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NUCLEIC ACID VACCINES FOR PREVENTION AND TREATMENT OF INFECTIOUS DISEASES: CURRENT APPLICATIONS

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***Abstract*— In terms of therapeutic applications, nucleic acid vaccines offer tremendous potential in the face of the difficulties brought on by infectious diseases. Nucleic acid vaccines differ from conventional immunizations in that they are both very effective and inexpensive. As a result, nucleic acid vaccinations may be beneficial for both the prevention and treatment of disease. However, the development of nucleic acid vaccines has been constrained by their low immunogenicity and instability. In order to enhance their immunogenicity and stability through better delivery techniques, numerous studies have been carried out, advancing research and development for clinical applications. The main focus of this article is a review of nucleic acid vaccines, including their benefits and drawbacks as well as their workings and methods of administration.**

***Keywords*— Nucleic acid; Vaccines; Therapeutics; Immunogenicity; Infectious disease.**

I. INTRODUCTION

Since the creation of the first vaccines more than 200 years ago, vaccinations have significantly reduced the morbidity and mortality brought on by infectious diseases in sizable human populations (Rappuoli et al., 2011; Koff et al., 2013). Additionally, in clinical practice, vaccines can be either preventative or therapeutic and can be generally categorized as toxoid vaccines (inactivated bacterial toxins), inactivated vaccines (killed microbes), live attenuated vaccines (weakened microbes), and subunit vaccines (purified antigens) (Wadhwa et al., 2020). Conventional vaccinations have significantly lowered the burden of many infectious diseases to date. For instance, they successfully eradicated smallpox and significantly decreased the prevalence of tetanus, polio, diphtheria, and measles globally (Younger et al., 2016). Despite these successes, there are still restrictions and possible issues with the traditional approaches. This approach is unfavorable for highly pathogenic viruses since there is a very low chance that attenuated antigens would revert to full potency. Additionally, live attenuated vaccines can only produce the necessary protective immunity to ward off evident illness symptoms in the host
animal under carefully regulated and described settings. Due to limitations in the way of presentation for inactivated vaccines, the immune response is only moderate and must be boosted with adjuvants or immunostimulants.
Additionally, the manufacture of live attenuated vaccines and inactivated vaccines may be difficult because to the need for strict biosafety standards and cultivation-specific laboratories. It can successfully prevent the addition of unwanted "foreign" protein from the culture medium, such as eggs, tissue culture, or simply culture medium, which may affect immunogenicity or be potentially allergic or reactogenic. Subunit vaccines and recombinant protein-based vaccines are often utilized in combination with adjuvants or delivery systems to induce a protective effect due to the insufficient immunogenicity of the protein antigen alone. Additionally, the ongoing introduction of novel diseases and the re-emergence of well-known pathogens necessitate the rapid development of safe and effective vaccines, which is why researchers must create new vaccines in this way. In order to battle infectious diseases and tumors, scientists have discovered that nucleic acid vaccines are developing into a reliable and adaptable scientific technique. Vaccines made from nucleic acids have the potential to be cost-effective, have a safety margin, and are efficient. Additionally, the immune responses brought on by nucleic acid vaccinations solely focus on the chosen pathogen antigen. Vaccines based on nucleic acids, such as DNA (as plasmids) and RNA [as messenger RNA (mRNA)], show remarkable potential for addressing a variety of indications and diseases. Additionally, cancer vaccines offer an alluring method that can trigger targeted and long-lasting immune responses against tumor antigens. Bacterial plasmids that encode antigens and immunostimulatory molecules are the basis for DNA vaccines (i.e., IL-2 and GM-CSF). In the 1990s, plasmid DNA encoding the influenza A nucleoprotein caused a protective and targeted cytotoxic T lymphocyte (CTL) response, which was the first instance of DNA vaccine-

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mediated immunity (Yankauckas et al., 1993). Additionally, a number of animal models have effectively shown how DNA vaccines can be used to prevent or treat cancer, infectious illnesses, allergies, and autoimmune disorder (Wolff, 1990; Ulmer et al., 1993; Fuller et al., 1994; Donnelly et al., 1996; Wang et al., 2008). Similar to this, in vitro transcribed (IVT) mRNA was first successfully used in animals in 1990. At that time, mice were given the gene encoding the mRNA sequence, and researchers later discovered the protein that had been generated. We first give a general summary of what is currently known about nucleic acid vaccines in this paper. The delivery and mechanism of action of nucleic acid vaccines were our next areas of emphasis. Finally, we elaborated on the possibilities and required advancements for the therapeutic applications of nucleic acid vaccines (Wolff, 1990).

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II. DNA VACCINES

DNA vaccines are created by introducing an antigen-coding gene into a plasmid obtained from bacteria. This process requires a strong promoter, which is typically the CMV-promoter (Leitner et al., 2001). By employing the prokaryotic origin of replication, DNA plasmids are replicated in bacteria that can be chosen based on antibiotic resistance via genes expressing resistance markers. Additionally, DNA vaccines can influence cellular immunity as well as humoral immunity. We have a good understanding of the functions of immune cells in the processing, presentation, and recognition of antigens, despite the fact that the precise mechanisms underlying the induction of an immune response to antigens expressed by host cells after DNA immunization have not yet been identified.

2.1. Mechanisms of Action

DNA can be administered in a number of different ways, including intramuscular (IM), intradermal (ID), mucosal, and transdermal administration. DNA vaccines can stimulate both humoral and cellular immune responses. After internalization for DNA vaccines, the DNA is sent to the nucleus for transcription before being translated into the cytoplasm (Bai et al., 2017).

The following three potential pathways for antigen presentation are put forth: (1) After internalization, somatic cells (such as myocytes) produce plasmid DNA, which is then presented to CD8+ T lymphocytes by the MHC class I complexes on the somatic cells; (2) antigen presentation is dependent on specialized antigen-presenting cells (APCs), such as dendritic cells (DCs), which are transfected by plasmid DNA at the injection site and subsequently present the produced antigens to T cells via MHC class I and II complexes; and (3) Professional APCs phagocytose somatic cells that have been plasmid-transfected, resulting in cross-priming and the presentation of antigens to both CD4+ and CD8+ T cells. Cross-presentation is likely the main pathway, possibly through the processing of apoptotic cell debris, at muscle locations because the presentation of antigens through MHC class II is required to trigger CD4+ helper T cells (Matthew et al., 1998). A gene gun can transfer DNA directly into Langerhans cells even in skin areas, which permit the direct presentation of antigens; cross-presentation from keratinocytes is likely the primary pathway (Stoitzner et al., 2006).

III. RNA VACCINES

There are now two types of mRNA vaccines that are commonly accepted, namely non-amplifying mRNA and self-amplifying mRNA, which are separated based on their different processes. Non-amplifying mRNA vaccines primarily have five structural components that are essential for the life cycle and expression: the "cap" [m^7Gp_3N(N: any nucleotide)], this is m7G (7-methyl-guanosine) residue bound to RNA transcript in the 5^{prime} end by a triphosphate bond (5^1–$5^{1)}$ with any of the nucleotide; a 5^I untranslated region (5^1UTR) which located immediately before the translation initiation codon; an (ORF) open reading frame which encodes the gene of interest (GOI); a (3^1 UTR) 3^1 untranslated region; and the [poly(A) tail] 100–250 adenosine residues tail (Banerjee, 1980; Wickens, 1990; Dominski and Marzluff, 1999). The cap structure, among these components, is crucial for protecting mRNA from exonucleolytic decay and facilitating translation (Ryner and Baker, 1991; Parker and Song, 2004; Yamashita et al., 2005). While the translational machinery recognizes the untranslated regions (UTRs), the poly(A) tail's specifics also influence mRNA stability and translation (ribosome) (Chang et al., 1990; Ryner and Baker, 1991; Zhong et al., 2018). Self-amplifying mRNA was created to increase the length and intensity of GOI expression in contrast to replication-deficient mRNA constructs. A self-amplifying RNA not only encodes antigens but also has a sequence resembling that of a replication-competent virus, allowing it to reproduce in cells and boost protein expression. This is in addition to the five required components described above. An -virus self-amplifying RNA, for instance, has non-structural genes (nsP1-4), a subgenomic promoter, and a changeable GOI that swaps out the coding sequence for the viral structural proteins.

3.1. Mechanisms of Action

Antigen-encoding mRNA can be successfully delivered to APCs directly via RNA vaccines in vivo. Effective methods of delivering antigen-encoding mRNAs into APCs, such as using nanocarriers, allow the mRNAs to be released and translated into corresponding antigenic proteins in the cytoplasm. After that, they undergo peptide epitope processing before being

coupled with the MHC class I through a cross-presentation pathway. In this section, the transfer of MHC-peptide complexes to the cell surface of APCs results in the activation of CD8+ T lymphocytes, which triggers an appropriate immunological response.

Both native mRNA and IVT mRNA have pharmacological activity in the cytoplasm. While IVT mRNA reaches the cytoplasm from external sources, native mRNA is translated from DNA in the nucleus and crosses the nuclear membrane (Miliotou and Papadopoulou, 2020). IVT mRNA will adhere to the same mechanisms that control the stability and translation of endogenous mRNA once it has been transported to the cytoplasm (Wadhwa et al., 2020). As a result, mature protein products that contain antigens are probably going to trigger cellular and humoral immune reactions that are particular to the pathogen (Maruggi et al., 2019).

IV. NUCLEIC ACID VACCINE DELIVERY

Therapeutic goals must be met in order to effectively deliver DNA or mRNA in vivo. The nucleus or cytoplasm, where transcription or protein expression, respectively, can occur, must be penetrated by nucleic acid vaccines.

As was already established, cross-membrane barriers cause limited immunogenicity, which is the biggest problem with nucleic acid vaccines. In order to improve delivery, DNA vaccines must break through the nuclear membrane barrier, while mRNA vaccines must cross the lipid-based plasma membrane as quickly as feasible. As a result, numerous techniques to improve cell transport and immunogenicity have been created.

4.1. Chemical and Physical Delivery Methods

Nucleic acid vaccines can be administered in a variety of ways, including intramuscular (IM), intradermal (ID), mucosal, and transdermal administration, just like traditional protein-based vaccines. Delivery by needle injection accounts for why nucleic acid vaccines fail to stimulate a robust immune response in people. At the moment, physical delivery techniques like a gene gun or intradermal electroporation may ease transfer and improve immunogenicity (Low et al., 2009; Dupuy et al., 2011; Bagarazzi et al., 2012; MacDonald, 2015; Grant-Klein et al., 2012; Grant-Klein et al., 2015). According to earlier research, using a gene gun or in vivo electroporation to transport mRNA can cause substantial immune responses in mice because it causes an increase in mRNA release into the cytoplasm when non-amplifying or self-amplifying mRNA is used as the delivery method. (Qiu et al., 1996; Aberle et al., 2005; Steitz et al., 2006; Kreft and Jetz, 2007; Johansson et al., 2012). Additionally, it's been noted that electroporation-enhanced DNA vaccination increases the quantity of polyfunctional CD8+ T cells in individuals who have received HPV DNA vaccines expressing the E6 and E7 genes of HPV16 and HPV18, respectively (van Voorhis et al., 2013).

Chemical delivery techniques can considerably increase the efficacy of nucleic acid vaccinations (i.e., nanocarriers). At the moment, the components of nanocarriers used in nucleic acid vaccines can be divided into lipid-based nanosystems (Li and Szoka, 2007; Han et al., 2008; Tseng et al., 2009; Gomes-da-Silva et al., 2012; Sato et al., 2019), polymeric nanomaterials (Tanner et al., 2011; Shim and Kwon, 2012), bioinspired nanovehicles (Li et al., 2017), and inorganic nanoparticles (Lin et al., 2016; Shen et al., 2018; Lin et al., 2020). Nanotechnology offers a diverse and focused technique for the effective and secure delivery of nucleic acid vaccines because of the increased permeability and retention (EPR) effect (Pecot et al., 2011; Hrkach et al., 2012; Zhong et al., 2018).

As a result, nanosystems not only shield DNA or mRNA from immune reactions and enzyme-mediated destruction but also encourage RNA accumulation in the tumor site (Pecot et al., 2011; Zhong et al., 2018), which aids in the prolonged release of vaccines that have been administered (Basarkar et al., 2007; García et al., 2009). The chemical delivery strategy offers nucleic acid vaccines a new direction in comparison to the physical delivery method. There have also been reports of other DNA formulations, including the integration of cationic lipids or cholesterol (Donnelly et al., 1996; Donnelly et al., 2005), absorption and presentation by expert APCs, and stimulation of the production of costimulatory surface molecules. For instance, alum, used as a universal adjuvant in vaccines since 1926, induces phagocytic cell death, which aids in the production of an immunological danger signal.

It has been suggested that using an alum adjuvant with a Toxoplasma gondii DNA vaccination can increase the survival rate of mice. Additionally, a variety of cytokine genes, PRR ligands, and immunostimulatory molecules encoded by vaccine plasmids all make use of recombinant DNA technology, enabling them to be administered alongside an antigenic DNA vaccine plasmid to specific cellular compartments or APCs to boost the immune response (Li and Szoka, 2007; Li et al., 2017). Exogenous RNA derived from viruses and synthetic double-stranded RNA were initially utilized as RNA adjuvants for mRNA vaccines, but serious adverse effects eventually prevented their further use (Field et al., 1967; Absher and Stinebring, 1969). According to studies, IVT mRNA can be stabilized through chemical or compounded synthesis and utilized as an adjuvant (Scheel et al., 2004). Additionally, RNA sensor receptors are efficient targets for adjuvants. They have developed innate and adaptive immune systems in concert to recognize and fend off viral infections (Sadler and Williams 2008). TLRs 7/8 and 3 in the endosome, respectively, identify single- and double-stranded RNA molecules (Alexopoulou et al., 2001; Diebold et al., 2004; Heil et al., 2008). TLR3 is still triggered and transcribed by mRNA secreted by cells or synthesized in vitro in addition to being activated by double-stranded RNA (Gauzzi et al., 2010). Therefore, a crucial adjuvant signal in initiating an immune response is the activation of TLR7 and maybe TLR3.

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V. USE OF NUCLEIC ACID VACCINES IN INFECTIOUS DISEASES

A number of DNA vaccines have been created and are currently in the clinical trial stage in response to a wide range of infectious diseases that harm humans. However, the majority of DNA vaccines that are formally certified for use on the market are intended for use in treating animals because some serious flaws have not been fixed. For instance, it has been claimed that vaccinating horses and dogs with the canarypox vaccine can effectively treat West Nile virus illness (Grosenbaugh et al., 2004; Karaca et al., 2005; Rau et al., 2006; Grant-Klein et al., 2012). However, no DNA vaccines have yet been authorized for use in human preventive medicine. In the first phase of I clinical study of DNA vaccines in humans, volunteers with and without HIV-1 infection participated in the testing of an HIV-1 vaccine candidate. This study found that the HIV env and rev genes encoded in the DNA vaccine were well tolerated during vaccinations and that no adverse effects or anti-DNA antibodies were seen. Further evidence that this DNA vaccination against HIV-1 was efficacious came from measurements of antibody-GMTs against gp120, CTL response and T lymphocyte proliferation in both HIV-1 infected and non-infected individuals (MacGregor et al., 1998). Since that time, other organizations have carried out clinical studies of further preventive and therapeutic DNA vaccines, including DNA vaccine trials for influenza, malaria, hepatitis B, and different forms of HIV-1 candidate viruses. These studies have shown that the DNA vaccine platform is safe and well-tolerated, however, the first-generation DNA vaccines did not successfully produce a significant amount of vaccination-specific immunity in humans. The development of DNA vaccines against a variety of uncontrollable viral pathogens, such as HIV, West Nile virus, and hepatitis C virus, as well as DNA vaccines capable of treating bacterial and protozoan diseases, such as tuberculosis and brucellosis, is a current research priority (i.e., leishmaniasis, malaria, and toxoplasmosis) (Chang et al., 1990; Ryner and Baker, 1991; Grosenbaugh et al., 2004). This article primarily distinguishes between three forms of DNA vaccines for the treatment of infectious diseases and reports representative clinical trials to show their immunogenicity and safety.

VI. SAFETY MEASURES OF NUCLEIC ACID VACCINES IN CLINICAL TRIALS

The stable integration of transfected DNA into the genome of somatic or germinal cells, which may result in dysregulation of gene expression and gene mutation, has long raised concerns regarding the safety of DNA vaccines. Adverse reactions to the DNA vaccine are restricted to local reactions at the injection site, and its general safety has been amply proven in a number of clinical trials (Fioretti et al., 2014). Therefore, proving the efficacy of DNA vaccines has become the primary research focus for clinical trials.

Although studies on humans have shown that DNA vaccines can successfully elicit cellular and humoral responses, the potency of these responses is typically insufficient to result in appreciable clinical effects. Additionally, DNA vaccines still need to be enhanced in terms of eliciting efficient antigen-specific cellular immune responses because of tumor immunological resistance to endogenous autoantigens. Therefore, the creation of a technique to get around immune tolerance is necessary for the development of DNA vaccines. DNA vaccines can also be combined with other cancer therapies to intensify the fight against malignancies (Yankauckas et al., 1993; Yang et al., 2014).

The production risk of mRNA is substantially lower than that of other vaccine platforms since it involves no harmful chemicals and is produced in an environment free of contamination by foreign viruses (i.e., live virus, viral vector, subunit protein vaccine, and inactivated virus). Contrary to DNA vaccination, there is no theoretical risk of infection or integration of the vector into host cells after vaccination (Dugger et al., 2018). mRNA vaccines are, in essence, a relatively secure type. From phase I to phase IIb, various distinct mRNA vaccines have been the subject of clinical trials, and the outcomes have demonstrated the safety and tolerability of these vaccines. But recent human studies have demonstrated that various mRNA platforms exhibit varying degrees of unfavorable reactions at the injection site or all over the body following vaccination (García et al., 2009) . Some platforms for mRNA vaccines can lead to strong type I interferon responses, which may be connected to inflammation and autoimmune disease (Abd El-Aziz and Stockand, 2020). Extracellular RNA, which can make endothelial cells more permeable and cause edema, is another safety concern (Fischer et al., 2007; Fioretti et al., 2014). Therefore, RNA's ability to transmit and transfect is inhibited by its own instability as well as the presence of other physiological barriers, which makes it difficult to use RNA clinically to treat cancer (Rosenblum et al., 2018). Exogenous RNA may also be eliminated by the body's immune system. However, since no delivery mechanism was used in the manufacture of the mRNA candidate vaccines tested in clinical trials, it is clear that the delivery method for mRNA vaccines needs to be improved (Wickens, 1990; Wadhwa et al., 2020). A nanoparticle-based delivery system has been investigated as a viable RNA delivery tool for preclinical applications in order to get over these challenges and guarantee the secure delivery of RNA therapies to target areas (Chen et al., 2017). This method has shown promise in preclinical studies, which were followed by cancer immunotherapy clinical trials using several RNA-mediated nano delivery systems (Lin et al., 2016; Lin et al., 2020).

VII. CONCLUSIONS

Currently, nucleic acid vaccines are being developed quickly for the treatment of malignancies and infectious disorders. Malignant tumors and pandemics like HIV, AIDS, Ebola,

COVID-19, breast cancer, and melanoma have made people more aware of the threats to human health around the world and encouraged the development of nucleic acid vaccine platforms, allowing researchers to meet the difficulties of difficult circumstances. Numerous preclinical and clinical studies show that nucleic acid vaccines are effective in the treatment of infectious diseases and have also shown promise in the treatment of cancer. Although nucleic acid vaccines have a few advantages over traditional vaccines, they still need to be improved before they are used as the major therapeutic approach for patients. This article provides an overview of three optimization techniques based on the drawbacks of nucleic acid vaccines: physical approaches, chemical methods, and adjuvants. As a result, it is possible to enhance the absorption and membrane-penetrating ability of nucleic acid vaccines, strengthening the immune response. The safety and acceptability of a growing array of molecular adjuvants, including adhesion molecules, cytokines, chemokines, and transcription factors, are being evaluated. Similar to this, the ongoing development of vaccine delivery techniques is encouraging and deserving of further study. Although it appears improbable that technology will be created that can offer proper treatment for every single patient, combining existing technology with continuously advancing knowledge of human immunology will result in stronger weapons to combat known and developing global dangers.

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Conflicts of Interest
The authors declare no conflict of interest.

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