DNA Vaccines in Poultry – Emerging trends

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One of the most exciting developments in biotechnology has been the resounding success of DNA vaccines, as protective and therapeutic agents. Using DNA, as a vaccine is a new approach that has the potential to eliminate many of the problems associated with traditional vaccines. There are three classes of traditional vaccines: attenuated, killed, and recombinant. Attenuated vaccines are live microorganisms with reduced pathogenicity and are generally the most effective vaccines. However, they can produce complications if the vaccine agent grows unchecked or reverts to a more pathogenic form. Killed vaccines require multiple injections, thereby increasing cost and creating logistical problems, and may contain incompletely killed microbes. Recombinant vaccines, in which an antigen from a pathogenic virus is engineered into a non-pathogenic vector, can only be used once because the immune system will also respond to the vector. To be effective, vaccines need to provide a sufficient dose of antigen for time periods long enough to induce a secondary (memory) response. This poses a problem for traditional vaccines; DNA vaccines, however, can effectively produce copies of pathogenic antigens for long periods of time, usually until the host cell is killed by the immune response it induced.

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DNA vaccines consist of plasmids that have been altered to carry genes specifying one or more antigenic proteins normally made by a selected pathogen, at the same time, excluding those genes that would enable the pathogen to reconstitute itself and cause disease. When these antigen-specifying genes get expressed within the host cells, the foreign protein that is produced will stimulate the required immune response by the host and hence protect against the disease, should the actual pathogen ever invade the host system in the future.

Considering the case of poultry industry, infectious diseases continue to cause enormous financial losses and a great effort has been launched in their prevention, using vaccines. DNA vaccines are still in the experimental stage of development, but they could provide livestock and poultry producers with a cost-effective alternative to other types of vaccines. Though no DNA vaccines are currently registered for human or veterinary applications, the first commercial products can be expected to hit the market anytime now.

Development of the vaccine

The use of DNA as a vaccine was first proposed in 1990 by Wolff *et al.* who demonstrated that purified bacterial plasmid DNA ('naked' DNA) injected into the muscle of a mouse resulted in the expression of an encoded reporter gene[1]. These experiments suggested that a simple 'vector less' vaccine could be created using a plasmid containing a cassette that included an eukaryotic promoter and a gene or genes encoding a protective antigen. Early DNA vaccine experiments used plasmids that encoded either the human growth hormone or the human a-1-antitrypsin gene. Mice inoculated with these plasmids developed antibodies against the encoded proteins[2]. Another DNA vaccine containing the gene encoding for the nucleo-capsid protein of influenza A virus when injected into mice was found to immunize them against the disease[3]. Since these early research reports on DNA vaccines has shown that the approach can be applied to many different pathogens and that there are multiple methods to alter and improve the immune response

to these vaccines.

Basic components of the vaccine

The plasmid DNA for gene vaccination has two major units[4]. They are: (i) plasmid backbone that delivers adjuvant and mutagenic activity via immunostimulatory sequences (ISS), and (ii) a transcription unit comprising upstream promoter and enhancer sequences recognized by host cell transcriptional machinery, coding sequences with in-frame ribosomal initiation and termination codons, and downstream polyadenylation and RNA termination sequences. An intron sequence either upstream or downstream of the insertion site of the foreign coding sequences can enhance the processing and transport of expressed mRNA.

Mechanism of action of DNA vaccines[5]

DNA vaccines work primarily by invoking 2 branches of the immune system– (i) Humoral arm – attacks pathogens outside of cells, and (ii) Cellular arm – eliminates cells that are colonized by an invader.

Immunity is induced with the entry of the DNA vaccine into a targeted cell, such as muscle and the cells' subsequent production of antigens normally found on the pathogen of interest. The antigens may be released from the cell as such to elicit a humoral response or may be fragmented within the cell to antigenic peptides which are displaced on the cell surface via MHC class I molecules to elicit a cell-mediated response.

Advantages of DNA vaccines

DNA vaccines have several potential advantages, some of which are listed below, that make them ideal vaccine candidates.

1. Allows presentation of pathogenic antigens to the immune system in a native form eliciting both cellular and humoral immunity.

2. Allows formulation of combination of immunogens that could be delivered in a single dose, i.e. multi-epitope DNA vaccines[7].

3. Devoid of the risk associated with live attenuated vaccines where the pathogen may mutate back to a virulent form.

4. Do not pose any special threat to young, old or immunocompromised animals and its immunogenicity will not be decreased by maternal antibodies.

5. Easy to design and to generate in large quantities using common place recombinant DNA technology.

Stable and can be stored at room temperature obviating the need for a cold chain, thereby reducing the vaccine distribution costs considerably.

DNA vaccines can be created against pathogens that do not grow well in laboratory or production systems.

The same plasmid DNA vector can be used multiple times without the host developing immunity to it.

9. Only genes encoding critical antigen regions for protective immunity are delivered, obviating the need to use carrier organisms with their own abundant genetic material.

DNA Vaccines in poultry

In comparison to protein vaccines, DNA vaccines could become easier to administer, depending on the cost-effective commercialization of gene gun delivery technology that injects the DNA vaccine into the skin. DNA vaccines are highly effective when delivered in the skin with needle-free devices, meaning no meat loss because of tissue damage. DNA vaccines are also advantageous from a safety perspective because they exist in the cells independently and do not enter the genetic material of the animals[8]. Also, the cells are targeted by the immune system and usually killed. So there should be no concern about whether people should consume meat from animals that have been administered a DNA vaccine.

Several DNA vaccines have already been developed against diseases for which no effective vaccine exists as yet. All candidate vaccines are in early stage trials examining safety and immune responses, and all have been well tolerated. No trials of effectiveness for disease prevention or treatment have begun. Most of the studies are ongoing. Vaccination of poultry with naked plasmid DNA has been successfully demonstrated with several different poultry pathogens, but the technology needs to be further developed before it can be practically implemented.

Newcastle disease virus

Immunization using plasmid DNA construct expressing the Newcastle disease virus F protein (NDV-F) under the control of the human cytomegalo-virus immediate early enhancer and chicken beta-actin gene promoter conferred efficient protection against the disease[9].

Avian influenza virus

Gene gun delivery of DNA encoding an H5 HA pro-

tein confers complete immune protection to chickens challenged with lethal H5 viruses. Perhaps most important, the HA-DNA vaccine conferred 95% cross-protection against challenge with lethal antigenic variants that differed from the primary antigen by 11 to 13% (HA1 amino acid sequence homology). Overall, the high levels of protection seen with gene gun delivery of HA-DNA were as good as, if not better than, those achieved with a conventional whole-virus vaccine, with fewer instances of morbidity and death[10].

Infectious bursal disease virus

Direct DNA inoculations were used to determine the efficacy of gene immunisation of chickens to elicit protective immune responses against infectious bursal disease virus (IBDV). Plasmids encoding VP2-VP4-VP3 induced IBDV-specific antibodies and protected the chicken from infection[13].

Infectious bronchitis virus

Recent advances in the control of Infectious Bronchitis in poultry include the use of a DNA vaccine based on the spike envelope protein of the virus[14].

Specific cytotoxic T-lymphocyte (CTL) responses to nucleocapsid of infectious bronchitis virus (IBV) were identified in chicken inoculated with a DNA plasmid encoding nucleocapsid proteins. IBV-specific CTL epitopes were mapped within the carboxyl-terminal 120 amino acids of the nucleocapsid protein. Furthermore, chickens immunized with a DNA plasmid encoding a CTL epitope(s) were also protected from acute viral infection[15].

Duck hepatitis B virus

The protective efficacy of DNA vaccines against duck hepatitis B virus infection was reported[16] wherein the anti-S antibodies induced by the S proteins-DNA construct were highly effective in neutralizing the virus infectivity.

Avian coccidiosis

Naked DNA immunization of chicken for controlling avian coccidiosis using pcDNA3-SO7' plasmid construct that encodes for a refractile body antigen (designated SO7') found in sporozoites of *Eimeria tenella* showed for the first time that a single coccidial antigen protects chickens (as measured by reduced lesion scores and high rate of growth) against cecal coccidiosis[18].

Chlamydial infection

The efficacy of the major outer membrane protein (MOMP) based DNA vaccination as a means of preventing severe clinical signs, lesions and chlamydia excretion in a turkey model of *Chlamydia psittaci* serovar A infection was demonstrated[19,20].

Engineering for optimal effect

Currently available eukaryotic expression vectors are not optimised for use in chickens[21], and significant improvements should be possible when the vectors are optimised for poultry.

Several approaches have been proposed for enhancing the overall immune

reactivity to DNA vaccines and for optimizing the ratio of cellular to humoral responses in the host[5,22]. Some of these are as follows:

1. Increasing the number of immunostimulatory sequences in plasmids might well amplify the immunogenicity of the antigenic codes in a DNA vaccine.

2. Incorporating genes for signalling molecules called cytokines into antigen carrying plasmids or into separate plasmids can boost overall immune response to DNA vaccines[23].

3. Genes for substances known as chemokines (a class of low molecular weight cytokines) that attract both antigen presenting cells and T cells to damaged or infected tissues may be incorporated in the plasmid.

4. Combining specific chemokine genes with related cytokine genes could help customize both the type and extent of immune responses elicited.

5. DNA vaccines could in theory even sidestep the need for classical APCs to prime CTLs if the gene for an antigen were bundled with the gene for a co-stimulatory molecule. Thus, DNA vaccines could facilitate both the priming and the activation of CTLs by displaying along with the antigen, the crucial second signal by the co-stimulatory molecule.

Conclusion

DNA vaccination is a new technology having great potential with numerous advantages over currently available vaccines. Although DNA vaccines have been used successfully in a wide variety of animals, including chickens, DNA vaccines in their present form are not cost effective nor do they elicit high enough protection for

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