production. Not to be overlooked, RNA vaccines have advanced at a breakneck pace in recent years, with the promise to solve the problem of low antigen expression. The majority of the molecular adjuvants mentioned above in the context of DNA vaccinations are also applicable to RNA vaccines. Given the high number of studies being undertaken in this field, the first human DNA vaccines are anticipated to be in the domain of therapeutic vaccines against cancer, but infectious disease applications, such as HIV, also appear promising.

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