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The past, current and future trends in DNA vaccine immunisations

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PEER REVIEW

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Comments

This is a valuable and systematic review in which the authors have clearly focused on the scientific and the clinical applications of DNA immunizations. The manuscript was witten in a way that was informative and unbiased with the general and current principles and concept of DNA vaccines technology and provided a summary of the novel approaches to the DNA vaccine, in parallel with its descriptive mechanism(s) of protective immunity induced.
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ABSTRACT

This review focuses on DNA vaccines, denoting the last two decades since the early substantiation of preclinical protection was published in Science in 1993 by Ulmer *et al*. In spite of being safely administered and easily engineered and manufactured DNA vaccine, it holds the future prospects of immunization by inducing potent cellular immune responses against infectious and non-infectious diseases. It is well documented that injection of DNA plasmid encoding a desired gene of interest can result in the subsequent expression of its products and lead to the induction of an immune response within a host. This is pertinent to prophylactic and therapeutic vaccination approach when the peculiar gene produces a protective epitope from a pathogen. The recent studies demonstrated by a number of research centers showed that these immune responses evoke protective immunity against several infectious diseases and cancers, which provides adequate support for the use of this approach. We attempt in this review to provide an informative and unbiased overview of the general principles and concept of DNA vaccines technology with a summary of a novel approach to the DNA vaccine, present investigations that describe the mechanism(s) of protective immunity provoked by DNA immunization and to highlight the advantages and disadvantages of DNA immunisation.

KEYWORDS
Plasmid, DNA, Clinical trials, Vaccine, Bioechnology, Immunotherapy

1. Introduction

One of the most astonishing and important applications in the field of immunology in the last century was the invention and development of the vaccines. It was a significant leap forward in the prevention of infectious diseases that saved the lives of millions of people. The principal study started in this field was the experiment conducted by Edward Jenner in 1798, when he demonstrated that inoculation with pus from cowpox lesions was conferring protection and assurance against smallpox infection. This was a milestone ever in the history of immunology[1]. Subsequently, this prompts to the extermination of the smallpox through an innovative contribution to immunization[2].

As a matter of fact these experiments establish the premise of vaccinology, the principle of isolation, inactivation, and administration of disease causing pathogens and hence treatment of infectious diseases. Later, there has been nonstop advance in producing safe and highly efficient vaccines against a number

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of common diseases. These vaccines contained bacterial toxoids (diphtheria and tetanus); killed entire organisms (*e.g.* typhoid, cholera, pertussis and the Salk polio vaccine); or live attenuated organisms (reduce its pathogenicity) (*e.g.* Bacillus Calmette Guerin, yellow fever, the Sabin polio vaccine, measles, mumps and rubella)[3].

Currently, with the advance in the biotechnology and the utilization of novel techniques in molecular biology, it is conceivable to make new vaccines. For instance, the utilization of yeast cell to express hepatitis B antigens was the first and strikingly fruitful recombinant protein vaccine. This vaccine has been highly effective in preventing hepatitis B viral infection and thusly became the first vaccine, which has the capability to prevent a human cancer, the hepatocellular carcinoma, associated with early-acquired, persistent hepatitis B infection[4].

The successful vaccine gives a fruitful opportunity to use it not just as a part of the term prophylaxis of infectious diseases but also to broaden their purposes in controlling existing and persisting infectious diseases. For instance, vaccines are being investigated as an approach to control HIV and other incessant viral infections as well as treatment of cancer and autoimmune ailments[5-7].

In spite of all these accomplishment underway in production of vaccines, there are major challenges facing with difficulties, constraints and drawbacks confronting vaccination.

Researchers were unable to produce a vaccine for pathogens with antigenic hypervariability including serogroup B meningococcus, HIV and HCV) or against pathogens with an intracellular phase, causing infections that are transcendently controlled by T cells, such as tuberculosis and malaria[8]. Likewise, development of conventional vaccination can be time and labor intensive, not permitting a quick action to the need of a new vaccine, as in the occurrence of an influenza pandemics. Also there are likewise hypothetical safety concerns linked with the approaches of using both non-live and attenuated concepts[9,10]. To overcome all these challenges, new approaches amid the most recent 30 years have been applied to vaccine advancement. These updated approaches in vaccination technology included recombinant DNA, polysaccharide chemistry and more recently reverse vaccinology, structural vaccinology, and synthetic RNA vaccines are all opening up the perspective for the outlining and advancement of "third generation" vaccines, beforehand characterized as impossible to make[11].

2. Conventional vaccine

Conventional vaccines or traditional vaccines based on inactivated or live attenuated microorganisms or on purged pathogen subunits, such as toxins, polysaccharides and proteins, have been extremely productive in averting infections of pathogens. The mechanism by which these vaccines works are fundamentally by inspiring functional antibodies that can neutralize viral invasion, neutralize bacterial toxins and induce opsono-phagocytosis or complement-dependent bacteriolysis[12,13].

Today most of the licensed vaccines are conventional vaccine. The current use of these vaccines leads to extraordinary accomplishments, such as the annihilation and the virtual disappearance of smallpox and diseases like diphtheria, tetanus, poliomyelitis, pertussis, measles, mumps, rubella and intrusive *Haemophilus influenzae* type B, increasing the life quality and expectancy[12,13]. However, using conventional vaccines was time consuming and taken a years or decades of research. Also, some microorganisms are difficult to cultivate or even to attenuate that brings about adverse or undesirable immune responses demonstrating that these approaches are unfeasible in some instances[14]. Moreover, the vast majority of the techniques used so far to acquire and purge the target antigen were failed which result in less suitable vaccine candidates[15].

3. Polysaccharide chemistry and glyco-conjugate vaccines

In the course of the most recent decades, capsular polysaccharides have been successfully used in the preparation of antibacterial vaccines. The commercialisation of several polysaccharide-protein conjugate vaccines was a breakthrough aimed at filling the gaps in many areas, which can prevent most childhood deaths. Immunisation by vaccines made out of plain bacterial polysaccharides has been acquainted subsequent to the 1970s to control diseases caused by clinically important bacteria such as *Haemophilus influenzae* type B, *Streptococcus pneumoniae* (*S. pneumoniae*) and *Neisseria meningitidis* (*N. meningitidis*) group[16-18].

Carbohydrates are substantial molecular destination in the evolution of vaccines against cancer, viral and bacterial infections, and many other diseases. However, one of the major immunological problems faced in the development of polysaccharide vaccines has been referred or due to that carbohydrates are usually poorly immunogenic and cannot induce a T cell-dependent immune response that is necessary for protective immunity and therefore, it is less effective especially in children (aged below two years) and infants who represent the main target population of vaccination[19,20].

To solve this dilemma of poor immunogenicity, the carbohydrate molecules have to be coupled to a carrier protein, to enhance their immunogenicity. By facilitating access to structures of increasing complexity many carbohydrate-protein coupling techniques have been applied to develop several polysaccharide-protein conjugate vaccines, which filled the gap in many areas, especially for children and infant vaccination. The current progress in glycol-chemistry has facilitated the design of adequate and highly sophisticated glyco-conjugate vaccines using synthetic saccharide components, which are imitative epitopes that naturally involved such protection[21].

One of these approaches is an establishment of vaccine based on the linkage of an orthogonal azido-protein (the univalent group N3- derived from hydrazoic acid) group to the carbohydrate molecule during and after their syntheses; such univalent group can be selectively reduced to a free amino group, to which a N-pentenoyl protecting group can be distinctly and region expressly linked. Since the azido group is orthogonal to most conversions that implicated in carbohydrate synthesis, it can be introduced at an early stage of the synthesis. Furthermore, the link between the 4-pentenoyl group and the carbohydrate molecule after synthesis would somewhat facilitate the synthetic design of complex carbohydrates, including the design of protecting strategy[22]. Subsequently, immunisation with protein–polysaccharide conjugate vaccines has the capacity to incite a long last immune response, with high affinity IgG antibodies and with the ability to be boosted by subsequent immunizations[18,23,24]. Protein–polysaccharide conjugate vaccines were introduced in the 1980s against *Haemophilus influenzae* type B, inducing a better and persistent antibody response in all age groups[25-27]. Today, different approaches to prepare conjugate vaccines can be followed and adequate glyco-conjugate vaccines are available for *S. pneumoniae* and the different strains of *N. meningitidis*[28,29].

On the other hand, although the advancement made in the innovation of glyco-conjugate vaccines made the successful control of distinctive bacterial infections conceivable, this approach could not be utilised to develop *N. meningitidis* type B (MenB) vaccine[30]. Regardless of the availability of effective antibiotics MenB, is a major cause of meningitis and sepsis subversive diseases that can kill children and young adults within hours[31]. It is a Gram-negative bacterium part of the commensal flora that colonizes the upper respiratory tract of healthy individuals. In a small proportion of cases, the bacterium can invade the host bloodstream and, after crossing the blood–brain barrier, cause meningitis[32,33]. The unsuccessful endeavor of developing a MenB vaccine in view of its capsular polysaccharide was to a great extent because of the fact that it is identical to the polysialic acid present in human glycoproteins, for example, neural cell adhesion molecule. Numerous endeavors coordinated to the development of a protein-based vaccine were all disappointed by the inconsistency of the data probably due to the utmost variability of the well-known surface proteins examined as vaccine antigens.

Generally speaking new strategies and approaches may open new perspectives in vaccine research devoted to prophylactic and/or therapeutic applications against bacterial, fungal, parasitic or viral infections, and certain cancers.

4. Reverse vaccinology

A critical upheaval in vaccine disclosure is connected to the approach of genome sequencing innovations that have changed the scene in the gradually advancing field of immunology. The defining moment was the publication documented in 1995 of the genome arrangement of the first living organism[34]. By sequencing the genome and by characterizing the entire antigenic repository of the infectious microorganism, several contender protective targets could be distinguished and tested for their suitability as vaccine. The technique, named reverse vaccinology, has implemented a change in the viewpoint of vaccine design. The thought of the reverse vaccinology was started to conquer the issues confronted to develop vaccine with high adequacy against MenB. The genome sequencing of the MenB pernicious strain MC58 permitted a selection to choice for the potential vaccine targets from the genomic data bank[35,36]. The precept at the foundation of the reverse vaccinology path was that, felicitous vaccine targets were proteins either expressed on the surface of the microorganism or excreted into the extracellular environs. About 600 surface-exposed proteins were predicted and successfully expressed using bioinformatics analysis. Of these, about 350 were cloned in *Escherichia coli* (*E. coli*), expressed and used to immunize animal model. The sera of such animals were examined using a bactericidal assay that is well known to correlate with protection. This screening procedure allowing a selection criteria which are necessary in order to select the most feasible candidates to be discarded are not satisfying quality benchmark. Therefore, the process elicited the identification of previously obscure vaccine candidates. Through this process three protective antigens that are common to multiple MenB strains have been filtered and characterised and named as factor H-binding protein, *Neisseria* adhesin A, and neisserial heparin-binding antigen and combined with a MenB outer membrane vesicle, resulting in the first universal vaccine against MenB[37,38].

This was the first vaccine developed using reverse vaccinology technology that holds a positive feedback from the European Medicines Agency and has been ratified with the commercial name of Bexsero®. Following the success of this project, the reverse vaccinology technology has been utilized in a wide range of other clinically important pathogens, such as *S. pneumoniae*[39,40], *Streptococcus pyogenes*[41], *Chlamydia pneumonia*[42], *Chlamydia trachomatis*[43], *Streptococcus agalactiae*[44], *E. coli*[45], and *Leishmania major*[46]. Consequently, the genome-based reverse vaccinology approach can rig out adequate and innovative strategies to design vaccines that were found to be difficult or even unattainable to develop using conventional approaches[46].

5. DNA immunization

Genetic immunization or DNA vaccination, a rapidly developing technology which has been described as a third generation of vaccines[47], offers new approaches for the prevention and therapy of several diseases of both bacterial and viral origin[48,49]. DNA

immunisation has also emerged in the last decade as a strikingly novel approach to immunoprophylaxis[50], and has been widely used in laboratory animals and non-human primates over the last decade to induce antibody and cellular immune responses[51].

Successful *in vivo* transfection of mammalian cells following injection of purified DNA was first reported over 40 years ago[52]. However, its potential went largely unrealised until 1990 when Wolff and colleagues demonstrated that a reporter gene encoding an enzyme protein could be expressed in murine skeletal muscle *in vivo* and the tissue retained its transgenic biological activity for up to 60 days after inoculation[53]. These observations were extended by several studies such as those of Tang *et al*., (1992) who demonstrated that mice injected with plasmid DNA encoding human growth hormone elicited antigen-specific antibody responses[54]. Based on these findings, it is concluded that this technology is promising as it can enhance both cellular and humoral immunity against parasites, bacteria and disease-producing viruses[55-57].

5.1. Advantages of DNA vaccines

The ability of plasmid DNA to induce both cellular and humoral immune responses after inoculation has been demonstrated in several animal models, and hopes have been raised that its applications will lead to new therapies for a range of human diseases[58,59]. Since the first published report on the protective immune responses against infectious diseases in animals, several studies were performed to evaluate the safety and immunogenecity of DNA vaccination in humans, and many studies are still ongoing up to date.

It is potentially cheaper to produce than recombinant protein vaccines. It is much easier to transport and use, especially in developing countries, DNA-based immunisation exhibits several important advantages over conventional immunisation strategies that involved live-attenuated or killed pathogens, proteins, or synthetic peptides. It incorporate many of the most attractive features of each approach. One of the important advantages of the DNA immunisation[60], is that the immune response to immunisation can be directed to elicit either humoral or cellular immune responses or both without the need for live vectors or complex biochemical production techniques. Other advantages of DNA vaccines are that they are highly specific and the expressed immunizing antigen is subjected to the same glycosylation and post-translational modifications as natural viral infection. Moreover, it is relatively easier to insert multiple variants of an antigen into a single array of plasmid vaccine[61-63]. Candidate bacterial antigens can now be chosen from genomic sequences and plasmid vaccines permit much simpler taking advantage of this new data than the alternative of developing a good expression system for each antigen and then setting up the recombinant protein. This is a considerable advantage for curative vaccination against tumor antigens which may be identified only as DNA sequences produced from both human and cancer genomes[61-63].

Logistic advantages of DNA vaccines include the relative ease and low cost of production and transportation making them more suited to production in the developing world than other systems. A summary of these perceived advantages of DNA vaccines is illustrated in Figure 1[64].

		DNA Vaccine	Live attenuated	Killed/protein subunit
Immune response				
(i) Humoral → B cells		+++	+++	+++
(ii) Cellular → $CD4^+$		Th2	Th1	Th1
(ii) Cellular → $CD8^+$		++	+++	-
		MHC class	**MHC class**	**MHC class**
Antigen presentation		I&II	I&II	II
(iii) Memory →	- Humoral	+++	+++	+++
(iii) Memory →	- Cellular	++	+++	+/-
Manufacturing	- Ease of development & production	++++	+	++
	- Cost	+++	+	+
	- Transport & Storage	+++	+	+

Figure 1. Summary of relative advantages of DNA vaccines over conventional vaccines[64].
MHC: Major histocompatibility complex.

5.2. Disadvantages of DNA vaccines

The disadvantages of DNA vaccines are based mainly on health and safety issues. Most of the safety issues concerning the system are based on the activation of oncogenes as a result of genomic incorporation of immunising DNA[65], as well as eliciting anti-DNA antibodies; however, this has rarely been detected in experimental studies[60]. While these issues are of concern and require careful monitoring, it would not be applied to DNA immunisation of captive animals to produce antibodies, particularly if gene gun is used. This is due to the likelihood of eliciting anti-DNA antibodies when use of the gene gun is minimised because it requires 100-fold less DNA than intramuscular injection to achieve equivalent seroconversion efficiencies[66]. Other drawback of plasmid vaccines is the reduced level of immunogenicity[67]. Therefore, adequate adjuvants will be necessary to overcome this impediment. One of the suggested solutions is to integrate the plasmid, genes for those cytokines such as interleukin 4 or granulocyte-macrophage colony-stimulating factor that enhances immune responses or for C3d oligomers as an adjuvant for B-lymphocyte cells. Other likely approach may include an ensuing booster immunisation with the relating antigen as a protein.

5.3. Principles of DNA immunisation

DNA vaccination involves the introduction of nucleic acid into host

cells where it directs the synthesis of its encoded polypeptide(s) and stimulates an immune response[68]. Unlike gene therapy, genetic integration is not intended. Indeed, the construction of a DNA vaccine is designed to permit localized, short-term expression of the target antigen.

Although several attempts have been made to study the cellular pathways for the processing of antigens and their presentation to T lymphocytes, the precise mechanism based on cellular and molecular events involved in the induction of immune responses following DNA immunisation are not fully understood[69]. However, it is well documented that the magnitude and type of immune response induced after DNA immunisation are influenced by a number of different parameters, some of which are represented by the type and components of the expression plasmid.

5.4. Essential components of a DNA plasmid

A typical "first generation" DNA vaccine plasmid requires (i) the incorporation of a strong viral promoter to achieve optimal expression in mammalian cells[70], such as cytomegalovirus or simian virus 40 which provide the greatest gene expression; (ii) an origin of replication allowing plasmid propagation in *E. coli*; (iii) a bacterial antibiotic resistance gene (this allows plasmid selection during bacterial culture); and (iv) a transcription-stop sequence such as bovine growth hormone 3'-untranslated region (Figure 2)[70].

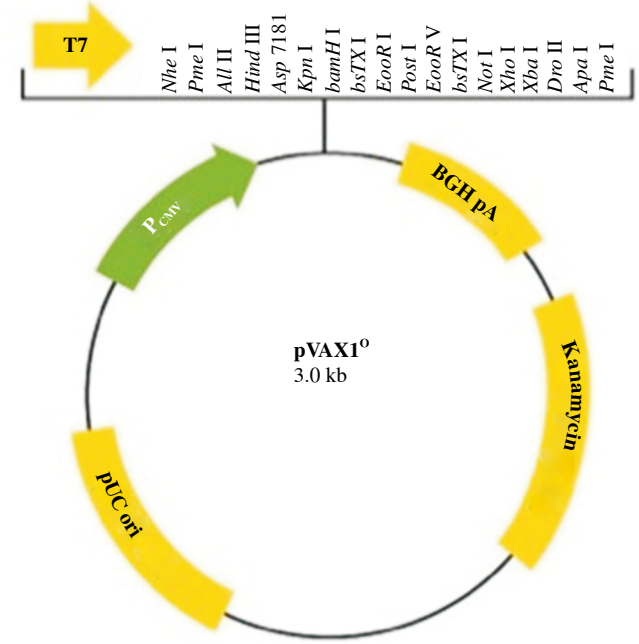

Figure 2. A schematic representation of a simple DNA plasmid[70].

5.5. The influence of mode and site of gene delivery

Several studies have shown that the type of immune responses induced by plasmid immunisation is significantly affected by (i) the mode and site of gene delivery, (ii) the dose of plasmid and (iii) the administration of booster injections and the interval between immunisations[71].

In general, immunisation with DNA can be accomplished in two fundamentally different ways. One approach is the use of needle injection into different tissues, the most effective route being intramuscular injection into the hind leg quadriceps or tibialis anterior[72,73], followed by intradermal injection[74-76]. These routes usually provoke strong, antigen-specific Th1-biased, humoral and cellular immune responses[76,77].

An improvement in efficacy of plasmid transfection was achieved by injection of DNA into regenerating skeletal muscle, achieved by prior injection of either cardiotoxin or local anaesthetic such as bupicaine[51]. Several methods have been investigated to improve delivery of DNA vaccines including (i) mechanical delivery consisting of microinjection by various types of needles including pressure injection, (ii) electrical delivery (electroporation, ionophoresis) and (iii) chemical (liposomes and various polymers) [78-82] and mucosal delivery (Figure 3). Each one of these methods of delivery introduces plasmid DNA into distinct areas of immune surveillance and therefore primes the immune system in distinct ways.

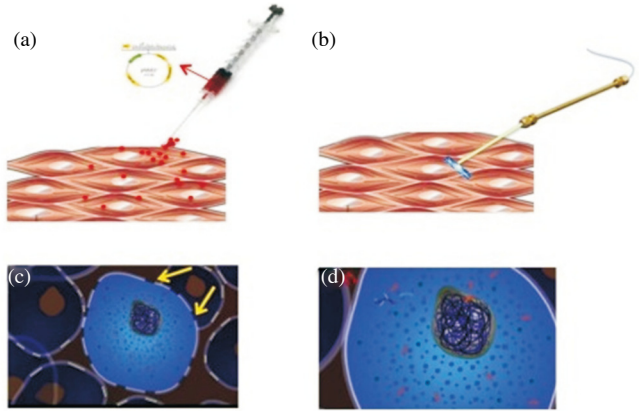

Figure 3. Schematic illustration of electroporation mediated transfection. a: Intramuscular injection; b: Electroporation; c: Transient increased permeability of cell membrane (yellow arrows) results in plasmid transfer into the cell; d: Resting of cell membrane (red arrow).

Gene gun delivery of DNA which propels the DNA-coated gold particles into the epidermis[83,84] resulted in a more Th2 biased antibody isotype response and efficient humoral and cellular responses[76,77]. The distinct Th1- or Th2-biased immune responses elicited by intramuscular injection or gene gun delivery, respectively, are not fully understood. Bacterial DNA contains CpG motifs that induce non-specific Th1-dominant responses. Gene gun delivery requires 100-1000 fold less DNA to stimulate immune responses to that achieved by intramuscular injection. The reduced number of Th1-promoting CpG motifs involved in gene gun immunisation may therefore explain the Th2-bias response to gene gun DNA vaccination.

5.6. Antigen presentation following DNA immunisation

An important step in the design of DNA immunisation constructs is

to understand the immune correlates of protection. Antigen peptides expressed after DNA immunisation are usually presented by antigen-presenting cells (APCs) in the context of either MHC class II or class I molecules to $CD4^+$ and $CD8^+$ T cells, respectively. There are at least three means by which MHC class I–restricted cytotoxic T-lymphocyte (CTL) might be elicited following administration of plasmid DNA: (i) transfection of professional APCs, (ii) antigen presentation mediated directly by transfected myocytes or, (iii) cross priming[64], as illustrated in Figure 4[69].

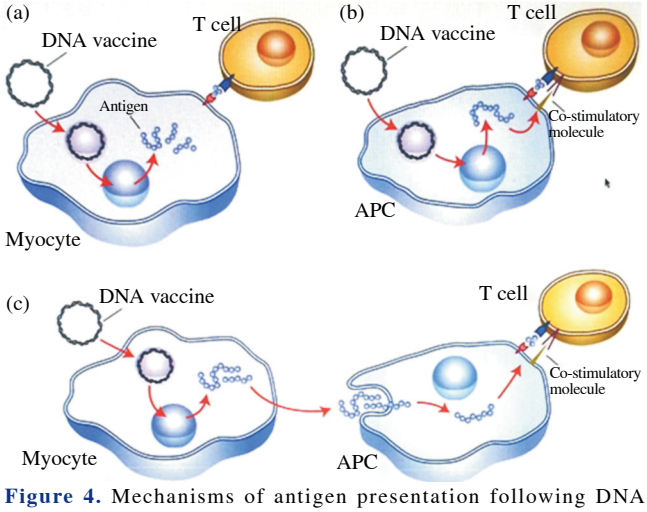

Figure 4. Mechanisms of antigen presentation following DNA immunisation[69].

a: Antigen presentation mediated directly by transfected myocytes; b: Transfection of professional APCs; c: Cross priming.

5.6.1. Transfection of professional APCs

The immune response following DNA immunisation was found to be dependent upon professional APCs, specifically bone marrow-derived dendritic cells (DC)[85]. In 1997, Fu and colleagues[86] demonstrated that when parental bone marrow chimeras were immunised with plasmid DNA encoding influenza nucleoprotein by intramuscular injection and gene gun, CTL responses were specific to the peptide presented by the MHC class I molecules found on the donor bone marrow[87] (Figure 4b)[69]. Furthermore, the same authors reported that, although only a small proportion of the DCs were transfected with plasmid DNA, it was noticeable that there was general activation (maturation) and migration of large number of DCs that had not been transfected. However, whether this generalised maturation of untransfected DCs could also present antigen via additional mechanisms remains uncertain.

5.6.2. Antigen presentation mediated directly by transfected myocytes

Ulmer *et al*. (1993)[88], by demonstrating that direct intramuscular inoculation of plasmid DNA induced a strong $CD8^+$ CTL to influenza nucleoprotein, provided the first evidence that cellular responses could be induced *in vivo* by DNA immunisation and the induced immune responses had a potentially important protective role. Subsequent experiments were then undertaken to directly test whether DNA-transcribed muscle cells alone are sufficient to prime immune response (Figuer 4a)[69]. However, Iwasaki *et al*. (1997)[89], reported that muscle cells failed to prime CTLs responses when injected with DNA plasmid encoding CD86 or granulocyte-macrophage colony-stimulating factor only (*i.e*., without antigen). To examine the contribution of both bone marrow- and non-bone marrow-derived cells to CTL priming, Agadjanyan *et al*. (1999)[90] found that antigen-specific CTL responses could be induced by non-bone marrow-derived (muscle) cells only when mice were immunised with DNA encoding either the antigen or CD86. Surgical ablation experiments have been used to identify the contribution of antigen expression in tissues subjected to DNA immunisation by distinct routes. Torres *et al*. (1997) demonstrated that removing the DNA-injected muscle bundle within 10 min of DNA injection had no effect on the longevity and magnitude of humoral and CTL responses[91], suggesting a rapid migration of transfected cells or plasmid DNA from the site of injection. These authors also found that excision of the epidermal site 24 h after gene gun bombardment abrogated the induction of CTL responses, suggesting that the immune response was dependent on the transfected epidermal cells. This finding indicates that intramuscular injected plasmid DNA is likely to gain rapid access to the lymphatic or circulatory system, thus obviating the need for transfection of muscle cells at the site of injection. In conclusion, these data indicate that DCs such as Langerhans and myocytes play a crucial role in the primary response triggered by DNA vaccines.

5.6.3. Cross priming

Cross priming has been suggested as a mechanism to explain antigenic transfer from DNA-transfected somatic cells to professional APCs (Figure 4c)[69]. The concept of cross priming, in which triggering of $CD8^+$ T-cells responses can occur without *de novo* antigen synthesis within the APCs, was first described by Ulmer (1996)[92] and Fu (1997)[86] and provides an additional mechanism by which DNA immunisation can enhance immune responses. During cross priming, antigens or peptides expressed by DNA-transfected myocytes or DCs presented in context of either MHC I or II can be taken up by professional APCs to prime T-cell responses. Thus, DNA-transfected myocytes or DCs may serve as antigen-producing "factories" which magnify and maintain the immune response via cross priming[93,94].

6. Strategy of DNA immunisation in the development of clinical trial

The remarkable advance and diverse applications of DNA immunisation attracted the attention of many researchers as an alternative procedure for analysing the structure and expression of genes in general[95], studies for improving the treatment of several diseases[96-99], and clinical trials soon ensued.

Owing to the promise of DNA vaccines in studies using small animal model, clinical trials were soon ensued. The primary of a

few of phase I trials, performed almost 2 decades back, assessed the adequacy of a DNA vaccine targeting HIV-1 for therapeutic and prophylactic usage[100]. Subsequent studies conducted after that focused on other diseases such as cancer, human papillomavirus, hepatitis, malaria, influenza, and other HIV-1 antigens.

Nonetheless, the results of these early clinical trials were thwarting. The DNA vaccines were intact and well abide, yet they turned out to be inadequately immunogenic. The antibody titers induced has been found to be very low or absent; CD81 T-cell responses were desultory, and CD41 T-cell responses were of low frequency. However, these studies provide substantiation of connotation that DNA vaccines could safely induce immune responses[100]. Numerous improvements have been integrated into the present DNA vaccines, and these improvements have assist to gleam a revival of interest in the dais. Although the subsequent or the second generation DNA vaccines seem to influence towards both humoral and cellular immune responses regardless of animal models used, researchers suggested that new modified DNA vaccines can be more efficient by broadly activate $CD8^+$ CTLs in larger animal models, compared with previous approved DNA methods[101]. The reduced level of immunogenicity of precocious DNA vaccines is speculated to stem, due to the inefficiency of cellular uptake of the inoculated plasmids.

Current research is focusing on developing neoteric approaches to promote transfection competence and improve other facets of the DNA platform. Such neoteric approaches involve optimization of the antigens encoded by the plasmids to increase antigen expression on a per-cell basis, enhance formulation, and inclusion of molecular adjuvants to promote and direct immune responses[99]. Up to date there are about 43 clinical trials evaluating DNA vaccines for viral and non-viral ailments recorded in the gene database clinical trials. The majority of the recorded trials are investigating vaccines for HIV and cancers. The remaining are investigating vaccines for human papillomavirus, malaria, influenza, and hepatitis B and C viruses[99]. Furthermore, in the available trials there is currently a lack of long-term follow up. Ideally, the availability of data from randomized clinical trials featuring robust end points such as biochemical response, progression free and overall survival will provide categorical evidence for DNA vaccination's potential.

7. Conclusions

Development of the vaccines is one of the most astonishing and important applications in the field of immunology in the last century. It was a major achievement in the prevention of infectious diseases that saved the lives of millions of people. currently most of the licensed vaccines are conventional. DNA vaccine is potentially cheaper to produce than recombinant protein vaccines. It is much easier to transport and use, especially in developing countries. Importantly, DNA-based immunisation exhibits several advantages over conventional immunisation strategies that involved live-attenuated or killed pathogens, proteins, or synthetic peptides. It incorporate many of the most attractive features of each approach. One of the important advantages of the DNA immunisation is that the immune response to immunisation can be directed to elicit either humoral or cellular immune responses or both without the need for live vectors or complex biochemical production techniques. The 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. However, these issues are of concern and required to be resolved based on both scientific and clinical research studies.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

The review is mainly focused on the scientific and clinical approach in both the conventional and the new approach of DNA vaccines. During the last two decades, there has been formidable progress in the field of DNA immunization. This has been an outcome of new and better vectors, distinctive types of delivery strategies, which is activated by either the expression of DNA plasmid itself and/or by encoded proteins. The present review brings together primary data and up-to-date summaries and outlines the great leaps forward in utilizing DNA plasmids for immunizations and immune-therapies.

Research frontiers

The present review depicts and illustrates the past and current trends of DNA vaccine, denoting the last two decades since the early substantiation of preclinical protection of vaccine. In general the present review brings together primary data and up-to-date summaries and outlines leaps forward in utilizing DNA plasmids for immunizations and immune-therapies. In addition the review gives sufficient information for the clinicians as well as the researchers to exploit the therapeutic drugs for the prophylaxis against the untreated diseases.

Related reports

A large number of articles are available on immunization and DNA vaccination. However, the present work is different from previously published reports and reviews in the fact that it gives detailed information of the scientific and clinical applications as well as the updated technology of both conventional and DNA immunization. Also it reflects the basis of recent and excellent research works in such field with an excellent flow of data since an early stages of immunization.

Innovations and breakthroughs

The authors have tried to present the history, current work and the future trends of immunisation with the new technology of DNA vaccine. The authors also illustrated the main advantages and

disadvantages of the old and new era of immunization and DNA vaccine in general, showing that the current conventional treatments are not satisfactory and are full of adverse effects. Moreover, the authors have taken note of the old and current reports with adequate references to build strong statements and polymerize our knowledge on the concept of DNA immunization by providing an excellent prediction of the future of DNA vaccination to understand. It is really interesting to see a review that summarizes and brings together all what has been said from an early stage of immunisation to the prediction of our future concept on DNA technology and vaccination.

Applications

From the literature survey, the authors tried to demonstrate the applications with their drawbacks based on DNA vaccination, and showed the future prospects that whether or not it is safe or need an attention to rectify any major or minor issues related to human health and safety.

Peer review

This is a valuable and systematic review in which the authors have clearly focused on the scientific and the clinical applications of DNA immunizations. The manuscript was witten in a way that was informative and unbiased with the general and current principles and concept of DNA vaccines technology and provided a summary of the novel approaches to the DNA vaccine, in parallel with its descriptive mechanism(s) of protective immunity induced.

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