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Review on Gene-Based Vaccines

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Abstract:

DNA vaccines are third generation vaccines, made up of small, circular pieces of bacterial DNA, plasmids. These plasmids are genetically engineered to produce specific antigens from a pathogen. The DNA is injected into the cells of the body where the host cell then interprets the DNA and uses it to synthesize the pathogen's proteins. An immune response is then triggered when the pathogen's proteins are recognized as foreign to the immune system. DNA vaccines have shown efficacy in preclinical animal models in preventing or even treating a variety of diseases caused by infectious agents, malignancies or immunological disorders. One of the main perceived advantages of DNA vaccines for use in less developed countries is their low cost. Nevertheless, in general, immune responses elicited by DNA vaccines are less potent than those induced by traditional vaccines or second generation viral recombinant vaccines.

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1. INTRODUCTION

Immunity is the defense mechanism that works in the body to fight against a disease or infection in order to over-come it. The principal architects of vaccination were Edward Jenner, Louis Pasteur, Robert Koch, Emil von Behring and Paul Ehrlich. Jenner established a relationship between cow pox

and small pox. He observed that exposure to cow pox was able to elicit immunity against small pox. In fact, the term vaccination was derived from "vacca" the Latin word for cow.

It was discovered almost 20 years ago that plasmid DNA, when injected into the skin or muscle of mice, could induce immune responses to encoded antigens. Since that time, there has since been much progress in understanding the basic biology behind this deceptively simple vaccine platform and much technological advancement to enhance immune potency. Among these advancements are improved formulations and improved physical methods of delivery, which increase the uptake of vaccine plasmids by cells; optimization of vaccine vectors and encoded antigens; and the development of novel formulations and adjuvants to augment and direct the host immune response. The ability of the current, or second-generation, DNA vaccines to induce more-potent cellular and humoral responses opens up this platform to be examined in both preventative and therapeutic arenas. This review focuses on these advances and discusses both preventive and immunotherapeutic clinical applications. In 1993, Ulmer et.al. worked on Heterologous protection against influenza by injection of DNA encoding a viral protein**.** Cytotoxic T lymphocytes (CTLs) specific for conserved viral antigens can respond to different strains of virus, in contrast to antibodies, which are generally strain-specific. The generation of such CTLs in vivo usually requires endogenous expression of the antigen, as occurs in the case of virus infection. This resulted in the generation of nucleoprotein-specific CTLs and protection from a subsequent challenge with a heterologous strain of influenza A virus, as measured by decreased viral lung titers, inhibition of mass loss, and increased survival [1].

In 2011 Liu and his coworker worked on an historical perspective and view to the future. This review provides a detailed look at the attributes and immunologic mechanisms of plasmid DNA vaccines and their utility as laboratory tools as well as potential human vaccines. The immunogenicity and efficacy of DNA vaccines in a variety of preclinical models is used to illustrate how they differ from traditional vaccines in novel ways due to the in situ antigen production and the ease with which they are constructed. This introductory article provides an overview of the field and highlights the observations of the articles in this special issue while placing them in the context of other recent publications [2].

Ledgerwood et.al [3] expressed that West Nile virus (WNV) is a flavivirus transmitted primarily by mosquitoes to a variety of vertebrate hosts. Flaviviruses are positive-stranded RNA viruses and include important human pathogens such as yellow fever virus, St Louis encephalitis virus, dengue virus, and Japanese encephalitis virus (JEV). WNV was initially isolated from a human residing in the West Nile district of Uganda in 1937. The virus is present throughout Africa, Asia, the Middle East, and the Americas. WNV was first recognized in the United States in 1999 when it caused an epidemic in New York state. Since 1999, WNV has spread throughout the Americas [2–4]. The incidence in the United States peaked at 9862 cases in 2003. The infection is now considered endemic in the United States and in 2009 there were 720 reported cases [5, 6].

WNV is an enzootic infection and is maintained in a mosquito–bird transmission cycle; incidental hosts have been identified, including humans, horses, and alligators [3, 8]. The principal form of transmission to humans is from the bite of a mosquito. Person-to-person transmission has been recognized, including blood transfusion, organ transplantation, breastfeeding, and transplacental or

laboratory acquisition [2, 9]. Human illness peaks in late summer or early autumn, reflecting peak viral amplification within the bird–mosquito–bird cycle [1].

WNV infection of humans has been associated with a variety of symptoms from asymptomatic to severe encephalitis. Central nervous system involvement occurs in 1 in 150 patients [10, 11]. Care is supportive but intravenous immunoglobulin, alpha interferon, and ribavirin have been investigated for severe cases [12, 13]. One investigational therapy with potential for benefit is a humanized monoclonal antibody, Hu-E16, which binds to the envelope protein of WNV and has shown efficacy in preclinical testing and safety in clinical testing [14–16].

As vaccines are developed, consideration for those at greatest risk is a priority. For WNV, advanced age is a risk factor for severe disease [14]; however, the mechanism for increased susceptibility in the elderly and immunocompromised remains unknown. Published data suggest a role for antibody in protection and clearance of flavivirus infections [12, 14]. In vitro data also implicate dysregulation of toll-like receptor 3 (TLR3) in macrophages in the elderly, leading to higher cytokine (interleukin [IL]-6, interferon [IFN]-β, tumor necrosis factor [TNF]-α) levels, which are associated with higher viral burdens in macrophages and facilitation of WNV entry into the cerebrospinal fluid secondary to blood-brain barrier disruption. In contrast, in young adults, TLR3 expression declines during WNV infection, diminishing WNV entry and cytokine release [10]. In general, vaccines induce decreased immunity in the elderly [3-7]. Taken together, these data describe immunosenescence, an age-related change in immunity, which may impact the predilection of the aged to become seriously affected by WNV and is a possible reason for the generalized decreased vaccine efficacy seen in older adults [15-16].

2. HISTORY OF DNA VACCINES

DNA vaccines first sparked the interested of the scientific community in the early 1990s, when it was reported that plasmid DNA, delivered into the skin or muscle, induced antibody responses to viral and nonviral antigens. The simplicity and versatility of this vaccine approach generated a great deal of excitement and inspired additional preclinical studies targeting a plethora of viral and nonviral antigens. In theory, DNA vaccines could generate broad immune responses, similar to the live-attenuated virus platform, without the need for a replicating pathogen. [6]

Owing to the promise of DNA vaccines in small animal studies, clinical trials soon ensued. The first of several of phase I trials, conducted almost 2 decades ago, evaluated the efficacy of a DNA vaccine targeting human immunodeficiency virus type 1 (HIV-1) for therapeutic and prophylactic applications. Other studies shortly followed that targeted cancer or other HIV-1 antigens, influenza, human papillomavirus (HPV), hepatitis, and malaria. However, the results of these early clinical trials were disappointing. The DNA vaccines were safe and well tolerated, but they proved to be poorly immunogenic. The induced antibody titers were very low or nonexistent, $CD8^+$ T-cell responses were sporadic, and $CD4^+$ T-cell responses were of low frequency. However, these studies provided proof of concept that DNA vaccines could safely induce immune responses (albeit low-level responses) in humans.

2.1. What is a DNA vaccine?

A DNA vaccine uses a gene from a virus or bacteria to stimulate the immune system. When the DNA vaccine is administered to a patient, the machinery in their cells makes a viral or bacterial protein which their immune system recognises as being foreign to the body. Like any vaccine the immune system will then recognise the bacteria or virus in the future – hopefully preventing illness. [4]

2.2. Recombinant DNA technology [9]

Joining together of DNA molecules from two different species that are inserted into a host organism to produce new genetic combinations that are of value to science, medicine, agriculture, and industry. Since the focus of all genetics is the gene, the fundamental goal of laboratory geneticists is to isolate, characterize, and manipulate genes. Although it is relatively easy to isolate a sample of DNA from a collection of cells, finding a specific genewithin this DNA sample can be compared to finding a needle in a haystack. Consider the fact that each human cell contains approximately 2 metres (6 feet) of DNA. Therefore, a small tissue sample will contain many kilometres of DNA. However, recombinant DNA technology has made it possible to isolate one gene or any other segment of DNA, enabling researchers to determine its nucleotide sequence, study its transcripts, mutate it in highly specific ways, and reinsert the modified sequence into a living organism.

2.3. Steps Involved

2.3.1. DNA Cloning

In biology a clone is a group of individual cells or organisms descended from one progenitor. This means that the members of a clone are genetically identical, because cell replication produces identical daughter cells each time. The use of the word *clone* has been extended to recombinant DNA technology, which has provided scientists with the ability to produce many copies of a single fragment of DNA, such as a gene, creating identical copies that constitute a DNA clone. In practice the procedure is carried out by inserting a DNA fragment into a small DNA molecule and then allowing this molecule to replicate inside a simple living cell such as a bacterium. The small replicating molecule is called a DNA vector (carrier). The most commonly used vectors are plasmids (circular DNA molecules that originated from bacteria), viruses, and yeast cells. Plasmids are not a part of the main cellular genome, but they can carry genes that provide the host cell with useful properties, such as drug resistance, mating ability, and toxin production. They are small enough to be conveniently manipulated experimentally, and, furthermore, they will carry extra DNA that is spliced into them [9-10].

2.3.2. Creating the clone

The steps in cloning are as follows. DNA is extracted from the organism under study and is cut into small fragments of a size suitable for cloning. Most often this is achieved by cleaving the DNA with a restriction enzyme. Restriction enzymes are extracted from several different speciesand strains of bacteria, in which they act as defense mechanisms against viruses. They can be thought of as

"molecular scissors," cutting the DNA at specific target sequences. The most useful restriction enzymes make staggered cuts; that is, they leave a single-stranded overhang at the site of cleavage. These overhangs are very useful in cloning because the unpaired nucleotides will pair with other overhangs made using the same restriction enzyme. The resulting molecule is called recombinant DNA. It is recombinant in the sense that it is composed of DNA from two different sources. Thus, it is a type of DNA that would be impossible naturally and is an artifact created by DNA technology.

The next step in the cloning process is to cut the vector with the same restriction enzyme used to cut the donor DNA. Vectors have target sites for many different restriction enzymes, but the most convenient ones are those that occur only once in the vector molecule. This is because the restriction enzyme then merely opens up the vector ring, creating a space for the insertion of the donor DNA segment. Cut vector DNA and donor DNA are mixed in a test tube, and the complementary ends of both types of DNA unite randomly. Of course, several types of unions are possible: donor fragment to donor fragment, vector fragment to vector fragment, and, most important, vector fragment to donor fragment, which can be selected for. Recombinant DNA associations form spontaneously in the above manner, but these associations are not stable because, although the ends are paired, the sugar-phosphatebackbone of the DNA has not been sealed. This is accomplished by the application of an enzyme called DNA ligase, which seals the two segments, forming a continuous and stable double helix.

The mixture should now contain a population of vectors each containing a different donor insert. This solution is mixed with live bacterial cells that have been specially treated to make their cells more permeable to DNA. Recombinant molecules enter living cells in a process called transformation. Usually, only a single recombinant molecule will enter any individual bacterial cell. Once inside, the recombinant DNA molecule replicates like any other plasmid DNA molecule, and many copies are subsequently produced. Furthermore, when the bacterial cell divides, all of the daughter cells receive the recombinant plasmid, which again replicates in each daughter cell.

The original mixture of transformed bacterial cells is spread out on the surface of a growth medium in a flat dish (Petri dish) so that the cells are separated from one another. These individual cells are invisible to the naked eye, but as each cell undergoes successive rounds of cell division, visible colonies form. Each colony is a cell clone, but it is also a DNA clone because the recombinant vector has now been amplified by replication during every round of cell division. Thus, the Petri dish, which may contain many hundreds of distinct colonies, represents a large number of clones of different DNA fragments. This collection of clones is called a DNA library. By considering the size of the donor genome and the average size of the inserts in the recombinant DNA molecule, a researcher can calculate the number of clones needed to encompassthe entire donor genome, or, in other words, the number of clones needed to constitute a genomic library.

Several bacterial viruses have also been used as vectors. The most commonly used is the lambda phage.

Isolating the clone

In general, cloning is undertaken in order to obtain the clone of one particular gene or DNA sequence of interest. The next step after cloning, therefore, is to find and isolate that clone among other members of the library. If the library encompasses the whole genome of an organism, then somewhere within that library will be the desired clone. There are several ways of finding it, depending on the specific gene concerned. Most commonly, a cloned DNA segment that shows homology to the sought gene is used as a probe. For example, if a mouse gene has already been cloned, then that clone can be used to find the equivalent human clone from a human genomic library. Bacterial colonies constituting a library are grown in a collection of Petri dishes. Then a porous membrane is laid over the surface of each plate, and cells adhere to the membrane. The cells are ruptured, and DNA is separated into single strands—all on the membrane. The probe is also separated into single strands and labeled, often with radioactive phosphorus. A solution of the radioactive probe is then used to bathe the membrane. The single-stranded probe DNA will adhere only to the DNA of the clone that contains the equivalent gene. The membrane is dried and placed against a sheet of radiation-sensitive film, and somewhere on the films a black spot will appear, announcing thepresence and location of the desired clone. The clone can then be retrieved from the original Petri dishes.

2.3.3. DNA SEQUENCING [11]

Once a segment of DNA has been cloned, its nucleotide sequence can be determined. The nucleotide sequence is the most fundamental level of knowledge of a gene or genome. It is the blueprint that contains the instructions for building an organism, and no understanding of genetic function or evolution could be complete without obtaining this information.

2.4. Recombinant vaccines:

Biotechnology sector has also played its part in developing vaccines against certain diseases. Such vaccine which makes use of recombinant DNA technology is known as **recombinant vaccines**. It is also known as **subunit vaccines.**

Recombinant vaccines can be broadly grouped into two kinds:
(i) **Recombinant protein vaccines**: This is based on production of recombinant DNA which is expressed to release the specific protein used in vaccine preparation
(ii) **DNA vaccines**: Here the gene encoding for immunogenic protein is isolated and used to produce recombinant DNA which acts as vaccine to be injected into the individual.

2.4.1. Steps involved:

Production of recombinant vaccines involves the following steps:
(i) First and foremost, it is important that the protein which is crucial to the growth and development of the causative organism be identified.
(ii) The corresponding gene is then isolated applying various techniques. Further to this, an extensive study of the gene explains the gene expression pattern involved in the production of corresponding protein.

(iii) This gene is then integrated into a suitable expression vector to produce a recombinant DNA.
(iv) This rDNA is used as vaccines or is introduce into another host organism to produce immunogenic proteins which acts as vaccines.

2.5. Characteristics of DNA vaccines

Viruses have highly evolved structures and mechanisms that enable them to introduce their genetic material into infected cells. Therefore, despite emerging evidence in the 1980s, it was not until a 1990 publication by Felgner and colleagues that the ability of simple plasmids of DNA (circular rings of DNA that exist extrachromosomally in bacteria) to directly enter mammalian cells when injected *in vivo* with ensuing synthesis of the protein they encoded was accepted. The plasmid required no formulation or alteration other than a promoter active in mammalian rather than bacterial cells DNA plasmids as gene delivery vehicles have a number of advantages over other systems which involve either removal of cells from an individual in order to transfect them *in vitro* prior to re-implantation of the transfected cells, or which require the manipulation of viruses and bacteria (which are themselves pathogenic, immunogenic or both) – a process significantly more complicated than manipulating and producing plasmids. But there were some concerns regarding their suitability and capability as vaccines. One of them arose from the original observation by Felgner and colleagues that the *in vivo* transfection of cells was still an inefficient process Moreover, the cell type that took up the DNA and produced the encoded protein most efficiently were muscle cells, a cell type that under normal conditions is not involved in the generation of immune responses.

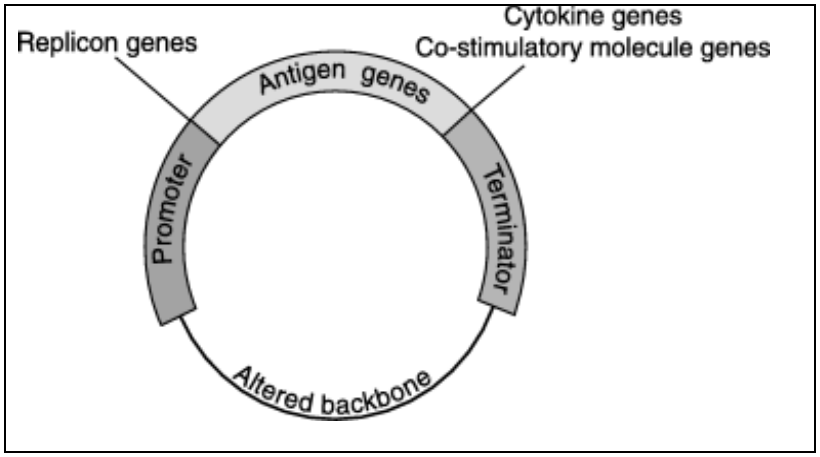

Fig 1:

A schematic representation of a DNA vaccine. DNA vaccines are bacterially derived plasmids containing a gene encoding the desired antigen. Expression is driven by a promoter active in mammalian cells (generally a strong viral promoter), a transcription terminator, and often an antibiotic resistance gene that facilitates the selection of the plasmid during production in bacteria. Sites for increasing the potency of DNA vaccines are shown. For example, additional genes encoding cytokines or co-stimulatory molecules can be added to the gene for the antigen. Genes encoding a

viral replicase has been shown to increase the potency of DNA vaccines. Alterations to the plasmid can also result in increased protein production, leading to increased immune responses.

2.6. Advantages of DNA vaccines

They can be made in a short time span – it is easier to make large amounts of a gene than make proteins or grow up bacteria or viruses. Speed is important when making a vaccine to strains of bacteria or virus that are constantly mutating and changing. DNA vaccines are easy to transport and store – DNA is a very stable molecule anddoes not need to be stored at low temperatures making transportation and storage cheaper and easier than conventional vaccines. DNA vaccines may be very cheap to make – it is relatively easy to make and purifylarge amounts of DNA. There is no risk to those who are making the vaccine - some conventional vaccinesrequire growing up the infectious bacteria or virus – and this carries a risk (all be it very small) to those who work making vaccine.

2.6.1. Disadvantages of DNA vaccines

Initial attempts to create DNA vaccines have not worked – they have not had a bigenough impact on the immune system.

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

2.6.2. How is the DNA vaccine administered?

Many DNA vaccines are injected into the muscle, however, a method using a ‘gene gun’ is being developed that uses helium to propel DNA into the cells of the skin. If this is successful it will provide a ‘needle free’ vaccine.

2.6.3. Application

2.6.3.1. Veterinary Applications

A successful species for DNA immunization has been the fish. DNA vaccination against infectious hematopoietic necrosis virus now takes place at commercial level; the DNA vaccine was licensed in 2005 as noted above. Additionally, immunization against a salmon viral hemorrhagic septicemia virus has been demonstrated experimentally. Means to improve uptake and persistence of DNA vaccines in fish by molecular adjuvants or carriers are ongoing.

2.6.3.2. Human Studies [15]

2.3.6.3. Cancer

When cancers result from infection with viruses, such as human papilloma viruses (HPV) or hepatitis B virus (HBV), it is possible to target viral proteins and by preventing infection, to decrease the incidence of the related cancer. Once infection has occurred, it may still be possible to protect against the development or progression of cancer, such as for HPV infection. A number of groups are now targeting the E6 and E7 proteins of HPV, as these so-called oncoproteins play a role in the transformation of infected cells into tumor cells. One such example utilizing DNA vaccines to target

E6/E7 antigens in patients with high-grade cervical lesions due to HPV, resulted in CD8+ T cell responses . Attempts to break tolerance to purely endogenous tumor antigens represented in large amounts on tumor cell surfaces such as alpha-fetoprotein (AFP), carcinoembryonic antigen (CEA) or prostatic acid phosphatase (PAP) have been more difficult to accomplish. Perhaps the most successful example has been Sipuleucel-T licensed in 2010 in the USA for prostate cancer . The patient's own dendritic cells are incubated ex vivo with an endogenous enzyme prostate acid phosphatase (PAP) and the immunostimulating agent GM-CSF; subsequently, the mixture is given back to the patient. For DNA vaccines, a veterinary cancer product is OnceptTM. It is a DNA vaccine encoding the human enzyme tyrosinase, and has been licensed for the treatment of melanoma, in dogs. The human tyrosinase differs from the canine version, enabling tolerance to be broken. In humans, a similar type of heterogenous tyrosinase vaccine, delivered with electroporation, showed increased CD8+ in 40% of the patients at the highest dose . A clinical trial of DNA encoding a modified CEA resulted in some immune responses but unclear tumor reduction . A clinical trial of a DNA vaccine encoding epitopes of Prostate Specific Acid Phosphatase (PSAP) linked to a fragment of tetanus toxin resulted in a doubling of the rate of rise of Prostate Specific Antigen (PSA, a marker for tumor cell growth) . However a separate study of a DNA vaccine encoding rhesus Prostate Specific Antigen did not result in any change in the rate of rise of PSA levels. DNA vaccines may have benefits for the development of idiotype-specific vaccines for B cell lymphomas because DNA vaccines could readily be made directed against the patient's idiotype. More recently, it has been shown in mice that a DNA vaccine could elicit cross-reactive anti-idiotype antibodies directed against human B cell lymphomas.

2.3.6.4. Influenza [7]

Every year, the scientific and medical communities are charged with the task of determining the appropriate influenza strains to include in the seasonal influenza vaccine. Current vaccine platforms require months to generate sufficient quantities of antigens because of the requirement for the growth of the virus in chicken eggs. This can delay the availability of viral stocks or result in a mismatch between the vaccine strains selected and the actual circulating strains. In 2007, the seasonal influenza vaccine coverage was estimated at only 30% because of mismatches between the strains that were expected to emerge and the strains that actually circulated. In contrast, development of a DNA vaccine for a particular influenza strain could shorten this timeline 2–4-fold and could potentially provide a product in a few months with little chance of mismatch. Influenza presents a particular challenge for the DNA platform because protection is specifically associated with antibodies, and induction of humoral responses was a shortcoming of the original DNA vaccines. New approaches incorporated into the second-generation platform have enabled the induction of humoral responses against a variety of antigens. Thus, the development a DNA vaccine for influenza has become a more reasonable goal. One preclinical study of an H5N1 influenza DNA vaccine showed that protective antibody titers were induced to multiple clades of H5N1 using a single consensus H5 antigen . In further support of this cross-protection approach, it has recently been shown that cross-protective titers can be achieved to viruses that circulated over 90 years apart; namely, the 1918 "Spanish Flu" and the 2009 "Swine Flu". The concept of cross-neutralization of different influenza strains may be of great significance in future influenza vaccines. Moreover, this concept applies not only to influenza strains with the potential to cause pandemics but also to

strains included in seasonal vaccines.The success of DNA vaccines against multiple strains of influenza in preclinical models has paved the way for their development for the clinic. To that end, there are currently several DNA-based influenza vaccines in various stages of phas I clinical trials, including vaccines against potentially lethal pandemic strains such as H5N1 (Inovio Pharmaceuticals) and H1N1 (National Institutes of Allergy and Infectious Diseases). A completed phase I clinical trial conducted by Vical demonstrated that formulation of a monovalent H5N1 DNA vaccine in Vaxfectin achieved protective hemagglutination inhibition titers or antibody responses in more than 47% of subjects, and H5-specific T-cell responses were detected in at least 75% of subjects .A phase 1 trial completed by PowderMed demonstrated reductions in disease symptoms and viral shedding in subjects who received a trivalent DNA-based seasonal influenza vaccine, delivered using the PMED device, compared with placebo. The ultimate success of these vaccines could reshape the way physicians and researchers view influenza vaccine development.

3. Conclusions

During the last few years, there has been immense progress in the field of DNA vaccines. This has been a result of new and better vectors, different types of delivery methods and devices, addition of immunologic adjuvants, and harnessing (or decreasing the activation of) the innate system, which is activated by the plasmid DNA itself, and can be further activated by encoded proteins. The combination of DNA vaccines with other vectors for heterologous prime-boost regimes, and selection of optimal diseases/antigens and vaccines also are important for making successful DNA vaccines. The present issue on “Research progress for gene-based vaccines” brings together primary data and up-to-date summaries of breakthroughs in using DNA plasmids for vaccines and immunotherapies.

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