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DNA VACCINES: CHALLENGES AND APPROACHES

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ABSTRACT
Since the discovery of the first vaccine about 200 years ago, improvement in vaccine development approaches has occurred over the years. Most notably, the emergence of DNA vaccines. DNA vaccines can evoke both humoral and cell mediated immunity, they are safe and have several advantages over other vaccines types. Despite this, poor immunogenicity produced by DNA vaccines in humans has called for novel strategies. This review highlight ways to improve the efficacy of DNA vaccines through plasmid modification, delivery systems, prime boost and addition of adjuvants. The review also discusses the potential of DNA vaccine in pandemic settings such as that of corona virus disease 2019 (COVID-19).

Keywords: DNA vaccine, Plasmid design, Adjuvant, Delivery systems, COVID-19, Pandemic settings

INTRODUCTION

The development of vaccines is a major stepping stone in the elimination and control of infectious diseases. Since the discovery by Edward Jenner in 1798 that inoculation using pus taken from cowpox lesion could protect people from smallpox (Ramezanpour *et al*., 2016), researchers have developed numerous vaccines for the treatment of many infectious diseases.

The history of vaccine development has witnessed tremendous success over time. Around the 18th and 19th century there was global adoption of smallpox immunization culminating in its eradication (Plotkin and Plotkin, 2011). Also within this time, Louis Pasteur spear headed the development of a vaccine against cholera. The vaccine was made by attenuation of the bacterium responsible for chicken cholera. The idea of attenuation arose by chance, when he observed that accidental exposure of avian cholera culture to air for a prolong amount of time resulted in reduced virulence of the microbes (Bonanni and Santos, 2011). He further went on to apply the idea of attenuation on anthrax bacillus, a bacteria that cause plaque in cattle and 'fixed viruses' of rabies (Berche, 2012). Many vaccines made today are from attenuated pathogens. The beginning of the 20th century, saw the development of vaccines for common fatal infection like diphtheria, and by the middle of the 20th century, vaccine development entered the golden age. This was a period were viruses were grown in culture and live attenuated vaccines against measles, polio, mumps and rubella were developed (Tahamtan *et al*., 2017).

Despite these achievements, limitations in the production of some desired antigens and tendency of attenuated pathogens to revert to their virulent form, drove the need for other vaccine development strategies. Thus, in the late 20th century, use of bacterial polysaccharide-protein conjugate and genetic engineering in vaccine development emerged. Vaccines against *Streptococcus pneumonia, Haemophilus influenzae* type b and *Neisseria meningitides* were produced (Hasson *et al*., 2015). The bacterial polysaccharide-protein conjugate is able to elicit an immune response in the host against bacteria encapsulated by the polysaccharide contained within the vaccine. Vaccine against Hepatitis B virus was the first to be produced by genetic engineering for human use (Plotkin and Plotkin, 2011). Other vaccines were; those against the human papilloma virus (HPV) types causing cervical cancer and HPV types causing genital warts (Bonanni and Santos, 2011). Recently, the use of DNA vaccines for the treatment of infectious diseases of both bacterial and viral origin are been described (Hasson *et al*., 2015). Immune responses are elicited by injection of engineered DNA sequence of an infectious organism into a host cell, where it is transcribed and translated. Although, a lot is still required to be done, DNA vaccines have become an important approach in the prevention and therapy of several infectious diseases, non-infectious disease like cancer and autoimmune diseases, several of these vaccines are in human clinical trials (Khan, 2013). The objective of this review is to present an overview of the challenges associated with DNA vaccines and the approaches to address these challenges. The potential of DNA vaccines in outbreak situation are also discussed.

DNA VACCINES

The concept of DNA vaccines was introduced by Wolff and his colleagues in 1990. They demonstrated that injection of naked plasmid DNA into the muscle of mice induced immune responses (Wolff *et al*., 1990). Their findings opened new possibilities for the induction of immunity in vaccine development. Research by other scientists, Tang *et al.* (1992) showed that, injection of human growth hormone also induced immune responses in mice. However, the first preclinical protection provided by a DNA vaccine was demonstrated by Ulmer *et al.* (1993). Since then, improvement in DNA vaccine development approaches has occurred.

The principle of DNA vaccination involves the injection of plasmid DNA containing immunogen into a host were it elicit specific immune responses. The immunogen is transcribed and translated in the host without being integrated into the host gene. This differentiate DNA vaccines from gene therapy. The immune response generated is similar to those induced by the pathogen during natural infection. DNA

vaccines are able to elicit both humoral and cell mediated immunity, though the mechanism by which immune response is triggered is complex and poorly understood. Once expressed as a foreign protein, the antigen binds to major histocompatibility complex (MCH) Class I or Class II molecules and stimulates $CD8^+$ and $CD4^+$ T cells respectively (Saade and Petrovsky, 2012). The presentation of antigens by antigen presenting cells (APC) for induction of immune responses is suggested to occur by either of three mechanisms. The first involve the transfection of somatic cells. When somatic cells (myocytes and keratinocytes) are transfected through sub-cutaneous or intramuscular injection, cell proteasomes process the antigens generated and present them to T cells through MHC class I molecule. However, these cells require the support of other cells such as professional APCs for efficient presentation of the antigens. The second mechanism involves the transfection of professional APCs. The principal APC are dendritic cells obtained from bone marrow. Dendritic cells present endogenous antigens through MHC class I molecule to $CD8^+$ T cells. In the third mechanism, the DNA vaccine is phagocytized by APC after transfection into somatic cells in a process known as cross priming. Thus, triggering $CD4^+$ and $CD8^+$ T cells (Yurina, 2017).

Although no DNA vaccines have been approved for human use, several have undergone clinical trials. Example are DNA vaccines against influenza, HIV-1, malaria, hepatitis b, cancer and currently, DNA vaccines against corona virus disease 2019 (COVID-19). However, there are several approved DNA vaccines for veterinary use. They include vaccine for the treatment of canine melanoma, vaccine against West Nile virus in horses, growth hormone releasing hormone (GHRH) for pigs and vaccine against haematopoietic necrosis virus in salmon (Kutzler and Weiner, 2008).

Routes of administration

The amount of DNA vaccine required to elicit an immune response, the type of immune response, and success of vaccination is influenced by the route of administration. Although, the common route of administration is through intramuscular injection, several other delivery routes have been used. They include intradermal, intravenous, oral, intraperitoneal, intranasal, intravaginal, and subcutaneous and have been extensively reviewed (Rosa *et al*., 2015). A large number of immune cells are located in the skin making it a major target for vaccine administration.

CHALLENGES

The major challenges of DNA vaccines for use in human is their poor immunogenicity and cellular uptake of DNA (Figure 1). This stems from poor delivery efficiency and barriers (both extracellular and intracellular) that hinder DNA from getting to and been incorporated into the nuclei. The mode of delivery affects DNA vaccines efficacy as only a fraction of the DNA is taken up by the cells and expressed. For example, DNA vaccine delivered by intramuscular injection is susceptible to phagocytic and DNase degradation, and inactivation through nonspecific interaction with other protein. As a result, only a small amount of the injected DNA is present at the site of injection (Lim *et al*., 2020). For oral delivery, the injected DNA vaccine must be protected from degradation by the harsh and variable environment of the gastrointestinal tract. DNA vaccines administered through intradermal and intraperitoneal is susceptible to degradation by ubiquitous nucleases. Therefore, the DNA vaccine vector must also have a method by which it can target specific cell types. Regardless of the route of administration, DNA vaccine must gain entry into the cytoplasm of cells (Li and Petrovsky, 2016). Following internalization and possible entrapment in the endosomal compartment, the naked DNA become susceptible to endonuclease degradation and very few escape into the nucleus. To achieve a successful immunization, DNA vaccines must be protected from degradation, uptake enhanced and immune response activated.

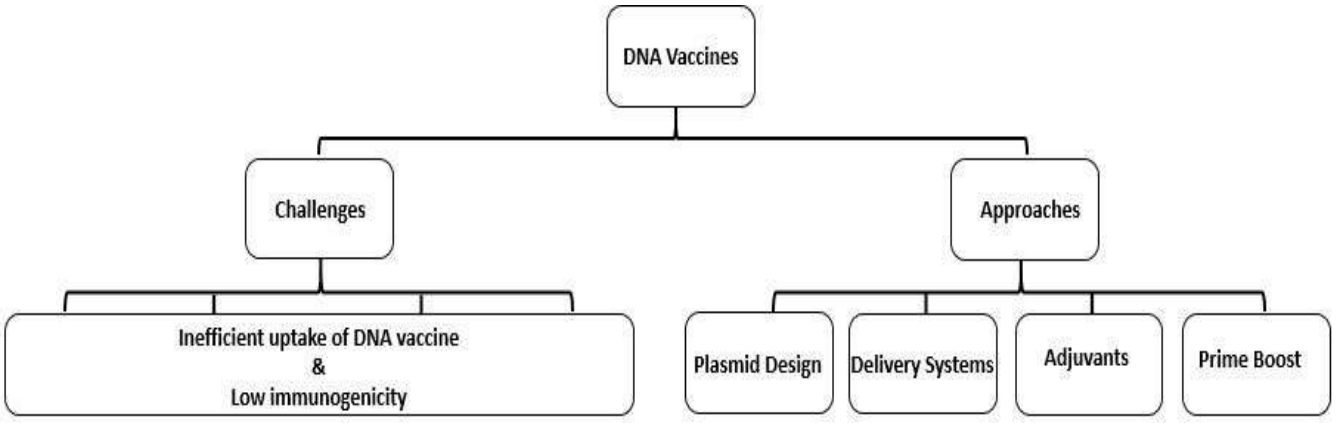

Figure 1: Challenges and approaches to improving DNA vaccine efficacy

APPROACHES

Strategies to tackle the challenges associated with DNA vaccines is currently on going to optimize plasmid design, delivery methods, adjuvants and prime boost in order to enhance antigen expression levels, immunogenicity and efficacy.

Plasmid Design

Once an immunogen/antigen gene for a particular pathogen is known, the next step is the design or construction of a DNA

vaccine plasmid. A typical DNA vaccine consist of plasmid containing genes that encodes one or more protein of a pathogen, an origin of replication so that it can replicate in a bacteria host, a strong promoter and an antibiotic resistance gene as selection marker as shown in Figure 2. Although antibiotic resistant genes like kanamycin in plasmid DNA vectors is important for stable plasmid uptake during bacterial growth they pose a serious health challenge, in that resistance to the antibiotic may be transferred to the host microbial flora. Initially, human oncogene viruses such as simian virus 40 (SV40) were used as promoters, however, other strong promoters like human cytomegalovirus (CMV) a non-carcinogen are also been used. These sequences control antigen expression in target tissues. CMV has the advantage that it allows high level transgene expression and do not suppress downstream read-through (Saade and Petrovsky, 2012). The use of non-viral promoter such as major histocompatibility class II (MHC II) promoter in addition to other viral promoter are been considered (Kutzler and Weiner, 2008). Codon optimization is also important as it increases gene expression by ensuring the translation and transcription of the antigenic protein. For example, the Kozak consensus sequence can be added and is recognized by mammalian ribosomes. It directs the efficient translation of the transgenes (Williams, 2013). In addition, immune response of certain DNA vaccine can be improved by co administering plasmid that encodes the antigenic protein with cytokines or chemokines since they enhance immune responses (Moss, 2009). It should be noted that the choice of vector used in DNA vaccine is dependent on the kind of immune response required.

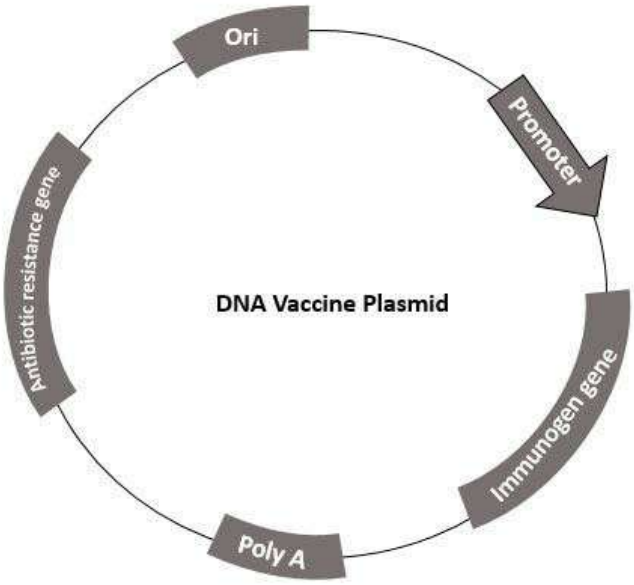

Figure 2: Schematic representation of a typical DNA vaccine plasmid.

In recent times, small bacterial RNA-based antibiotic free selection markers have been developed to address the problems associated with antibiotic resistant gene. Since these vectors contain noncoding RNA marker, they are not expressed in host cells after vector transfection or horizontally transmitted to host bacteria. In addition, the small size of these vectors also allow higher transfection efficiency which may be due to resistance to associated shear force among other factors (Suschak *et al*., 2017).

Delivery methods

Ability of DNA vaccine to elicit strong immune response in humans and other animals is also affected by delivery methods. To ensure optimal expression of the antigenic protein, the DNA must be delivered in cell and incorporated into the nuclei. Earlier methods in DNA vaccination using needles deposited DNA vaccines in cellular spaces instead of within cells, thus, leading to poor immune responses. Physical methods like particle bombardment using gene gun were then approached. Here, the DNA of interest is directly shot into the target cell using a gene gun unlike in intramuscular injection. Other promising physical approaches are electroporation and the use of ultrasound. In electroporation, transient pores are created in skin using electrical impulses. This allows the entry of DNA into the cells. Studies have shown that use of electroporation enhanced antigen production and DNA vaccine potency (Otten *et al*., 2004; Hirao *et al*., 2008). However, the challenge with electroporation is that the high voltage can result in physical pain and cell death (Lim *et al*., 2020). In ultrasound, transient disruption of cell membranes by ultrasonic energy allow entry of DNA into the cells (Saroja *et al*., 2011).

Currently, the use of needle free approach as a method for DNA vaccine delivery is gaining increasing popularity. Needle free approach allow effective mass immunization, prevent accidental transmission of diseases from needle sticks and create new ways for delivering DNA vaccines and other biopharmaceuticals that cannot be delivered orally. DNA vaccines are delivered into host skin or muscles tissues using jet injectors. This approach have shown great promise in human and animal clinical trials (Aguiar *et al*., 2001; Ledgerwood *et al*., 2012).

Addition of adjuvant

The use of effective adjuvant play a critical role in vaccine formulation. Adjuvants are compounds that when co administered with antigen can enhance or modulate DNA vaccine- induced immune responses. The delivery of DNA vaccine plasmid into host cell and their incorporation into host nuclei is hindered by barrier such as phospholipid cellular membrane, premature degradation by endosomes, lysosomes and even by cytosolic nucleases (Suschak *et al*.,

2017). Introduction of adjuvant enhance DNA escape from degradation and allow nuclear entry of DNA. Adjuvants can increase immune responses by detecting foreign signals, augmenting antigen-immune accessibility and immune stimulation (Kaurav *et al*., 2018). Traditional adjuvants include aluminum based minerals, oil emulsions and bacterial components polysaccharide. These adjuvants acts as delivery systems and/or immune stimulators (Saade and Petrovsky, 2012).

Over the past decades, the discovery of new antigens have called for improvement in vaccine formulation and the need for new generation of adjuvants that elicit specific immune responses. Liposomes, microparticles including DNA plasmid encoding immunostimulatory proteins like cytokines and chemokines have been employed and they show great potential as adjuvants in enhancing immune responses (Aiyer-Harini *et al*., 2013). Liposomes are spherical vesicles composed of phospholipids and cholesterol, forming a lipid bilayer. Liposomes not only serve as adjuvant they also act as delivery vehicle. The DNA plasmid is either encapsulated or bound to the surface of liposomes. Cationic liposomes can condense DNA construct thereby allowing them to enter into cell. They also allow DNA to escape degradation by bypassing the endosomal-lysomal route in cell (Wallis *et al*., 2019). Microparticles like nanoparticles are biodegradable polymers that can serve as adjuvants/ delivery systems. Microparticles can enhance DNA vaccine immunogenicity by protecting DNA plasmid from premature degradation, ensure efficient uptake of the antigen by APCs including enhancing antigen specific immune responses. The plasmid DNA is protected by been entrapped in the nanoparticle DNA plasmid encoding immunostimulatory cytokines like interleukin (IL)-2, IL-10, IL-12 and chemokines are used as adjuvants among others. When expressed, the cytokines act as sites for antigen expression. They provide longer immune stimulation since the cytokine is expressed at the same duration as the antigen. Adjuvants stimulates immune responses by binding to Toll-like receptors.

Prime Boost

Another approach to improve immune response is by using DNA vaccines as priming vaccines. DNA is used to prime certain antigen specific immune responses such as CD_4^+ and CD_8^+ T cells which are then boost with a recombinant protein or viral vector which codes for the same antigen (Moss, 2009). Since different types of immune responses are elicited, the immune response is generally improved. Several studies using the prime boost approach have been carried out. For example, Leong *et al*. (1994) observed an increase in immunogenicity in mice primed with DNA vaccines and later boosted with a recombinant viral vector encoding the same antigen, compared to mice immunize with each vaccine alone. In another study performed by Catanzaro *et al*. (2007), priming with DNA vaccine encoding HIV antigen followed by a boost with adenoviral vector encoding the same antigen showed an increase in T-cell responses unlike those administered with DNA vaccine or adenoviral vector alone. This approach has also been applied in the development of vaccines against CMV, tuberculosis, malaria among others (Nascimento and Leite, 2012; Bolhassani and Yazdi, 2009)

POTENTIAL OF DNA VACCINES IN PANDEMIC SETTINGS

The world has experienced a number of pandemic threats, epidemic diseases that have spread over a wide region causing severe illness and hundreds of millions of deaths. The number of new diseases have increased over the past few decades. Since the millennium pandemic threats like influenza (flu), severe acute respiratory syndrome (SARS), Middle East respiratory syndrome (MERS), ebola virus disease, dengue fever, zika virus disease and most recently the COVID-19 pandemic have occurred.

COVID-19 is an emerging infectious disease and an acute respiratory infection that have threatened global population. COVID-19 is caused by a novel corona virus known as severe acute respiratory syndrome corona virus 2 (SARS-CoV-2) that belong to a family of single stranded RNA viruses. COVID-19 outbreak occurred in Wuhan, China in mid-December, 2019 and have spread to several countries around the world. Although bats are considered as natural reservoir host, further confirmation is required to ascertain if infection with SARS-CoV-2 is transmitted directly from bats or through intermediate host (Wu *et al*., 2020). The pathogen is also endemic in many animal species and have the tendencies to jump from animals to humans. The route of transmission of COVID-19 is majorly through human-human interaction involving direct contact or transmission through respiratory droplets. It is also suggested that they may be a possible faecal to oral transmission. The virus enter human cells by binding to angiotensin-converting enzyme 2 (ACE2) receptors found in human respiratory airways (Silveira *et al*., 2021). Symptoms in majority of the cases are fever, dry cough and fatigue. For many viruses and emerging pathogens, monitoring their transmission and evolution go a long way in helping to provide protection against the disease. One of such means is through genome sequencing which have allowed rapid sequencing of the first SAR-CoV-2 virus (Shang *et al*., 2020).

A great deal of effort has been made in the development of vaccine against the SAR-CoV-2 virus. However, development of conventional vaccine using attenuated pathogens may not be suitable in pandemic settings. Convectional vaccine take longer time to produce and there may be possible risk of reversion. In addition, cultivation of the whole viral pathogen limits production as it may require high biosafety level and highly specialized labs (Rauch *et al*., 2018). One appealing vaccine strategy that can tackle challenges of outbreak situations is the use DNA vaccines. DNA vaccine is safe, can be rapidly produce and stored on a large scale.

Several DNA vaccines candidates against COVID-19 are currently been investigated but only a few have reached clinical trials. For example, Inovio pharmaceuticals developed an electroporated DNA vaccine (INO-4800) encoding SARS-CoV-2 spike (S) protein which is currently in phase 1 clinical trials. Immunization of mice and guinea pigs with the vaccine showed the induction of both cellular and humoral immune responses, the antibodies generated neutralize the viral infection and prevented the binding of S protein to ACE2 receptors (Smith *et al*., 2020). Another DNA vaccine candidate in clinical trial is the AG0301-COVID19 developed by AnGes Inc. and Osaka University. The vaccine candidate, like other DNA vaccines against COVID-19, target the S protein. Dosage is achieved using a two immunization scheme, first with a low dose and then a high dose within a two week interval and it's administered intramuscularly (Silveira *et al*., 2021). Although no DNA vaccine against COVID-19 have been authorized for use at the moment, tremendous progress has been made in the development of another type of nucleic acid vaccine, mRNA vaccines. This is a novel and alternative approach that deliver

genetic information to produce antigen in the host, thus inducing an immune response. The mRNA vaccine encodes the S protein of SARS-CoV-2, and it's encapsulated in a lipid nanoparticle that serve as delivery vehicle. Following intramuscular injection and successful uptake of the vaccine by the cell of an individual, the mRNA is translated into the S protein (antigen). The immune system of the individual recognizes the antigen and induce immune response by triggering both cellular and humoral immunity (Anand and Stahel, 2021). Some of the mRNA vaccines currently approved for emergency use authorization include BNT162b2 produced by Pfizer-BioNTech and mRNA-1273 produced by Moderna.

Nucleic acid vaccines have shown great potential in meeting the challenges of emerging pandemics. However, there are some limitations and safety concern. The ability of COVID-19 to spread rapidly around the world along with the emergence of new SARS-CoV-2 variants could potentially limit vaccine efficacy. In addition, there are concerns that antibody dependent enhancement (ADE) or enhanced respiratory disease (ERD) may occur. In ADE, the binding of a virus to antibody generated in response to the viral entry can lead to enhancement of viral replication. Thus, immune response is exacerbated and symptoms could become more severe. However, there are no evidences to show that COVID-19 vaccines can cause ADE in humans as of this moment (Bettini and Locci, 2021)

CONCLUSION

Vaccines have played vital role in the control and elimination of infectious diseases. However, development of DNA vaccines against certain disease that affect humans is slow. Efforts are currently being made in developing approaches to tackle the limitations and challenges faced in DNA vaccine uptake and immunogenicity. Nucleic acid vaccines are an appealing strategy in outbreak situation since they can be produce earlier while convectional vaccines are stilled being manufactured. It is expected that, in the nearest future, DNA vaccines against currently difficult pathogens will become readily available.

CONFLICT OF INTEREST

No conflict of interest

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