DNA Vaccines – Progress and Updates

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1. Background

The vaccination process is among the most successful applications of immunological principles to human health. Millions of lives have been saved by vaccines and vaccinations, and public health has changed forever. It is believed that vaccines save at least 2–3 million lives per year worldwide. Even so, vaccines still need to defeat an array of diseases. Each year, millions of people, including millions of children die from infectious diseases for which there are no effective vaccine. Besides this, all the available vaccine's efficacy needs periodic review and the safety of the vaccine is paramount. In addition, immunotherapeutic vaccines are critically needed to treat certain diseases such as cancer. It has been felt that the inability of previously existing technologies to develop the required vaccines is because of the different types of immune responses that has to be generated for certain diseases including the unique pathophysiological characteristics of those diseases. This urges the scientific community to discover and invent new effective vaccines.

Key words: Vaccines, Vaccination, DNA, DNA vaccines, Immunotherapy, Immunity.

2. Past, present and future:

History of vaccination dates back to 1000 A.D, when India, Chinese and western Asia used of variola scabs insufflate into the nose to immunize against smallpox, this was followed by the successful observations of Edward Jenner on cow pox virus that can cause mild illness in humans, could prevent smallpox. His revelation has revolutionized the health sector with the concept called vaccination. Louis Pasteur, a French Chemist and a renowned microbiologist was instrumental in defining the principles of vaccinations and promoting it to the next level. His work on chicken cholera, currently known as *Pasteurellamultocida*, led to a concept of attenuation. Attenuation is the process of decreasing the virulence or pathogenicity of a pathogen. From these observations Pasteur constructed the hypothesis that pathogens could be attenuated by exposure to environmental insults such as high temperature, oxygen and chemicals. His ensuing work on anthrax and rabies confirmed the hypothesis (Plotkin, 2005).

The vaccines developed over the first two hundred years since Jenner's lifetime have accomplished striking reductions of infection and disease wherever applied. Pasteur's early approaches to vaccine development, attenuation and inactivation, are even now the two poles of vaccine technology. Today, purification of microbial elements, genetic engineering and improved knowledge of immune protection allow direct creation of attenuated mutants, expression of vaccine proteins in live vectors, purification and even synthesis of microbial antigens, and induction of a variety of immune responses through manipulation of DNA, RNA, proteins and polysaccharides. Both non-infectious and infectious diseases are now within the realm of vaccinology.The profusion of new vaccines enables new populations to be targeted for vaccination, and requires the development of routes of administration additional to injection. With all this come new problems in the production, regulation and distribution of vaccines. Paul Ehrlich and Ilya Metchnikoff have developed the idea of antibodies and cellular immune responses (Plotkin, 2005). This is the way that the vaccine technology has advanced. Different types of vaccines were described in figure 1 (Tianhun and Jhang, 2021).

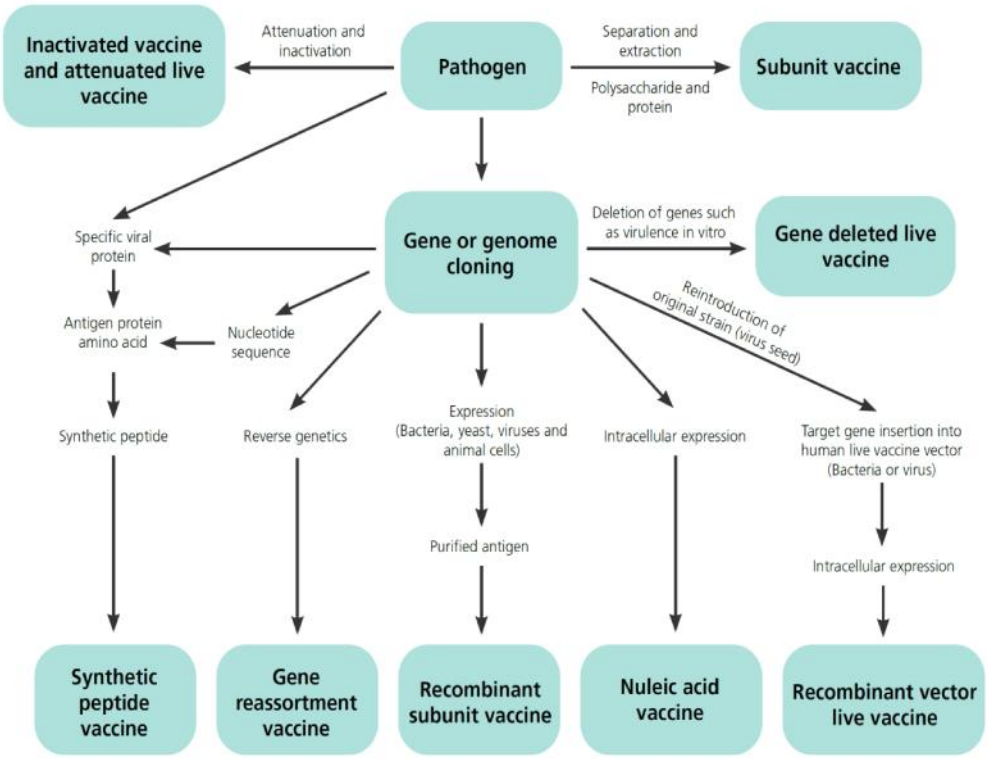

Figure 1: Types of Vaccines

3. Journey of Vaccine technology

When it comes to time line, the first golden age of vaccines started when Pasteur, Koch, Ramon, and Mérieux established the germ theory and developed vaccines based on live-attenuated or inactivated (killed) pathogens and on inactivated toxins (toxoids). These vaccines protected against rabies, diphtheria, tetanus, pertussis, and tuberculosis in infants. The second golden age of vaccines was a consequence of innovation in cell culture technologies in the second half of the 20th century. The 'cell culture revolution' allowed for effective inactivated vaccines to prevent polio (IPV) and hepatitis A, and live-attenuated vaccines against polio (OPV), mumps, rubella, measles (MMR), rotavirus, and varicella. Progress in microbiology led to the development of polysaccharide vaccines against some strains of pneumococcus and meningococcus. The vaccine development time line has depicted in figure 2 (Delany *et al*., 2014).

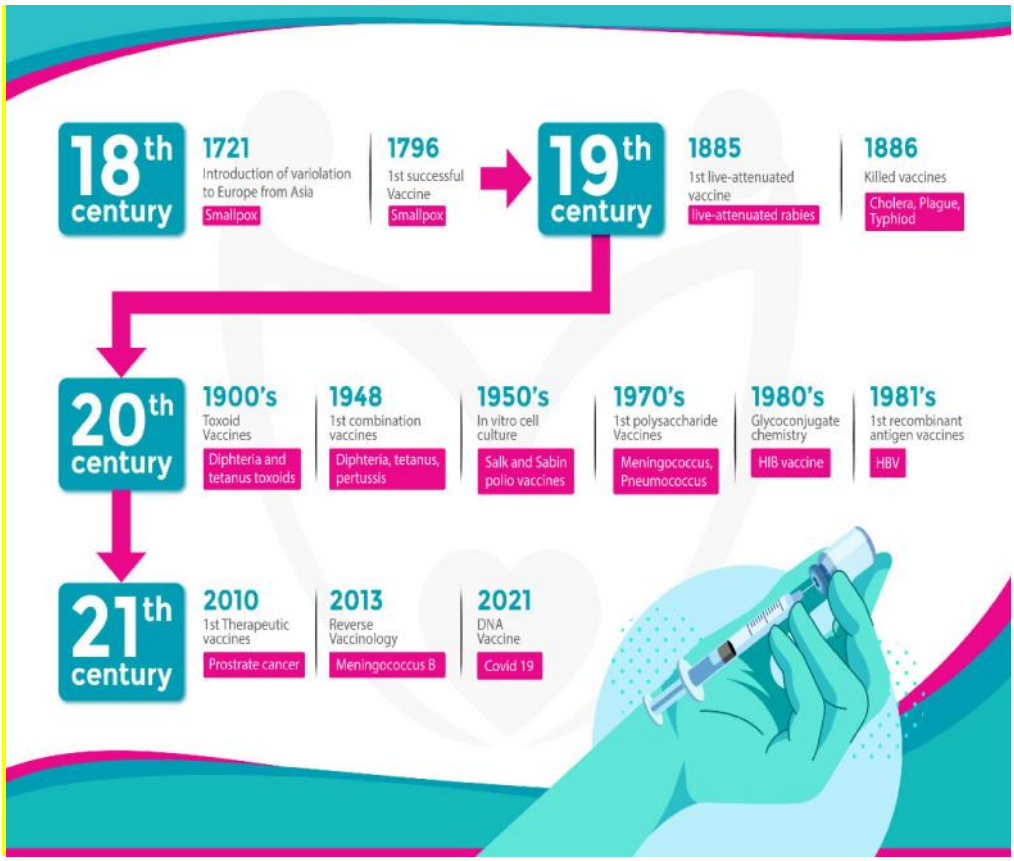

Figure 2: Journey of vaccine technology

4. Formulation:

Vaccines are sterile mixtures of several components, and proper formulation is fundamental to a vaccine's success or failure. Apart from the antigen, the basic components of a vaccine may include stabilizers, preservatives, adjuvants, and carrierparticles (Levine and Sztein, 2004).

4.1.Stabilizer for vaccine:

Vaccine stability is key to efficacy. Stabilizers such as $MgCl_2$ for oral polio vaccine (OPV), $MgSO_4$ for measles, lactose-sorbitol for chickenpox, and sorbitol-gelatine for measles, mumps, and rubella are used to protect the delicate antigen from degradation during storage. This is especially important if the chain of cold storage cannot be guaranteed during shipping of a vaccine. Correct choice of stabilizer depends on the antigen, and omitted or improperly

chosen stabilizers can lead to vaccines that lose their potency and become ineffective (Levine and Sztein, 2004).

4.2.Preservative for vaccines:

For vaccines in multidose containers, a preservative may be required to prevent microbial growth. Thiomersal, formaldehyde, or phenol derivatives are most common (Levine and Sztein, 2004).

4.3. Adjuvants for vaccines:

Inactivated antigen vaccines and sub-unit vaccines generate a relatively weak immune response and require properly formulated 'helpers' called adjuvants to provide robust and long-lasting immunity. The most common adjuvant used in human vaccines is alum, which comprises particles of insoluble aluminium salts. The development of new products requires an understanding of the pathogen, the immune system, and a deep understanding of vaccine formulation principles—from adjuvants to finished product. However, there are several other commercial examples of adjuvants (Levine and Sztein, 2004).

5. DNA Vaccines an overview:

We arein an era of unprecedented scientific advancements in vaccine technologies (Ilie and Cardoza, 2018). DNAvaccination is a technique for protecting an organism against disease by injecting it with genetically engineered DNA to produce an immunological response (Khan, 2013). It is a rapidly developing vaccine platform for combatting cancer, infectious and noninfectious diseases. Plasmid DNA used as immunogens that encode proteins to be synthesized in the cells of the vaccine recipients. Introduction of DNA vaccines into the host induces antibody and cellular responses against the encoded protein. In this way, the induction of immune response mimics the events occurring during natural infection with an intracellular pathogen (Ljungberg and Isaguliants, 2020). Mode of Immunization and Immunotherapy of DNA vaccine is depicted in Figure 3 (Gary and Weiner, 2020).

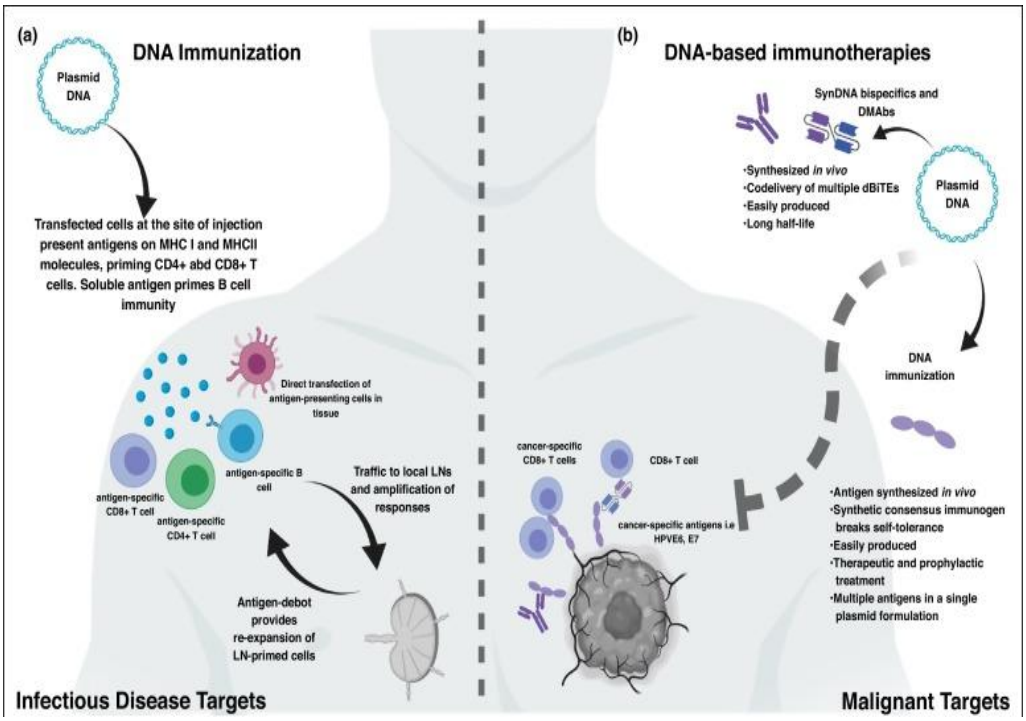

Figure 3: Immunization and Immunotherapy of DNA vaccine

However, the developments of new vaccines face some challenges. This is due to multiple factors including the high cost of their development which drives a focus on larger markets, slower than optimal time lines for vaccine advancement to clinical testing, among other complexities. Recently newer synthetic DNA vaccines have been rapidly advanced to clinical study and have demonstrated an impressive degree of immune potency and tolerability (Gary and Weiner, 2020). Immunization of animals with naked nucleic acid encoding antigens under the control of a variety of gene regulatory elements has been described as“polynucleotide immunization,” “DNA-based vaccination” (Liu, 2003). DNA vaccines against influenza have been in development since the 1990s, but the initial excitement over success in murine model trials has been tempered by comparatively poor performance in larger animal models. In the intervening years, much progress has been made to refine the DNA vaccineplatform therational design of antigens and expression vectors, the development of novel vaccine adjuvants, and the employment of innovative gene delivery methods (Lee, 2018).

Immunization with nucleic acids has received considerable attention in the field of new generation vaccines (Silveira*et al*., 2020). DNA vaccines offer simple yet effective means of inducingbroad-based immunity (Khan, 2013). They include diseases such as HIV/AIDS and ancient scourges such as malaria. The breadth of applications for DNA vaccines ranges from prophylactic vaccines to immunotherapy for infectious diseases, cancer, and autoimmune and allergic diseases (Smith *et al*., 2020). These vaccines function by generating the desired antigen inside the cells, with the advantage that this may facilitate presentation through the major histocompatibility complex (Khan, 2013).

6. DNA Vaccine for Covid 2019

Here a special mention is required for the novel coronavirus, and associated COVID-19 disease, as it become a global pandemic with a significant morbidity and mortality toll. Advanced research activities must be pursued in parallel to push forward protective modalities in an effort to protect billions of vulnerable individuals worldwide (Nascimento and Leite, 2012). It is also known as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which is associated with several fatal cases worldwide. To address the urgent need for a medical countermeasure to prevent the further dissemination of SARS-CoV-2 we have employed a synthetic DNA-based vaccine approach. It is an unusual global health threat wherein the vaccine is needed immediately. Some successful DNA vaccines expressing S, M, and N proteins have been developed against SARS-CoV. The obtained results confirmed the strong protective humoral and cellular immune responses in mice, macaques, and camels (Silveira *et al*., 2020).

7. Construction of DNA vaccines:

The feasibility of using DNA as a treatment has been demonstrated in animal models, but clinical applications of this form of technology remain elusive. When gene therapy does come into wide medical use, it may be as a vaccine. Difficulties in developing vaccines against chronic infections with viral agents such as the human immunodeficiency virus (HIV), herpes simplex virus, and hepatitis C virus are partly due to the poor immunogenicity of standard vaccines; these problems have spurred the development of new vaccine strategies that use DNA instead of protein. DNA vaccines contain the gene or genes for an antigenic portion of a virus, such as the core protein or the envelope protein (Pachuk *et al*., 2000). The vector structure contains a mammalian promoter that enables triggering the transcription of the gene used

for the vaccine through the host's cellular machinery (Silveira *et al*., 2020).Host cells take up the foreign DNA, express the viral gene, and make the corresponding viral protein inside the cell.An important advantage of this system is that theviral protein enters the cell'smajor histocompatibility complex (MHC) class Ipathway (Smith *et al*., 2020). Vaccination with DNA is a recent technology possessing distinct advantages over traditional vaccines (killed or attenuated pathogens) and the more recently developed subunit vaccines. Unlike most subunit vaccines, DNA vaccines can efficiently induce both the humoral and cellular arms of the immune response. DNA can be introduced by viral or bacterial vectors or through uptake of 'naked' or complexed DNA. The stimulation of both arms of the immune system is important not only for the prevention of many diseases including AIDS, but also allows the use of a vaccine for therapeutic purposes. While the traditional attenuated pathogen vaccines are also able to elicit both cellular and humoral immune responses, there is a risk of reversion from the attenuated state to the virulent state. This risk does not exist with DNA vaccines. DNA vaccines can be manufactured and formulated by generic processes. DNA vaccine technology, however, is still in its infancy and much research needs to be done to improve the efficiency with which these vaccines work in humans. While continued efforts toward improving both DNA expression and DNA delivery are equally important for increasing the utility of DNA vaccines (Lewis and Babiuk, 1999). DNA vaccines possess numerous properties ideal for influenza control and have been trialled for a range of diseases, including viral and bacterial infections, and some cancers. Their production does not require the growth of live virus and can be rapidly up scaled in response to emerging pandemic influenza (Lee *et al.*, 2018).

8. Mode of delivery:

Improvements in DNA delivery over prior needle and syringe approaches include jet delivery, gene gun delivery, among others. Among the most effective of these new delivery methods, advanced electroporation (EP). In the past, several clinical trials using plasmid DNA vaccines demonstrated a good safety profile and the activation of a broad and specific immune response (Gary and Weiner, 2020). The various methods involved in the process of DNA based vaccines, such as injection-grade plasmid preparation and delivery of DNA vaccines, are described(Figure 4).Plasmid DNA containing any of a variety of eukaryotic gene regulatory sequences, in the appropriate context of a gene, or genes, encoding antigen, is injected intramuscularly orintradermal (Lee *et al.*, 2018).

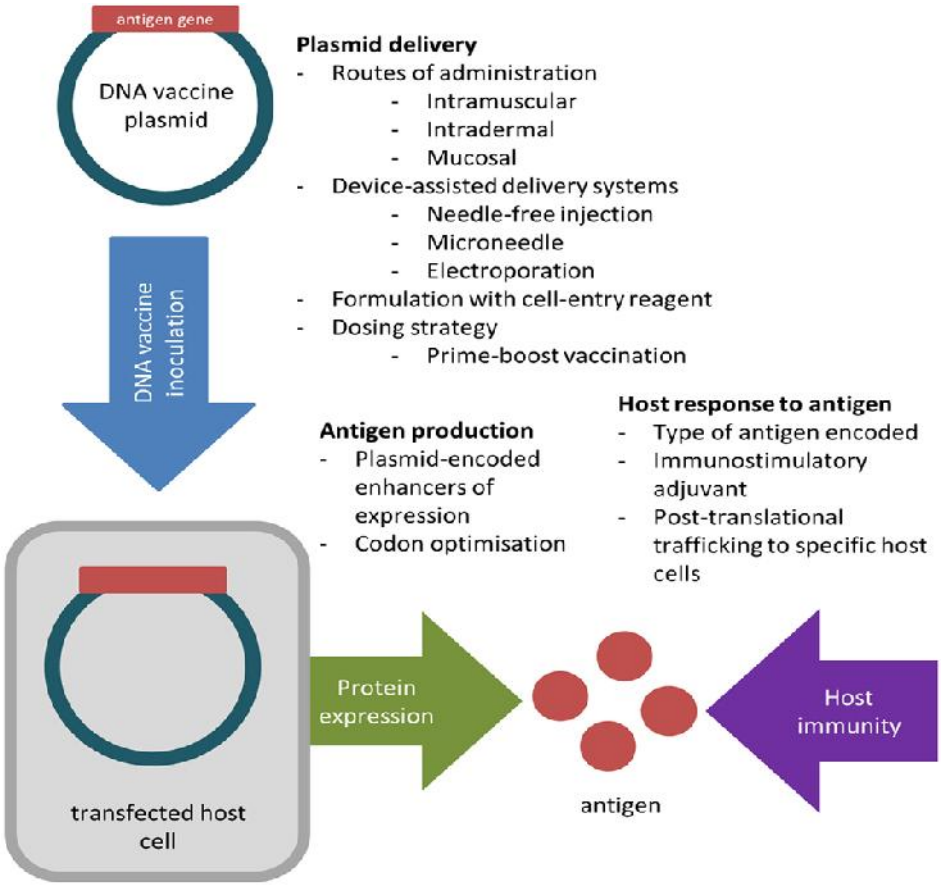

Figure 4: Plasmid DNA delivery

Theroute of administration is critical to vaccine effectiveness as it dictates the cell types that will be transfected. DNA vaccines were initially tested in the murine model using intramuscular injection of naked plasmids to produce antigens in passively transfected myocytes (muscle cells). This method relies on the influx of leucocytes following local inflammation to expose the immune system to DNA vaccine antigens. Outside of the murine model, effective intramuscular administration of plasmids depends on adjuvants and delivery systems to achieve sufficient immunogenicity. More recently, cutaneous delivery has become a highly desirable route for DNA vaccines, as the epidermis is abundant in Langerhans cells, which can efficiently transport and present DNA vaccine-encoded antigens in the lymph node (Lee *et al.*, 2018).

9. Antigen presentation

The primary goal of vaccine research progress in developing new vaccines is based on improved understanding of the molecular pathology of human disease and of the immune response in mammals. DNA (deoxyribonucleic acid) vaccination is a relatively new technology which utilizes genetically engineered DNA to produce an immunologic response. An important strategy to achieve this aim is to use DNA plasmids having antigens encoded on them. This antigen-encoding DNA plasmid can induce humoral and cellular immune response against parasites, bacteria and disease-producing viruses. The expression of the antigen-encoding gene can be controlled by a strong mammalian promoter which can be used on a plasmid backbone of bacterial DNA.Moreover various promoters, enhancers, and other elements were designed to elevate expression of the encoded protein in vaccine recipients. An important role in inducing immunity is played by professional antigen presenting cells (APCs), which are known to migrate to the primary lymphoid organs when directly transfected in the skin or muscle. In these organs they initiate an immuneresponse (Delany *et al.*, 2014). DNA vaccines are typically comprised of plasmid DNA molecules that encode an antigen(s) derived from a pathogen or tumor cell. Following introduction into a vaccine, cells take up the DNA, where expression and immune presentation of the encoded antigen(s) takes place (Lewis and Babiuk, 1999).

A DNA vaccine consists of a plasmid produced in bacteria that encodes the protein of interest (an antigen) in the presence of a mammalian promoter. It is placed in a way that it reaches the cell nucleus, enabling the transcription and translation in the transfected human cells (step 1).After the plasmid uptake in vivo, the encoded protein is expressed in the host's cells, and the vaccine antigen can be then presented to antigen-presenting cells (APCs), such as dendritic cells (DCs), through the major histocompatibility complex (MHC) pathways and be presented to activate naïve T cells.CD8+ T cell immunity is predominantly activated by endogenously expressed antigens presented on MHC class I molecules (step 2a). The active CD8+ T cell stimulates the release of cytokines (e.g., interferon-gamma [IFN-γ] and tumor necrosis factor-alpha [TNF-α]) that inhibit viral replication and increase the expression of MHC I molecules. Therefore, macrophages are also activated to support cell-mediated immune responses (step 2b).However, CD4+ T helper cell activation is triggered through MHC class II from APC (step 3). In case the vaccine proteins are secreted, these targets are recognized by B cell receptors in naïve B cells, which also use MHC-II to get activated (step 4). In this immune pathway,

activated B cells will produce different classes of antibodies (mainly IgG) to protect against the disease (step 5). Furthermore, immunization with DNA vaccine expresses proinflammatory cytokines and chemokines.DCs are responsible for producing IL-10, IL-12, and TNF-α that induce the cellular response by activating CD8+ T and IL-4 is involved in activating CD4+ T. In addition, myocytes have been reported to play a crucial role. Thus the immune response is generated (Figure 5) (Silveira *et al*., 2021).

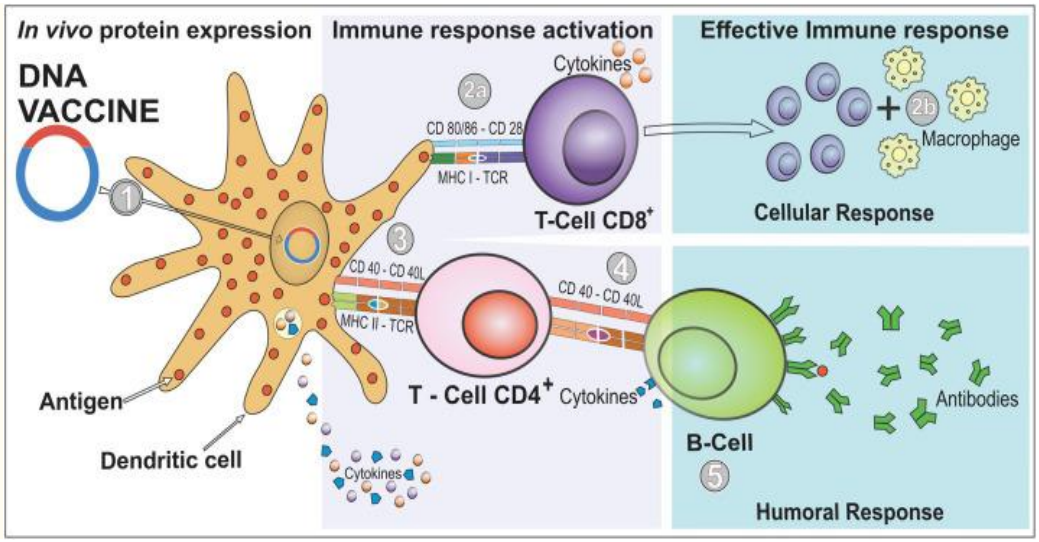

Figure 5: DNA vaccine expression and immunization.

10. Advantages of DNA vaccine:

i. Significant advantages of these vaccines include the cheapness, simplicity of production and consumption, transport and higher resistance.

ii. It is potentially cheaper to produce than recombinant protein vaccines. It is much easier to transport and use, especially in developing countries, DNA-based immunization exhibits several important advantages over conventional immunization strategies that involved live-attenuated or killed pathogens, proteins, or synthetic peptides.

iii. DNA immunization offers many advantages over the traditional forms of vaccination. It is able to induce the expression of antigens that resemble native viral epitopes more closely than standard vaccines do since live attenuated and killed vaccines are often altered in their protein structure and antigenicity.

iv. Another advantage of DNA vaccines istheir safety becausethe plasmid DNAis stable in biological systems and avoids using whole infectious organisms (Munita and Arias, 2016).

v. DNA vaccines are easy to transport and store – DNA is a very stable molecule and does not need to be stored at low temperatures making transportation and storage cheaper and easier than conventional vaccines.

11. Limitations of DNA Vaccine:

✓ Disadvantages of DNA vaccines are based mainly on the activation of oncogenes as well as elicitation of anti-DNA antibodies and low Immunogenicity in vaccines.

✓ No DNA vaccine has been licensed for use in humans yet - although some DNA vaccines are now in clinical trials, none are licensed for use – so they are an unproven method.

12. Conclusion

Vaccines areamong the most important medical interventionsin human history. Development of the vaccines is one of the most astonishing and important applications in the field of immunologyin the lastcentury. It was a major achievement in the prevention of infectious diseases that saved the lives of millions of people. The success of these vaccines has been demonstrated by the number of clinical trials conducted in humans, and by DNA vaccines already licensed in the field of infectious diseases and cancer immunotherapy in the veterinary field. A major advantage of DNA vaccination is the ability to induce both humoral and cellular immune responses. Though DNA vaccines are in the initial phase, it has proven to be promising and effective in many trails against variety of ailments. In the future it can be a boon in addressing several medical disorders.If DNA vaccines prove to be successful, this is really the future of vaccinology.

References

Plotkin, S. A. (2005). Vaccines: past, present and future. *Nature medicine*, *11*(4), S5-S11.

Tianhua, W., and Zhang, N. (2021). https://www.an.shimadzu.com/webinar-Streamline_and_Optimize_your_Analytical_Workflow_for_Vaccine_Testing.html?utm_source=

Delany, I., Rappuoli, R., & De Gregorio, E. (2014). Vaccines for the 21st century. *EMBO Molecular Medicine*, *6*(6), 708-720.

Levine, M. M., & Sztein, M. B. (2004). Vaccine development strategies for improving immunization: the role of modern immunology. *Nature Immunology*, *5*(5), 460-464.

Ilie, C., García, J., & Cardoza, G. (2018). Women's Social Progress in Latin America: Towards the Construction of Equal Societies. *Available at SSRN 3126911*.

Khan, K. H. (2013). DNA vaccines: roles against diseases. *Germs*, *3*(1), 26.

Ljungberg, K., & Isaguliants, M. (2020). DNA Vaccine Development at Pre- and Post-Operation Warp Speed.

Gary, E. N., & Weiner, D. B. (2020). DNA vaccines: prime time is now. *Current opinion in Immunology*, *65*, 21-27.

Gary, E. N., & Weiner, D. B. (2020). DNA vaccines: prime time is now. *Current opinion in Immunology*, *65*, 21-27.

Liu, M. A. (2003). DNA vaccines: a review. *Journal of Internal Medicine*, *253*(4), 402-410.

Lee, L. Y. Y., Izzard, L., & Hurt, A. C. (2018). A review of DNA vaccines against influenza. *Frontiers in Immunology*, *9*, 1568.

Silveira, M. M., Moreira, G. M. S. G., &Mendonça, M. (2020). DNA vaccines against COVID-19: Perspectives and challenges. *Life Sciences*, 118919.

Smith, T. R., Patel, A., Ramos, S., Elwood, D., Zhu, X., Yan, J., ...& Broderick, K. E. (2020). Rapid development of a synthetic DNA vaccine for COVID-19.

Nascimento, I. P., & Leite, L. C. C. (2012). Recombinant vaccines and the development of new vaccine strategies. *Brazilian Journal of Medical and Biological Research*, *45*, 1102-1111.

Pachuk, C. J., McCallus, D. E., Weiner, D. B., & Satishchandran, C. (2000). DNA vaccines--challenges in delivery. *Current opinion in Molecular Therapeutics*, *2*(2), 188-198.

Lewis, P. J., & Babiuk, L. A. (1999). DNA vaccines: a review. *Advances in Virus Research*, *54*, 129-188.

Cedillo-Barrón, L., Mellado-Sánchez, G., & Gutiérrez-Castañeda, B. (2012). Prospects for DNA vaccination and viral diseases. In *Plasmids: Genetics, Applications and Health*.

Munita, J. M., & Arias, C. A. (2016). Mechanisms of antibiotic resistance. *Microbiology spectrum*, *4*(2), 4-2.

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