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A review on immunization of DNA vaccine

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Abstract

DNA vaccines represent a cutting-edge approach to immunization by utilizing genetic material to induce an immune response. Unlike traditional vaccines that use weakened or in activated pathogens, DNA vaccines deliver a plasmid containing genes encoding specific antigens into the host cells. These cells then produce the antigens, stimulating a protective immune response. This method offers several advantages, including rapid development, ease of production, and the ability to target a wide range of diseases. DNA vaccines have shown promise in preclinical and clinical trials for various infectious diseases, cancer, and genetic disorders. Ongoing research focuses on optimizing delivery systems, improving efficacy, and ensuring safety to enhance their potential as a transformative tool in modern medicine. Electroporation and nanoparticle-based systems, to improve the effectiveness and safety of DNA vaccines

Keywords: DNA vaccine; Vaccine preparation; Delivery method; Immunization

1. Introduction

A eukaryotic plasmid containing a gene encoding an antigen, which is transcribed and translated into the matching protein upon transference into the host cell, makes up a DNA vaccine [1].The first demonstration of the usefulness of the vaccine DNA was done by injection of a human growth hormone encoding plasmid (HGH) to the mouse. In this first test, the hGH gene was injected into the skin of the ear to produce its protein for treatment [2] Third-generation vaccines known as DNA vaccines are made of a plasmid that has been genetically altered and adjusted to elicit an immune response. DNA vaccines are also known by other names such as genetic, somatic transgene, polynucleotide and nucleic acid vaccines. World Health Organization (WHO) has declared the term nucleic acid vaccines as official [3] .Another group of scientists injected influenza virus protein in the mouse muscle and it triggered an immunologic response and thus prevented the infection in mouse. These findings were published in 'The Science' in the year 1993 and it marked the beginning of nucleic acid vaccines or DNA vaccines [4] .DNA vaccines are third generation vaccines, and are made up of a small, circular piece of bacterial DNA (called a plasmid).The vaccine DNA is injected into the cells of the body, where The "inner machinery" of the host cells "reads" the DNA and Converts it into pathogenic proteins. Because these proteins are recognized as foreign, when they are processed by the host cells and displayed on their surface, implies; the immune system is alerted, which then triggers a range of immune responses [5].

2. Historical Development

2.1. Early Research

- **1993**: The seminal study by demonstrated the potential of DNA vaccines in mice. They showed that plasmids encoding the herpes simplex virus glycoprotein induced protective immunity in the animals. The foundation for the development of DNA vaccines was established by this research. [4]

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2.2. Advancements

- **2004**: The development of DNA vaccines for infectious diseases progressed significantly, with increasing emphasis on optimizing plasmid design and delivery systems
- **2008**: Research demonstrated that DNA vaccines could be effective against cancer by encoding tumour specific antigens, showing their versatility beyond infectious diseases

2.3. Recent Developments

2.3.1. COVID-19

The COVID-19 pandemic accelerated the development of DNA vaccines. While mRNA vaccines received much attention, DNA vaccines also showed potential. A number of potential DNA vaccines were explored, and a few of them proceeded to advanced clinical trials. Compared to their mRNA counterparts, these vaccines have the advantage of a simpler manufacturing method and a possibly longer shelf life. [6]

2.3.2. Cancer Immunotherapy

There is growing interest in using DNA vaccines as a cancer immunotherapy technique. For instance, personalized DNA vaccines that target specific tumor antigens are showing potential in clinical trials for various cancers. These vaccines aim to stimulate a robust immune response specifically against cancer cells. [7]

2.3.3. Infectious Disease Prevention

DNA vaccines have made significant strides in the prevention of infectious diseases. For example, research has progressed in using DNA vaccines for diseases like Zika virus, influenza, and tuberculosis. The success of these vaccines can be attributed to improve plasmid design and adjuvants that boost the immune response. [8]

2.4. Advantages of DNA vaccine

- Rapid Development: Once the genetic sequence of the pathogen is known, DNA vaccines may be created fast. This rapid development process is crucial in responding to emerging infectious diseases. Traditional vaccine development, which often involves growing large quantities of pathogens, is typically much slower **[9]**
- **Safety Profile:** DNA vaccines do not contain live pathogens, which eliminates the risk of causing the disease in vaccinated individuals This safety feature is particularly advantageous for developing vaccines against pathogens that can be dangerous or difficult to handle
- **Strong Immune Response:** DNA vaccines have the potential to induce both robust antibody responses (humoral immunity) and strong cellular immune responses (T-cell responses). This dual capability can be effective in generating comprehensive protection against infections **[10]**
- **Stability and Storage**: DNA vaccines are generally more stable than some traditional vaccines, which can require refrigeration or freezing. This stability makes them easier to store and transport, particularly in areas with limited cold chain infrastructure
- **Simplified Manufacturing:** The manufacturing process for DNA vaccines is relatively straightforward and less costly compared to traditional vaccine production, which often involves growing live pathogens. This can reduce production costs and facilitate scaling up **[11]**

2.5. Disadvantages of DNA vaccine

- **Limited Clinical Experience**: Despite their potential, DNA vaccines have not been as extensively tested in humans as traditional vaccines. This limited clinical experience means that long-term safety and efficacy data are still lacking. **[12]**
- **Delivery Challenges**: Efficiently delivering DNA into cells remains a significant challenge. Methods like electroporation or gene guns are used, but they can be invasive and may cause tissue damage.
- **Potential for Insertional Mutagenesis**: There is a theoretical risk that the DNA from the vaccine could integrate into the host genome and disrupt normal genes, potentially causing harmful effects or cancer. **[13]**
- **Immune Response Variability**: The immune response to DNA vaccines can vary significantly between individuals, potentially due to differences in the efficiency of DNA uptake or varying immune system responses.
- **Regulatory and Manufacturing Hurdles**: DNA vaccines are complex to manufacture and require rigorous regulatory oversight. This can make them more expensive and time-consuming to develop and bring to market. **[14]**

- **Potential for DNA Integration**: Though rare, there is a potential for the introduced DNA to integrate into the host's DNA, which raises concerns about genetic stability and unintended effects. **(15)**
- **Long-Term Efficacy**: The long-term durability of the immune response induced by DNA vaccines is still under investigation. There is a need for more research to confirm how long protection lasts and how it compares to traditional vaccines. **(16)**

3. Mechanisms involved in DNA vaccine (17)

Like all vaccines, the mechanism by which DNA vaccines generate immunogenicity is by activating the adaptive immune response. Delivered in close proximity to a cell, a plasmid can be taken up (passively or through facilitation) and its DNA identified and expressed by the cell's own machinery, producing the target antigen in the process. From there, antigens (usually varying lengths of peptides) are presented on the cell surface for interaction with the immune cells by one of two pathways, either the major histo compatibility complex (MHC) class I (MHC I) or class II (MHC II) pathways (Figure no 1). MHCI, which is present in all nucleated cells[18], is most frequently thought to be the presentation mechanism for endogenous antigens (most commonly peptides), while MHCII is thought to be the classical pathway for the expression of exogenous antigens, such as bacteria, fungi, protozoa and free viruses that the cell has endocytosis [19,20,21] . Because plasmids are taken up by the cell and the antigen to be presented is then generated intracellular through the transcription and translation of the delivered DNA, the most common mechanism of the antigen presentation in DNA vaccination is MHC Class I .[22,23]

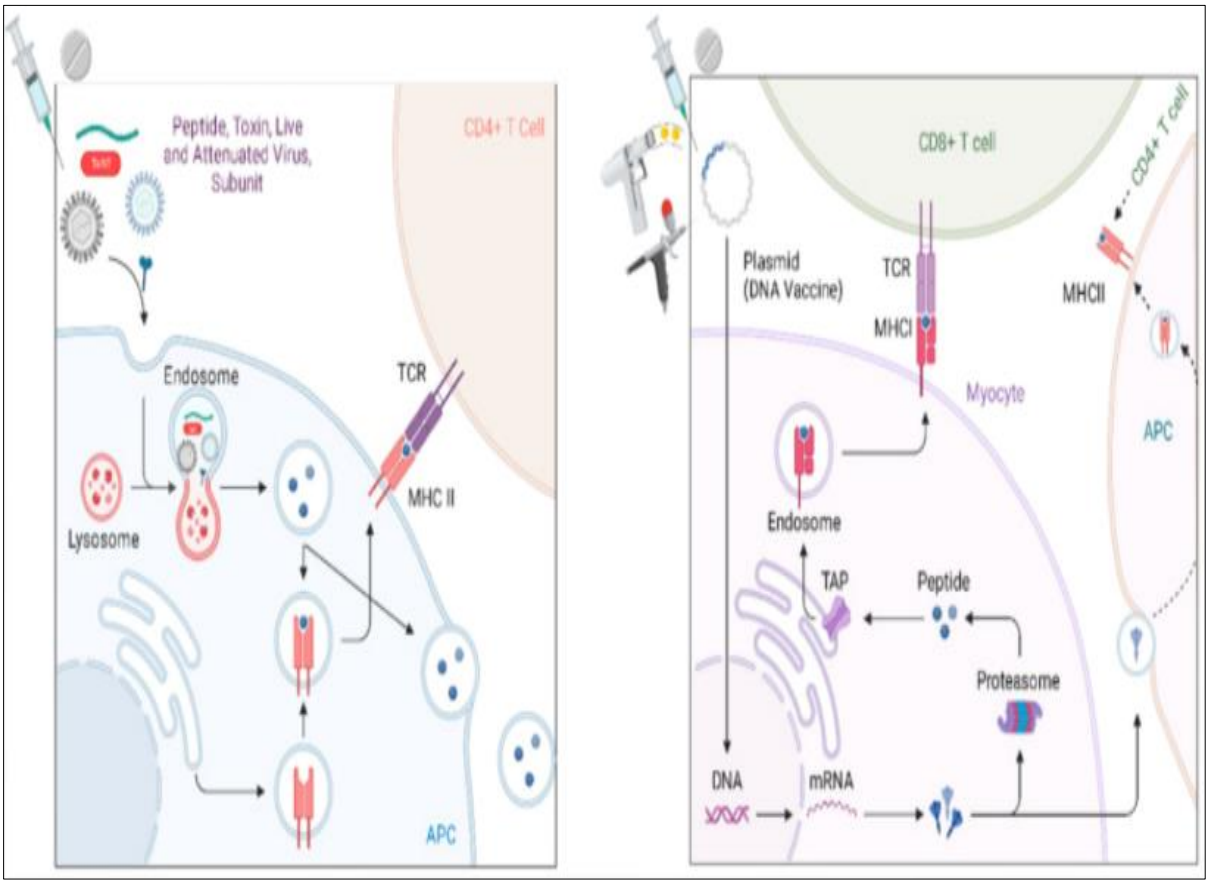

Figure 1 Conventional vaccine mechanism and DNA vaccine mechanism. Conventional vaccine (on the left)including peptide, subunit, live and attenuated viruses and toxins require endocytosis and intracellular processing of the pathogen in exogenous to the presenting cell, it is processed through the MHC2 pathway, which preferentially engages CD4+cells.DNA vaccine (on the right) can be endocytosis or can be engineered to passively cross the phospholipid membrane . the nucleic acid then locates to the nucleus and transcription occur as if the DNA were native, which leads to presentation of the peptide through the MHC 1 pathways, preferentially activating CD8+ cells, additionally, the same peptide is exocytosed and then taken up by nearby cells, which then present the peptide via the MHC2 pathway

3.1. Steps involved preparation of DNA Vaccine

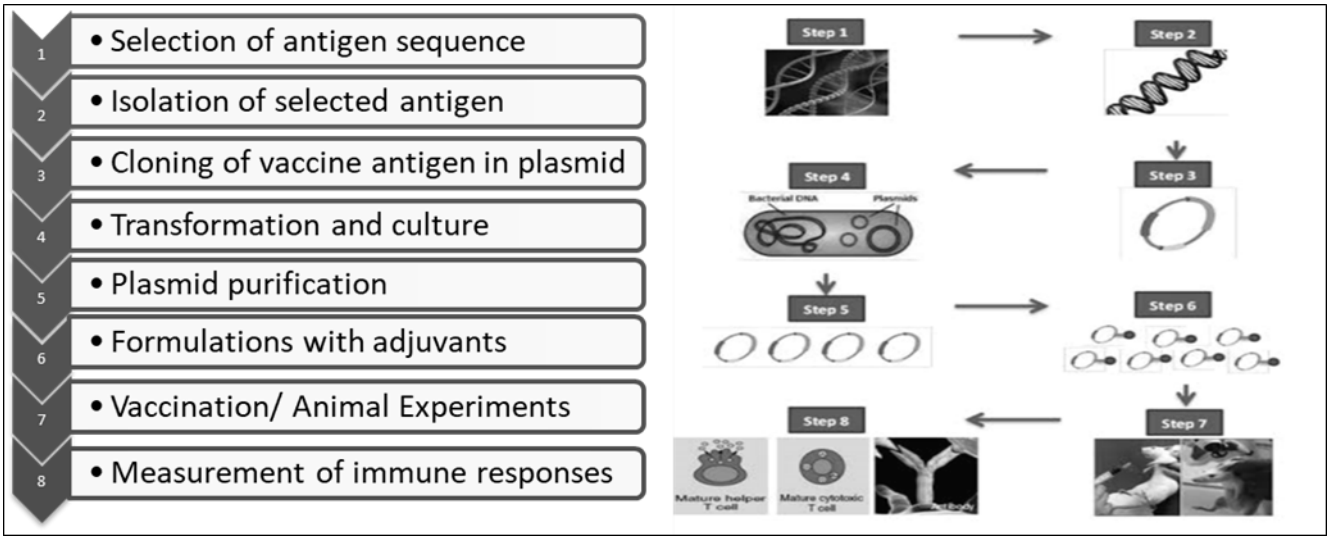

Figure 2 Steps involved in preparation of DNA vaccine

3.2. DNA vaccine against infective diseases [25]

3.2.1. DNA vaccines against cancer

Cancer is a worldwide leading cause of death, and several malignancies are incurable by conventional therapies. Therefore, new anti-tumor immune therapies are necessary to improve the outcome of patients with advanced cancer, and DNA vaccines are reliable forms of immunotherapy. Since DNA, vaccines are durable, safe, and simple to make, they are an effective type of antigen-specific immunotherapy. Moreover, tumor-specific antigens are expressed for a longer period of time as compared to RNA or protein-based vaccines. [26]

Using DNA vaccination to produce vaccines against cancer, especially cervical carcinoma (CC), has proven to be an effective approach. Persistent infection with human papilloma viruses (HPV) is the main etiological factor in cervical cancer, the second most common cancer in women worldwide [27]

3.2.2. DNA vaccines against tuberculosis

Tuberculosis (TB) remains a major worldwide health problem .TB is driven by the acquired immune response to the tubercle bacillus *Mycobacterium tuberculosis*. One promising tactic to combat tuberculosis is the use of therapeutic DNA vaccines. The expression of the HSP65 fusion gene and IL-2 in DNA vaccines was investigated. It improved the HSP65-DNA vaccine's immunogenicity, therapeutic benefits, and protective qualities against tuberculosis in mice. This was achieved by improving the Th1-type response.[28] Addition of immune stimulatory motifs in the transcribed region of a plasmid DNA vaccine elevated Th1 immune responses and the therapeutic effect against *Mycobacterium tuberculosis* in murine model [29]

*3.2.3. DNA vaccines against **Edwardsiella tarda***

Gram-negative Enterobacteriaceae bacteria include *Edwardsiella tarda*. It is a pathogen with a broad host range that includes humans, animal, and fish [30, 31]. As a human pathogen, *E tarda* is known to cause gastroenteritis and is implicated in septicemia, meningitis, and wound infections [32]. The antigens present in E tarda are FliC and Eta6. These two antigens are homologues of the FliC flagellin and an ecotin precursor, respectively. They were recognized as a vaccination made of chimeric DNA. Using the aforementioned data, pCE6, which encodes an Eta6 fused in-frame to FliC, was created. Compared to pEta6, PCE6 was shown to evoke higher levels of protection. [32].

3.2.4. DNA vaccines against HIV

One of the biggest risks to world health is the human immunodeficiency virus (HIV), which is the source of acquired immunodeficiency syndrome (AIDS). Today there are no vaccines to prevent HIV infection. To the best of this author's knowledge, every candidate that has been investigated thus far is in the experimental phase. HIV-negative people were used to study the effect of preventive vaccine candidates to see if they can prevent infection [33 The DNA vaccine platform

is a strong contender for an efficient HIV-1 vaccine due to its safety, stability, and capacity for repeated homologous immunization. The immunogenicity of DNA vaccines for HIV has been increased through improvement of the DNA vector, through the inclusion of molecular adjuvant, heterologous prime-boost strategies, and delivery with Electroporation [34].

3.2.5. DNA vaccines against anthrax

Bacillus anthracis, an encapsulated spore-forming bacteria, is the causative agent of anthrax, an infectious zoonotic illness. In human beings, three forms of anthrax have been recognized. They are cutaneous, gastroenteritis and pulmonary forms. [35]This disease is not common in western countries but the countermeasures against this disease are important because the spores of *B anthracis* can be used as bio-terror weapons. [36]The immunogenicity and efficacy of an anthrax/plague DNA fusion vaccine in a murine model has been described. [37]

3.2.6. DNA vaccines against influenza

The World Health Organization (WHO) suggests that influenza viruses be added to influenza vaccines every year, especially in the months of February and September, in preparation for the upcoming winters in the Northern and Southern hemispheres respectively. In general, influenza vaccinations are frequently modified to maximize their efficacy against recently discovered strains of human influenza viruses that are anticipated to become active during the upcoming influenza season. [38] Human morbidity and mortality are significantly increased by influenza viruses A and B. Hemagglutinin (HA) and neuraminidase (NA), the two main surface glycoproteins found on influenza virions, are the virus's main antigens. A number of influenza genes, such as HA, NA, matrix protein (M1), nucleoprotein (NP), and nonstructural protein (NS1), have been investigated as possible candidates for DNA vaccines. [39]

3.2.7. DNA vaccine against dengue

Dengue is a mosquito-transmitted infectious disease. Globally, it has a significant effect on human health as well.This disease has increased dramatically in the past century throughout the globe, and is now among the most common causes of febrile illness in travelers.[40] The human immune system generates antibodies directed against C, prM, E, NS1, NS3, NS4B, and NS5, among other dengue proteins. The E protein has been linked to the majority of the epitopes of anti-dengue neutralizing antibodies. For this reason, the E gene has been selected when creating DNA vaccines. Additionally, it has been stated that the prM gene is necessary for the correct folding and processing of the E protein; as a result, the prM gene has also been included. [41, 42]

3.2.8. DNA vaccine against typhoid

Salmonella infection is a food borne infection. [42] Typhoid fever is a prolonged febrile illness caused by bacterium *Salmonella typhi*. Typhoid can be treated by using antibiotics. [43]

4. Vaccine Formulations and Their Delivery Methods

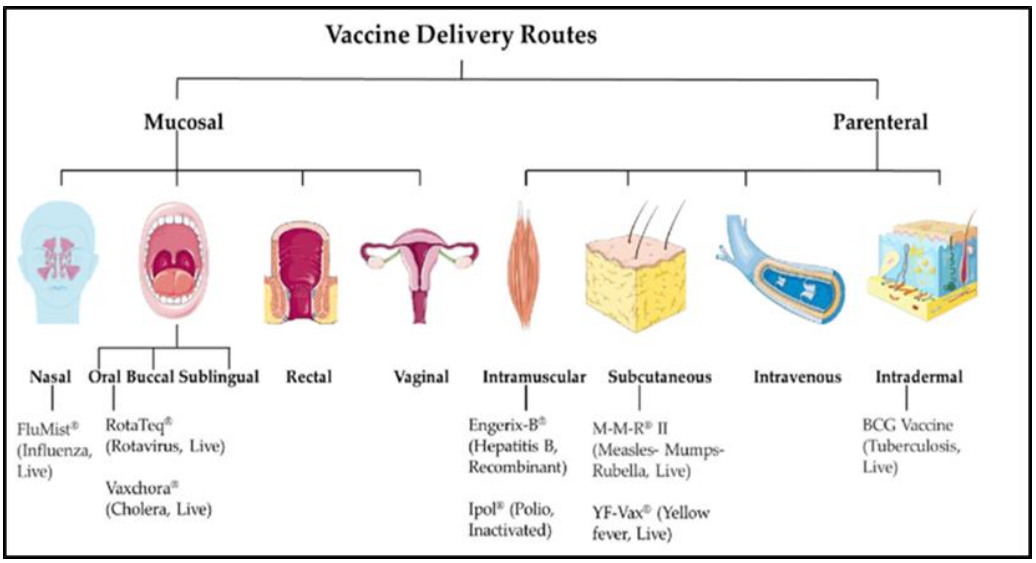

Figure 3 Delivery routes of DNA Vaccines

A vaccine's intended product profile is crucial in the early stages of development. The selection of vaccination dose types is influenced by various factors, including vaccine antigen classes such as live attenuated, inactivated, subunit, and, more recently, mRNA-based.The resulting formulation development ultimately affects its efficacy. The chosen method of administration must be taken into account while creating the formulation. The optimal balance between systemic and mucosal immune responses has always been taken into account when choosing the vaccination delivery method. Tolerance is necessary for the mucosal immune response, while the immune system's preparedness is necessary for systemic immunity. The current approaches involve mucosal and parenteral delivery of vaccines (Fig:3&4) The mucosal sites include nasal, oral, buccal, sublingual, rectal and vaginal. Moreover, intramuscular, subcutaneous, intravenous, and intradermal are among the parenteral locations. The site of infection, transmission route, type of vaccine, and type of immune response expected several factors that contribute to deciding the vaccine delivery route. ([44-45])

4.1. Mucosal Route

Mucosal vaccination involves administering vaccines through mucosal locations such as the nasal, oral, buccal, sublingual, rectal, and vaginal. Mucosal tissues cover a considerable portion of the body's surface, exposing it to numerous pathogenic pathogens. Mucosal infections include respiratory tract diseases such as COVID-19, influenza, respiratory syncytial virus; sexually transmitted diseases such as gonorrhoea and other genital tract infections; digestive tract infections such as rotavirus. Because many infections arise at mucosal locations, it is critical to develop techniques for neutralizing these infectious agents at the point of entry. Thus, for localized immune response, mucosal immunization would be an attractive route, as it would mimic the natural infection [(46)] Mucosal immunization also induces immune responses at other mucosal sites and/or systemically.[(47)] Furthermore, the mucosal interface contains well-organized lymphatic tissue, referred to as mucosa-associated lymphoid tissue (MALT). MALT includes the immune system's innate and adaptive arms. [(48)]

4.2. Nasal Route

Nasal vaccine delivery, a kind of mucosal delivery, stimulates nasal-associated lymphoid tissues (NALT) containing specialized M-cells to produce immunological responses, specifically innate immunity and IgA humoral and mucosal antibodies [(49)] Nasal drops or sprays offer a non-invasive, painless alternative to traditional routes. The intranasal method requires smaller amounts of antigen and has higher antigen stability. Both mucosal and systemic immune responses are induced upon intranasal vaccination. [(50)] Pulmonary vaccine delivery against measles and rubella has been studied. Pulmonary vaccines include aerosol or dry powder inhaler systems. Dry granules can be reconstituted into nasal drops. Commercially, there is only one licensed nasal spray flu vaccine FluMist Quadrivalent® (live attenuated influenza vaccine), that provides protection against influenza A (H1N1, H3N2) and influenza B. [(51)]

4.3. Oral Route

Oral vaccination stimulates the immune system in the Peyer's patch and mucosa associated lymphoid tissue (MALT) in the gut wall. [(52)] It stimulates mucosal as well as systemic immune sites. The oral route is safe, patient-friendly, simple to administer, and does not require a healthcare practitioner. However, oral vaccine development has challenges. Several protein-based antigens would be degraded by mucosal enzymes in the hostile gut environment. Thus, oral vaccine antigens lack stability.

4.4. Buccal and Sublingual Route

Vaccine administration via sublingual and buccal channels (mucosal distribution in the mouth) has recently received attention. Sublingual mucosa includes the ventral area of the tongue and area under the tongue, buccal delivery includes the cheeks, gums, upper and lower inner lips. These regions are rich in antigen-presenting cells like Langerhans cells, myeloid dendritic cells, and plasmacytoid cells. [(53)] Upon vaccination, the vaccine antigen will be captured by the APCs. APCs will then migrate to the draining lymph nodes. In the lymph nodes, APCs will engage with CD4 and 8 T cells, triggering an adaptive immune response. The advantages of vaccine distribution through these locations are similar to those of intranasal delivery. This technique also requires a lower amount of antigen than oral immunization. [(54)] Sublingual vaccination against influenza is found to protect against flu [(55)]

4.5. Rectal Route

To date, mucosal vaccination delivery routes have been devised, both nasal and oral. However, these vaccines, often subunits, require adjuvant combinations and there have been reported instances of neurological adverse reactions. [(56)] Therefore, to tackle these side effects, an alternative mucosal vaccine delivery route, the rectal route, has been proposed for the immunization against diverse microbial strains. Rectal vaccination against Chlamydia infection was found to provide protection following a challenge study.[(57)]

4.6. Vaginal Route

Recent studies focused on the vaginal route of vaccine administration for genital infections and cancers such as the human papilloma virus (HPV) and cervical infection. Topical vaccination for genital infections would allow for a localized immune response. The genital mucosa generates specific immune responses after vaccination with inactivated and live-attenuated vaccines.[58] Another study demonstrates outer membrane vesicles (OMV) with T-helper cells driving adjuvant and interleukin-12 intravaginal vaccine approach against gonorrhoea.[59] These studies are successful on experimental animal models that are not yet applied in human.

4.7. Parenteral Route

There are four routes for parenteral medications (also see Figure 3). Each form of injection necessitates a distinct skill set to guarantee that the drug is correctly prepared and delivered to the appropriate site.

The four types of injections are:

- **Subcutaneous (SC):** This injection places medication/solution the loose connective tissue just under the dermis.
- **Intradermal (ID):** This injection places the medication into the dermis just under the epidermis.
- **Intramuscular (IM):** This injection places the medication into the body of a muscle.
- **Intravenous (IV):** This injection places the medication/solution into a vein through an existing IV line or a short venous access device (saline lock). Intravenous medications can be administered as a bolus, intermittently (piggyback), or as a large volume continuous infusion.

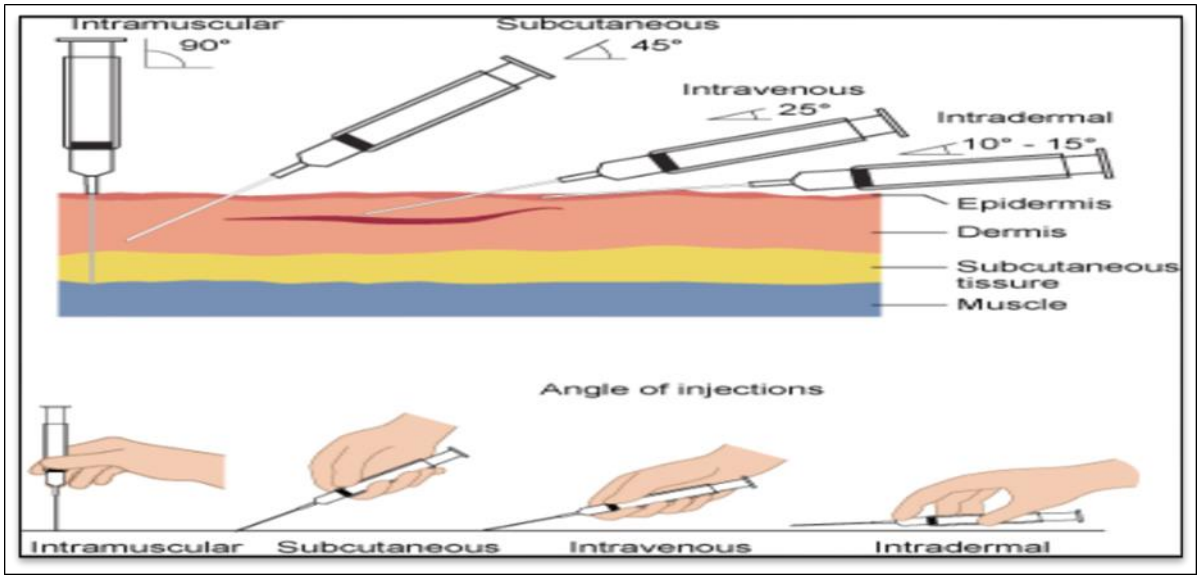

Figure 4 Insertion angles in parenteral route

Most of the vaccines available in the market are administered through the parenteral route (Table1). [60] Recently developed vaccines against COVID-19 are also delivered through the parenteral route. This route offers various approaches. such as intramuscular (IM) slow sustained release), subcutaneous (SC) (slow sustained release), and Intradermal (targeting antigen-presenting cells in the dermal region).[61] Depending upon the type of immune response desired, the vaccine can be delivered to the dermis, muscle, SC region, or veins.[62] Burst release of antigen can be helpful to induce an innate immune response. However, recent research focuses on needle-free delivery devices, which allow a longer period of gradual, sustained antigen release and more priming to the innate immune system, resulting in optimal adaptive immune responses. A prolonged release of antigens is beneficial because it ensures that APCs identify and take up the antigens over time, resulting in a powerful adaptive immune response Microneedles also prevent needle-stick injuries, which require a medical specialist and generate biohazardous waste. [63] (Listed in table 1).

Table 1 Diseases, Marketed DNA Vaccines, and their routes

Route	Vaccine	Disease
Oral	Dukoral , shanchol, and Euvichol	Cholera
	Rotarix, RotaTeq	Rotavirus
	Typhim Vi	Typhoid
Nasal	FluMist	Influenza
IM	Havrix(HepatitisA),Engerix (Hepatitis B)	Hepatitis A, Hepatitis B
	Gardasil	Human Papillomavirus(HPV)
	Menactra, Trumenba, Bexero	Meningococcal
SC	M-M-R 2	Measels,mumps&rubella
	Varivax	Varcella(var)
Intradermal	BCG Vaccine	Tuberculosis

5. Immune responses initiated by DNA vaccination

DNA vaccines induce an immune response by introducing plasmid DNA encoding an antigen into host cells. These cells produce the antigen, which is then presented on their surface. The immune system recognizes this foreign antigen, activating both antibody production (humoral response) and cytotoxic T-cell responses (cellular response). This process primes the immune system to recognize and combat the actual pathogen in the future.[64]

5.1. Characterization of immune response after DNA vaccination (65)

5.1.1. DNA Vaccination and Immune Response

- DNA vaccination often induces strong antigen-specific T cell responses, particularly type 1 cytokines and IFN-γ.
- Prominent IFN-γ and CD8 cytotoxic T lymphocyte (CTL) responses are noted.
- Protective immunity in mice is correlated with early IFN-γ production and broad epitope recognition by T cells.

5.1.2. Epitopic Diversity

- DNA vaccination results in a broader T cell epitope recognition compared to natural infection.
- CD4 T cells from DNA-vaccinated mice recognize more epitopes (e.g., 7 vs. 5) and CD8 T cells also show broader epitope recognition compared to infected mice.\

5.1.3. Subdominant Epitope Protection

Vaccination with subdominant epitopes, like those from ESAT-6, can be protective, while dominant epitopes may not be as effective.

5.1.4. Role of T Cell Subsets

- CD4 T cells producing IFN-γ are crucial for protection against tuberculosis.
- Protection from DNA vaccination was shown to be independent of CD8 T cells in some studies, though the role of CD8 T cells warrants further investigation.

5.1.5. Cytotoxic T Cells and Protection

- CD8 T cells that produce IFN-γ and are cytolytic are important for protection.
- CD8 T cells that are IFN-γ producing but non-cytolytic do not confer protection.

5.1.6. Persistence of Protective Immunity

- Protective immunity from DNA vaccination can last beyond typical study periods (4–6 weeks).
- Long-term protection has been observed in some studies, suggesting extended effectiveness of DNA vaccines.

5.2. Immunology of the immune response from DNA vaccine (66)

5.2.1. Preclinical Efficacy

- **Disease Models:** DNA vaccines have shown effectiveness in models of infectious diseases, cancer, allergies, and autoimmune diseases.
- **Responses:** Different immune responses (CTL, antibody, T-cell helper) vary by disease, antigen, animal model, and administration route.

5.2.2. Humoral Response

- **Antibody Production:** DNA vaccines can induce antibody responses, particularly in mice. These responses can be protective but are often weaker than those from protein are or live virus vaccines.
- **Antibody Kinetics:** Peak antibody response typically occurs 1-3 months after vaccination. The response can be long lasting (up to 1.5 years).
- **Comparison:** DNA vaccines generally produce lower antibody titers compared to live virus and protein-based vaccines. However, in some cases, such as with ovalbumin, antibody levels were comparable.

5.2.3. Cellular Immune Response

- **CD4 T-cells:** Can be Th1 (producing IFN-γ) or Th2 (producing IL-4, IL-5, IL-13). DNA vaccines generally skew responses towards Th1 due to CpG motifs in plasmid DNA.
- **CTL Response:** DNA vaccines can induce CTL responses comparable to live virus vaccines. They are effective against both dominant and subdominant epitopes.
- **Memory Response:** Long-term CTL responses have been observed, with significant immune activity persisting for up to 70 weeks post-vaccination

6. Conclusion

DNA vaccines offer a revolutionary approach to immunization by directly introducing genetic material encoding antigens into cells, which can induce strong and long-lasting immune responses. They have shown considerable promise in preventing infectious diseases and in cancer immunotherapy. Advantages include rapid design and production, and stability in storage and distribution. However, challenges such as optimizing delivery methods and ensuring long-term safety remain. Advances in electroporation and nanoparticle delivery systems are addressing some of these issues. Continued research is essential to refine these technologies, improve vaccine efficacy, and expand their applications. As the field evolves, DNA vaccines have the potential to become a pivotal tool in both responding to emerging pathogens and managing complex diseases, offering significant benefits over traditional vaccine platforms.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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