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A Review on DNA Vaccines

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Abstract A DNA vaccine uses foreign DNA to express an encoded protein and stimulate the body's immune system. It represents a new approach to immunization which is potentially less expensive than the old vaccines. The development of DNA vaccines against the pathogens that use veterinary species as host is completely feasible and going on and it is a promising technology in veterinary sciences. There is promising approach for generating desired immunity: cytolytic T lymphocytes(CTL). Certain methods like gene gun and electroporation are used to deliver DNA vaccines but phage mediated immunization is the recent technology to deliver the DNA vaccines that offers an extremely elegant and rapid method for identifying potential vaccine candidates for newly emerging diseases or for pathogens for which protective antigens have not yet been identified. Antigen presenting cells like dendritic cells (DCs) play an important role in increasing the potency of DNA vaccines. Anti-angiogenic DNA vaccine may prove promising strategy for the treatment of the cancer. DNA vaccines undergo through clinical trials to find the way to treat autoimmune diseases, hepatitis, mycobacterial diseases, allergy and malaria.

Keywords DNA Vaccine, Phage Mediated Immunization, CTL, Anti-Angiogenic DNA Vaccine

1. Introduction

Vaccines have prevented more disease than any other modern medical intervention in the medical history. First-generation vaccines were developed by Louis Pasteur by using attenuated and killed forms of microorganisms; the second was the use of defined natural or recombinant components of whole organisms. Both these formulations contain protein or proteinaceous substances. Even if polysaccharides or small organic molecules were used, they were coupled to carrier proteins. Nucleic acid is considered as the "third generation of vaccines"[1].

1.1. Why Need for DNA Vaccines?

Before DNA vaccines there were basically 3 types of vaccines that are also in use today. First are the killed vaccines that are preparations of the normal (wild type) infectious, pathogenic virus that has been rendered non-pathogenic. The immunity induced by these vaccines frequently decreases during the life of the host and may require additional boosters to achieve life long immunity. Second are attenuated vaccines that are live virus particles that grow in the vaccine recipient but do not cause disease because the vaccine virus has been altered (mutated) to a non-pathogenic form. Although live attenuated preparations are the vaccines of choice they do pose the risk of reversion to their pathogenic form, causing infection. Third are the subunit vaccines that are the purified components of the virus, such as a surface antigen but there is potential risk (http://pathmicro.med.sc.edu/lecture/vaccines.htm).

When the immunological issues of the vaccines are considered then it is shown that new efforts to make vaccines emphasis inducing CD8+ cytolytic T lymphocytes (CTL) responses. The typical inactivated virus vaccine cannot produce the desired CTL response and the reason behind this is that generally such a vaccine is taken up by the antigen presenting cell into the endolysosomal system and after degradation it is targeted to major histocompatibility complex (MHC) class II molecules. In order to generate the CTLs, protein synthesized within a virally-infected cell enters a cellular processing pathway from the cytoplasm that results in peptides associating with MHC Class I molecules. These in turn are recognized by the appropriate cytolytic T cells that then can be activated to kill the infected cell. Thus, if one could deliver a gene encoding an antigen into a cell (as a virus does during infection), the protein (in this case an antigen) following synthesis would be in the cytoplasm where some of it would enter the intracellular processing pathway resulting in the presentation of its relevant peptides on MHC Class I molecules for the stimulation of CTL. Deoxyribonucleic acid vaccines are vaccines that, rather than consisting of the antigen itself, provide genes encoding the antigen. The development of DNA vaccines grew from efforts to generate MHC class I-restricted CTL responses by capitalizing on the understanding of different intracellular Ag-processing pathways. It had become understood that proteins synthesized in somatic cells could generate peptides

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that would associate with MHC class I molecules for presentation to $CD8^+$ lymphocytes with their subsequent activation[2].

1.2. Construction of DNA Vaccines

DNA vaccines are composed of bacterial plasmids. Expression plasmids used in DNA-based vaccination normally contain two unites: the antigen expression unit composed of promoter/enhancer sequences, followed by antigen-encoding and polyadenylation sequences and the production unit that is composed of bacterial sequences necessary for plamid amplification and selection[3]. The construction of bacterial plasmids with vaccine inserts is accomplished using recombinant DNA technology. After constructed, the vaccine plasmid is transformed into bacteria, where bacterial growth produces multiple plasmid copies. The plasmid DNA is then purified from the bacteria, by separating the circular plasmid from the much larger bacterial DNA and other bacterial impurities. This purifies DNA acts as the vaccine

1.3. Mechanism of Action

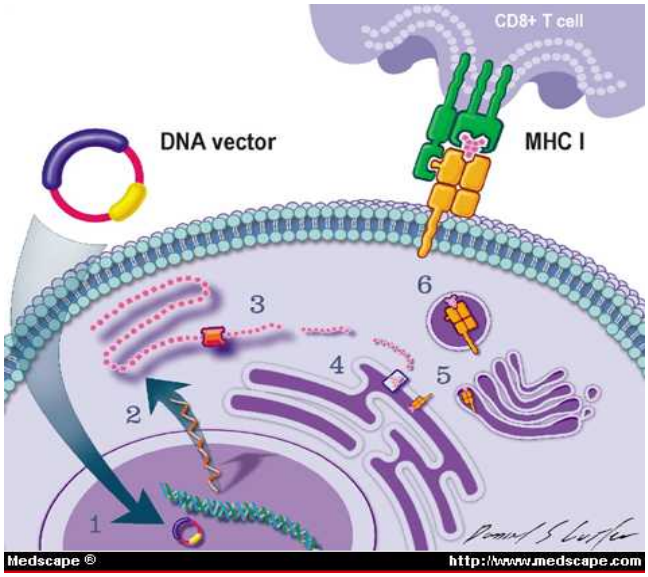

Figure 1. Mechanism of action of DNA vaccines. DNA vaccines favor a cell-mediated immune response. DNA plasmid vector vaccines carry the genetic information encoding an antigen, allowing the antigen to be produced inside of a host cell, leading to a cell-mediated immune response via the MHC I pathway. The plasmid DNA vaccine (above) carries the genetic code for a piece of pathogen or tumor antigen. The plasmid vector is taken up into cells and transcribed in the nucleus (1). The single stranded mRNA (2) is translated into protein in the cytoplasm. The DNA vaccine-derived protein antigen (3) is then degraded by proteosomes into intracellular peptides (4). The vaccine derived-peptide binds MHC class I molecules (5). Peptide antigen/MHC I complexes are presented on the cell surface (6), binding cytotoxic CD 8+ lymphocytes, and inducing a cell-mediated immune response. Because DNA vaccines generate cell-mediated immunity, the hope is that they will be effective against some difficult viruses even as standard vaccines have failed to work
(http://www.medscape.com/viewarticle/408733_8)

The principle of DNA vaccination is quite simple. DNA is isolated and then eukaryotic promotor and terminator are added and after this DNA or an immunogenic gene is inserted into an expression plasmid, which is inserted into cultured cells. The cells are screened for expression of the gene protein and then cultured. The plasmid DNA is then extracted from the cells and purified before being used to immunize a host.

DNA vaccines contain the nucleotides encoding an antigenic portion of the virus such as the viral core region or envelope region. The DNA is taken up into the host cell, translated, and the protein product expressed. Viral protein is made intracellularly and the protein is processed via the endogenous MHC class I pathway. More specifically, the plasmid DNA vaccine carries the genetic code for a segment of pathogen or tumor antigen. The plasmid vector is taken up into cells and transcribed in the nucleus. The single stranded mRNA is translated into protein in the cytoplasm. The DNA vaccine-derived protein antigen is then degraded by proteosomes into intracellular peptides. The vaccine-derived peptide binds MHC class I molecules. Peptide antigen/MHC I complexes are presented on the cell surface where they bind cytotoxic CD 8+ lymphocytes and induce a cell-mediated immune response(as depicted in figure 1). Because DNA vaccines generate cell- mediated immunity, the hope is that they will be effective against some difficult viruses even when standard vaccines have failed to work.

1.4. Safety Issues

The following issues have been raised with regard to DNA vaccines.

Integration into cellular DNA: DNA vaccines currently being tested rarely integrate into cellular DNA. However, as vectors are modified or adjuvanted which are used to strengthen the immune response to a vaccine and have been critical in modern vaccine development, the likelihood of integration could increase. The concern is that an integrated vaccine could result in insertional mutagenesis through the activation of oncogenes or inactivation of tumor suppressor genes. It may result in chromosomal instability through the induction of chromosomal breaks or rearrangements. Food and drug administration (FDA) continues to recommend integration studies for new DNA products[4]**.** It is recommended to perform the preclinical safety studies on every novel DNA vaccines or DNA vaccine/adjuvant combination. These studies are conducted for each plasmid vaccine product using accepted assays in animals prior to the initiation of human trials. Typically, if integration is detected at all, it is found to occur at rates that are orders of magnitude below the spontaneous mutation frequency. Development of autoimmunity: Studies in mice have shown that systemic autoimmunity is unlikely to result from DNA vaccination, and early human studies did not detect increases in antinuclear or anti-DNA antibodies[5]. Participants in human trials of DNA vaccines are followed for possible signs and symptoms of autoimmunity, and laboratory markers of autoimmunity are sometimes monitored as well. To date there has been no convincing evidence of DNA vaccine-associated autoimmunity. Antibiotic resistance: Part of

the production process of DNA plasmids involves selection of bacterial cells carrying the plasmid. This selection is accomplished by culturing the cells in the presence of an antibiotic to which resistance is conferred by a gene in the plasmid. Concern has been raised that resistance to the same antibiotic might be introduced in participants when the plasmid is used in clinical trials. Two precautions should be kept therefore. First, the antibiotic resistance genes contained by vaccine plasmids are driven by a bacterial origin of replication sequence (not a mammalian one) and are therefore expressed only in bacteria, not in host cells. Second, the antibiotic resistance employed does not involve antibiotics commonly used to treat human infections (http://chi.ucsf.edu/vaccines/vaccines?page=vc-01-01).

2. Mechanism of Delivery

There are certain ways to inoculate with plasmid-based vaccines. The first involves direct inoculation into muscle tissue, with the plasmid DNA suspended in a saline (salt) solution ("naked" DNA). Raz, (1998) demonstrated that skin and mucous membranes being considered the best site for immunization due to the high concentrations of dendritic cells (DC), macrophages and lymphocytes. The DNA is eventually taken up into nearby cells and processed to express the encoded antigen. The other method uses a high-pressure device, a so-called gene gun, to propel DNA-coated gold particles into cells in the skin. This method is sometimes referred to as biolistic particle inoculation. Both methods are widely used, and newer methods for the delivery of plasmid DNA vaccines are currently in development. Plasmid DNA can be diluted in distilled water, saline or sucrose and there has also been positive demonstration of proinjection or codelivery with various drugs. Widera *et al* used the technique of electroporation to facilitate the DNA delivery *in vivo* and using this technique resulted in increased expression and elevated immune responses[6]. To monitor the distribution and duration of gene expression of a DNA vaccine in living organisms, the naked DNA encoding firefly luciferase (F*luc*) as an imaging reporter gene has also been used[7].

2.1. DNA Vaccines and Phage Mediated Immunization

Bacteriophage (phage)-mediated immunization is a novel and exciting vaccine delivery technology. And this technology has been invented and developed by scientists of Moredun Research Institute and which is now generating both scientific and commercial interest. Using this new technique, 'vaccine' DNA is inserted into the genome of a bacteriophage vector (virus of bacteria), which is then used directly to immunize the host. Thus it is the genetic 'instructions' instead of the vaccine antigen itself which is being used as the immunogen. Phage appear to be targeted to the antigen presenting cells of the immune system, increasing the efficiency of the system. Once taken up, the phage coat is removed, the vaccine DNA component is expressed and vaccine protein is made within the host, leading to significant immune responses within a few weeks (as depicted in fig 2).

The use of phage-mediated immunization to deliver DNA vaccines offers many advantages. First one is the cost benefit that the phage are easy and cheap to produce and purify using relatively simple technology and second is the stability and that are naturally highly stable under fairly extreme conditions, and also offer a large cloning capacity meaning that several different vaccines can theoretically be delivered using the same phage particle. The technique also offers a rapid method for identifying potential vaccine candidates for newly emerging diseases or for pathogens for which protective antigens have not yet been identified. This 'whole- library' screening approach has already been used to identify a possible candidate vaccine against the serious respiratory disease contagious bovine pleuropnuemonia (CBPP), and both the technique and vaccine candidate are now the subject of a patent application by Moredun[8].

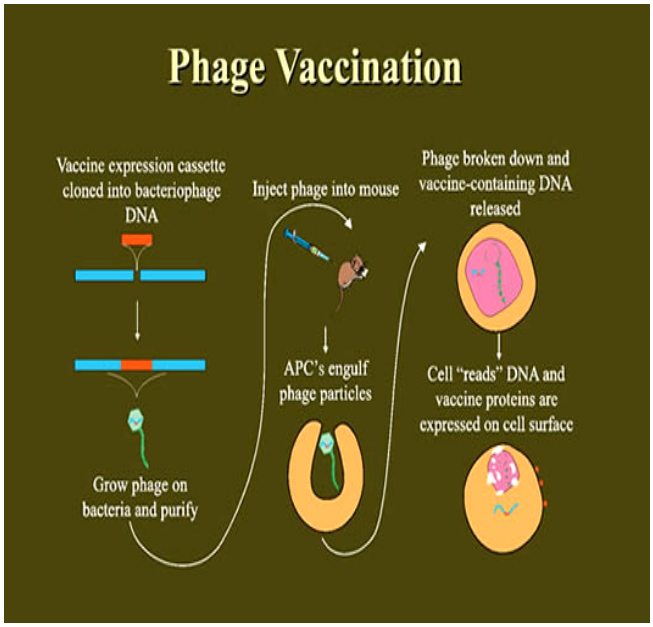

Figure 2. acteriophage mediated immunization.ebsite: http://www.mri.sari.ac.uk/bacteriology-reports-13.asp

3. DNA Vaccine Potency and Antigen Presenting Cell

Advances in the knowledge of the adaptive immune system have indicated that antigen-presenting cells, especially dendritic cells (DCs), play a very important role in the generation of antigen-specific immune responses. When the properties of DCs are modified then it represents an important strategy for enhancing the potency of DNA vaccines. It has been established that there are certain strategies to increase the number of antigen-expressing DCs, enhance antigen expression, processing and presentation in DCs, promote the activation and function of DCs, and improve DC and T-cell interaction, in order to optimize DNA vac-

cine-elicited immune responses. Continuing progress in the understanding of DC and T-cell biology serves as a foundation for further improvement of DNA vaccine potency, which may lead to future clinical applications of DNA vaccines for the control of infectious diseases and malignancies[9].

4. DNA Vaccines in Veterinary Sciences

DNA vaccines offer an attractive approach to vaccination for veterinary species. The main problem in development of the animal vaccines is the lack of available data on immune responses to pathogens that infect veterinary species. The commercial immunological reagents and the references on many pathogens are poorly available and this makes the DNA trials more difficult in these species than in mice. The protective immunity by naked DNA can be determined by clinical parameters such as mortality, fever, weight loss and pathogen titration. Naked DNA vaccination usually induces a humoral immune response, characterised by the production of antigen specific antibodies. The antibody level is very low to undetectable after the first DNA injection but increases both with the number of injections and the amount of injected DNA. Neutralising antibodies have been detected against the following viruses: bovine herpesvirus-1 (BHV-1)[10], equine herpesvirus-1 (Ruitenberg *et al.,*2000), bovine viral diarrhoea virus (BVDV), canine distemper virus (CDV), classical swine fever virus (CSFV), cottontail rabbit papillomavirus (CRPV), foot and mouth disease virus (FMDV), duck hepatitis B virus (DHBV), infectious bursal disease virus (IBDV), infectious hematopoietic necrosis virus (IHNV), influenza viruses, Japanese encephalitis virus (JEV) , porcine reproductive and respiratory syndrome virus (PRRS), pseudorabies virus, rabies virus (RV), vesicular stomatitis virus (VSV)[11] and viral hemorrhagic septicemia virus (VHSV). This technique is also suitable for the development of vaccines against intracellular bacteria for which T cell mediated immunity is required. DNA vaccines produce immune responses against protozoa and parasites as well. For example IgG1 antibodies as well as lymphoproliferative responses have been induced in cattle against *Anaplasma marginale*[12]. However vaccination against the tick *Boophilus microplus* by two injections of DNA only induced very low immune responses in sheep[13].

The commercial development of DNA vaccines against certain pathogens that use veterinary species as their specific host, such as BHV-1, RV and PRV, is completely feasible. Efforts now are concentrated on improving immunity in animals, by searching for appropriate adjuvants and the optimal route of administration[14].

5. DNA Vaccines –A Ray of Hope of the Treatment of Diseases

5.1. Hepatitis B

Due to the induction of strong CTL, DNA vaccines may be effective for the treatment of chronic carriers of HBV. Encke *et al.* found DNA based immunization is a promising antiviral approach for the development of therapeutic and prophylactic vaccine against HBV and HCV[15]. Michel *et al.,* 2001 found that the in mice and in various other animal models for hepadnavirus infection, DNA vaccines specific for hepatitis B virus (HBV) antigens induce a strong humoral and cell-mediated immunity that confers protection in some models[16]. Although there are effective prophylactic vaccines already available for HBV, there is currently no effective treatment for chronic HBV infection. Patients with HBV-associated liver disease are at increased risk of developing hepatocellular carcinoma and would greatly benefit from the availability of a therapeutic vaccine against HBV. By inducing immune responses closely related to those involved in clearing virus from the host, DNA vaccines may represent an alternative therapeutic approach for chronic HBV infection. Their results were further confirmed using a model for duck HBV (DHBV). Thermet *et al.,* 2003 evaluated the long-term therapeutic efficiency of DNA vaccine in a group of chronic DHBV carrying ducks[17]. They were immunized with plasmid encoding the large envelope protein. The results showed the DNA immunization against the large envelope protein was able to significantly decrease and even completely eliminate viral replication in chronically DHBV-infected ducks. This point to the possibility of designing a more effective way to treat HBV using DNA based vaccination. A Phase I/II trial is currently underway to determine whether the DNA vaccination of patients with chronic HBV infection treated with nucleos/tide analogs can lead to a T-cell restoration and delayed viral reactivation after treatment discontinuation[24].

5.2. Anti-Angiogenic DNA Vaccines and Cancer

A group of researchers at The Scripps Research Institute (TSRI) have developed a novel DNA vaccine that helps the body resist the growth of cancerous tumors by blocking the tumors' blood supply. A strategy is described to treat cancer by targeting endothelial cells that proliferate to form new blood vessels rather than by targeting the tumor cells. It is quite effective because endothelial cells supply blood to tumour and are pivotal for the tumor cells. The DNA vaccine uses an antigen "marker" known as vascular-endothelial growth factor receptor-2 that is upregulated on endothelial cells--particularly those that are undergoing angiogenesis due to nearby cancer tumor growth. This antigen DNA is inserted into a "targeting vector," the replication-deficient *Samonella typhimurium* bacteria, which direct the DNA to lymph nodes in the gut the so-called Peyer's patches. Once there, the bacteria die and release the bits of DNA, which are taken up by professional antigen-presenting dendritic cells and macrophages. Within these cells, the DNA is translated into protein and then presented to T cells. Once the T cells see the growth factor receptor, they are activated and will circulate through the bloodstream targeting potential tu-

mor-supporting angiogenic endothelial cells that display it. Novel DNA vaccines have been developed for the treatment of lung cancer which is based on the antiangiogenic DNA vaccine strategy. This approach focuses on stimulating T cell-mediated killing of key proteins in the proliferating endothelial cells in the tumor microvasculature and stromal fibroblasts, which is expected to result in the eradication of metastatic tumor cells. This may play an important role in treating lung adenocarcinoma[18]. It has been demonstrated that angiostatin receptor angiomotin (Amot) can be targeted by DNA vaccine which inhibits angiogenesis and suppresses tumor growth[19]. Results showed that therapy of attenuated *S. typhimurium* vaccine strain encoding murine vascular endothelial growth factor (VEGF) receptor-2 (flk1) combined with the plasmid DNA vector encoding the murine interferon-induced protein of 10 kDa (IP-10 or CXCL10) gene IP-10 gene has significant synergistic effect against tumors[26].

5.3. Autoimmune Diseases

It has been demonstrated that DNA immunization can protect animals against the autoimmune central nervous system inflammatory disease, experimental autoimmune encephalomyelitis (EAE). And many other autoantigens have now been identified; the application of this technology to other autoimmune diseases warrants investigation[20]. Several experimental DNA vaccines for HIV/AIDS have been produced and tested in small animals and non-human primates. In general, the results of these studies have been quite promising. DNA vaccines delivered intramuscularly or by gene gun have been shown to induce both neutralizing antibodies and CTL responses against HIV and SIV antigens. In one study, DNA immunization induced neutralizing antibodies and a vigorous CTL response, however, this immunization did not protect rhesus macaques from infection or disease upon subsequent challenge with a pathogenic SIV after peak CTL and neutralizing antibody titers had waned. DNA immunization was, however, successful in protecting chimpanzees against a non-pathogenic HIV infection and in protecting rhesus macaques against a non-pathogenic chimeric virus (SHIV). Clinical trials of candidate plasmid DNA HIV vaccines have begun. Results of a Phase I trial of a therapeutic DNA vaccine was reported by MacGregor *et al*.[5]. A Phase I clinical trial of two DNA candidate vaccines, one containing an HIV-1 Env and rev the other a Gag-Pol construct are underway through NIAID's (National Institute of Allergy and Infectious Diseases) intramural program and the AVEG (AIDS Vaccine and Evaluation Group). Additional trials are being planned; Phase I studies of Apollon HIV Gag/Pol DNA vaccines are in development through the AVEG. Cellular immunity may be enhanced by using DNA vaccine expressing HIV-1 gp120/immunoglobulin fusion protein[27].

5.4. Mycobacterial Diseases

Cell-mediated immunity is very important for the control of mycobacterial infections. $CD4^+$ T cells are very important in the control of *Mycobacterium tuberculosis*, but $CD8^+$ T cells also play an important role, and combination of the two T-cell subsets is necessary to bring about the protection. DNA vaccines were rapidly considered for use against mycobacterial infections and a considerable number of preclinical studies on the subject have been published in recent years. So far, DNA vaccines have been tested in experimental animal models for human and bovine tuberculosis (TB), leprosy, Buruli ulcer, and some nontuberculous mycobacterial infections[21].

It has been suggested that multi-T-cell-epitope based DNA vaccine induced T cell response to multiple T cell epitopes and led to enhanced protection against mycobacterial challenge[25].

5.5. Allergy

DNA vaccines have the ability to stimulate Th1 type reactions that's why act as a promising tool for immunotherapy of type I allergy. Recently, the strategies for up-to-date anti-allergic DNA-based immunization have been described which include the codon optimization of allergen genes and CpG-enrichment of plasmid vectors for enhanced Th1-bias. Recently, the replicase based vaccines are also introduced to overcome some of the deficiencies of conventional DNA and RNA based vaccines, including poor efficiency and low stability. At ultra low doses these vaccines can bring about the desired cellular and humoral immune responses. Additionally by the apoptosis of transfected cells the replicase-based vectors induce "self-removal" of the vaccine[22].

5.6. Malaria

Malaria, caused by infection with Plasmodium spp. parasites, has been treated effectively by the drugs. But the use of drugs results in the spread of drug resistant malaria therefore the construction of effective vaccine for the treatment of malaria is very critical. DNA vaccination provides a stable and long-lived source of protein vaccine capable of inducing both antibody- and cell-mediated immune responses to a wide variety of antigens. Injected DNA enters the cells of the host and makes the protein, which triggers the immune response. DNA vaccines with genes coding for different antigenic parts of malaria proteins have been created and presently some of these are undergoing field trials[23].

6. Future Prospects

Scientists are looking for the DNA vaccine which is devoid of any safety problem, DNA vaccines must surmount major safety concerns, including a theoretical potential for integration into host genome and insertional mutagenesis and induction of autoimmunity, immunological tolerance or a prolonged allergic reaction to an encoded protein in which synthesis is not readily terminated. There is a need to understand to a great extent the distribution, cellular uptake and expression of DNA vaccines to know the limitations to transfection *in situ*. Strategies to increase DNA uptake by

muscle cells or to facilitate DNA entry into the nucleus of APC are likely to increase the potency of DNA vaccines. Similarly, scientists are working to develop vaccines to completely treat rabies and other veterinary-related pathogens. If these efforts are successful, humans could enjoy healthier pets and live stock, and human disease arising from pathogens which have a wildlife reservoir could be prevented Strategies like phage mediated immunization to deliver DNA vaccine, anti- angiogenic DNA vaccine to treat cancer and replicase based vaccines for the allergy treatment need to be more investigated. Finally, promising DNA vaccines should be tested in animal models that closely mimic the human disease and efforts should be made to reduce the cost of DNA vaccination to make it commercially viable for use in higher animals.

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