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New Concepts on Vaccine Development for the Poultry Diseases

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ABSTRACT : Vaccination is one of the most important and cost-effective methods of preventing infectious diseases. Over the past decade, scientific in molecular biology and immunology have improved understanding of many diseases and led to the development of novel strategies for vaccination. An ideal vaccine would induce effective immunity specific for the type of infection, have long duration, require minimal or no boosters, have safety, would not induce adverse reaction, and be easy to administer. The desire to meet these criteria has resulted in the development of vaccines that do not depend on the use of the viable disease agent. It is not the intent of this review to give an extensive review of the field of vaccinology, but rather to address characteristics of conventional and genetically engineered vaccines.

(Key words : viral vaccine, recombinant protein, DNA, RNA)

INTRODUCTION

Poultry disease is a constant threat on the poultry industry. Worldwide, poultry industry losses to Newcastle disease that is a highly contagious and fatal viral infection affecting poultry and wild birds are estimated in the billions of dollars each year. Good management and biosecurity including vaccination have to apply to the farms to prevent economic losses from diseases and to increase the production rate. And research in poultry disease is essential to the economic survival of the industry.

The effectiveness of vaccines for prevention and control of poultry infectious diseases have been well recognized. Prevention of bacterial and viral infections through vaccination is very useful in reducing mortality, animal suffering and economic losses. Furthermore, it is estimated that vaccination has had a greater impact on the economics of the poultry production than all other therapeutic and prophylactic treatments combined.

Immunization through vaccine has been archived by using killed or attenuated conventional vaccines. Indeed, these vaccines were able to use even without fully comprehending the epidemiology and pathogenesis involved in the disease process. With the recent advances in an understanding of pathogenesis, primarily due to advances in immunology and molecular biology, the genetic engineering technology has revolutionized the research approach and accelerated the development of safer and more effective vaccines to help further reduce diseases in animals as well as humans.

New modern vaccines are mainly based on viral or bacterial vector vaccine with genes coding for desired antigens, subunit vaccine, peptide vaccine, nucleic acid vaccine and edible vaccine. This review will present the types of vaccine applied in poultry industry. And the present review will focus on the different types of genetically engineered vaccines for bacterial and viral diseases of poultry.

BACTERIAL AND VIRAL DISEASES OF POULTRY

1. Bacterial Diseases

Mycoplasma gallisepticum (MG) and *Mycoplasma*

synoviae (MS) infected in chickens are egg-transmitted pathogens. MG causes respiratory disease and MS causes respiratory disease and synovitis in growing chickens, although subclinical infections are common. MG is responsible for substantial economic losses from decreased egg production and hatchability, downgrading and condemnations of carcasses, and decreased feed efficiency. Strategies to minimize or eliminate the impact of MG infection in commercial poultry include surveillance, control and eradication programs. Control by vaccination with live vaccines and bacterins is confined to MG and, to a lesser extent, MS infections.

The live MG vaccine strains most commonly used are F, ts-11 and 6/85 strains. It has been known that F strain is more virulent than ts-11 or 6/85 strains. Although the F strain is relatively nonpathogenic, it is somewhat virulent for young chickens and is fully virulent for turkeys. In addition, the F strain elicited a stronger antibody response, provided better protection against airsacculitis, and persisted at higher levels in the upper respiratory tract than did the less virulent ts-11 and 6/85 strains. The F strain has been shown to offer protection against colonization by more virulent challenge strains, and it is known to displace field strains when it is used continuously on multi-age poultry farms. MG bacterins contain inactivated whole cells suspended in either oil emulsion or aluminum hydroxide adjuvants. Adverse tissue reactions and failure to stimulate an adequate local immune response following parenteral administration of oil adjuvants have prompted investigation of possible alternatives. The advent of novel adjuvants such as ISCOM may enhance the efficacy of vaccines.

Over 2400 different *Salmonella* serotypes have been identified. A relatively small number of these are known to be host-adapted, characteristically producing severe systemic disease in poultry. Infections of poultry with *Salmonella* can be grouped into three diseases, pullorum disease, fowl typhoid and paratyphoid infections. Pullorum disease caused by *S. pullorum* is an acute systemic disease of chicks and poults. Fowl typhoid caused by *S. gallinarum* is an acute or chronic septicemic disease that most often affects adult chickens. Both of these diseases have been responsible for serious economic losses to poultry industry.

Pullorum disease is controlled by test and slaughter method and there is very little incentive for the production of vaccines to control it. For the control of fowl typhoid, killed and live vaccines have been used in the poultry industry. Attenuated strains of *S. gallinarum* strain 9 have been assessed extensively as live vaccines for chickens since the 1950s. An *aro* A mutant of *S. gallinarum* strain 9, avirulent for 2-week old chickens, was effective as a vaccine but conferred less protection than the 9R vaccine.

Current bacterial vaccines have several disadvantages. The important thing is limited and incomplete protection against field infections. And chickens vaccinated with killed or live vaccines cannot be differentiated from naturally infected chickens, creating difficulties in routine screening procedures. Specifically deleting a gene in the vaccine strain and developing a serological test based on the deleted antigen could allow this distinction to be made.

2. Viral Diseases

Many viral agents cause the diseases in poultry and result in economic losses. The representative viral diseases are Newcastle disease (ND), infectious bronchitis (IB), infectious bursal disease (IBD), infectious laryngotracheitis (ILT) and highly pathogenic avian influenza (HPAI). These diseases have been controlled by vaccination with killed or live attenuated vaccines except for HPAI.

ND is well known as one of the most disastrous diseases in poultry industry. Virulent ND virus could be caused 100% mortality in flocks of susceptible chickens. In Korea, ND is major poultry disease like as other countries. All strains of ND virus are morphologically, structurally, and serologically indistinguishable. Using live and killed vaccines has controlled ND.

According to the virulence of vaccine strains, ND live vaccine strains are divided into two groups, lentogenic and mesogenic. Lentogenic strains are included Hitchner B1, La Sota, F, V4, VG/GA, and Ulster 2C,

which have been widely used in Korea. B1 and LaSota are prototype of live vaccine strains. However, mesogenic strains, Komarov, Mukteswar and H strains, suitable only for secondary vaccination of chickens due to their greater virulence, have not been permitted in Korea. B1, La Sota, F and V4 strains were isolated from field and were not attenuated for the reduction of virulence. These lentogenic vaccine strains could be administrated to chickens by various routes. Probably the most common method of application is via drinking water. And intranasal, intraocular, and oral routes could apply for vaccination. Spray and aerosol are used to mass application of live vaccines.

IB is highly contagious disease of the respiratory and urogenital tract of chickens. Economic losses from IB are resulted from decreased egg production, egg quality, feed efficiency and growth rate. In addition, IB virus cause mixed infections that produce airsacculitis that may results in increased condemnation rate in processing plants.

Many serotypes of IB virus are recognized and have practical significance in the control of IB, because immunity following infection or vaccination with one serotype often is not protective against infections with unrelated serotypes.

IBD referred to Gumboro disease is immunosuppressive disease in young, sexually immature chickens. Two serotypes of IBD virus (IBDV) are described. Serotype I viruses are pathogenic for chickens, whereas serotype 2 viruses are not. Antigenic variation among serotype I isolates of IBDV has been shown in the US since 1985. These antigenic variants were of different subtypes compared with classical strains. Vaccination with serotype 1 does not protect chickens from infection caused by antigenic variants. Since 1986, a highly pathogenic strain of IBDV was isolated from broilers and layers in many countries in Europe, the UK, Middle East and South Africa. These pathogenic variants of IBDV produced 90 to 100% mortality in experimentally infected SPF chickens. And these strains have the ability to break through an even higher level of maternal antibody. Some new vaccine strains have been developed to control infection of highly virulent IBDV. Generally, IBD live vaccine strains are divided into classic or mild, intermediate, and intermediate plus or hot according to virulence of vaccine strains.

ILT is an acute respiratory disease of chickens characterized by dysponea, gasping, coughing, expectoration of bloody exudate, decreased egg production and varying levels of mortalities. ILTV strains are a single serotype and attenuated ILT vaccines have been widely used as a preventive measure.

CONVENTIONAL VACCINES

1. Live Vaccines

Live vaccine strains are produced by naturally low virulent strain or attenuated wild-type virus which was isolated from outbreaks of disease and passaged in vitro through one or more cell types to attenuate its virulence. Live vaccines elicit broader immune response and require low doses to produce the immune response.

Live vaccines are relatively inexpensive and easy to administer and apply to mass vaccination. Local immunity is stimulated by infection with live viruses, and protection occurs very soon after vaccination. However, the vaccine may cause disease, depending on environmental conditions and the coinfection of other disease. The vaccine strains may be infected from vaccinated chickens to unvaccinated susceptible chickens and acquired virulence by mutation or recombination of virulence related genes through bird-to-bird passages. The immune responses of live vaccine may be interfered by maternal antibody.

2. Killed or Inactivated Vaccines

Killed vaccines are usually produced from infective allantoic or culture fluid treated with inactivated reagents such as formalin, binary ethylene imine or (-propiolactone to kill the agent and then mixed with a carrier adjuvants.

Killed vaccines are easier to handle and product than live vaccine, which may be easily inactivated by heat

Table 1. Potential advantages and disadvantages of general vaccine technology

	Live	Subunit	Genetic
Immune response	Broad	Mostly antibody	Most cellular
Cost	Very low	Variable	Low
Safety	Variable	Excellent	Good
Efficacy	Often high	High to low	Remains to be shown
Product analysis	Very difficult	Usually good	Easy
Correlate of efficacy	Uncertain	Usually identified	Uncertain
Enabling technology	Stability	Adjuvants delivery	Formulation

and can be contaminated other agents, if not carefully controlled during production. Killed vaccines are expensive to produce and to apply because of the labor needed for their application. Killed oil emulsion vaccines are as adversely affected by maternal antibody as live vaccine.

GENETICALLY ENGINEERED VACCINES

The overall technologies for developing a vaccine may be divided into three categories: live, subunit (inactivated or killed) and genetic (nucleic acid-based). Each of these categories is further divided into multiple categories, which include recombinant-derived as well as native microorganisms and their components.

1. Recombinant Vaccines

Application of a recombinant DNA strategy to develop new vaccines is performed by identifying the specific component(s) that can elicit the production of protective antibodies, and then cloning and expressing the gene encoding that protein and assembly of a

Table 2. Pathogenicity of TK gene-deleted recombinant ILTVs in SPF chickens [1)]

Virus	Dose [2)]	Mortality (%)	ITPI [3)]
Parent	3.3	50	2.5
TK gene-deleted	3.0	0	0.3
Vaccine A	3.5	0	0.8
Vaccine B	3.4	0	0.3

1) Chickens were intratracheally inoculated with ILTV and were observed daily for 14 days.
2) log $TCID_{50}$/100 ul.
3) Intratracheal pathogenicity indices (ITPI) were calculated by scoring clinical signs. 0 = normal, 1 = mild (sneezing, coughing), 2 = severe (dyspnea, mouth breathing), 3 = death. Indices were determined by dividing the sum of the scores by the total number of observed chickens.

Table 3. Protective efficacy of recombinant infectious laryngotracheitis virus

Virus	Inoculation route	Dose[1)]	Re-isolation of challenge virus	Protection rate (%)[2)]
TK- mutant	Intraocular	1.0	5/5[3)]	0
		2.0	5/5	40
		3.0	1/5	100
	Intratracheal	2.0	0/5	100
Vaccine A	Intraocular	3.5	0/5	100
Vaccine B	Intraocular	2.8	1/5	100
Control		-	5/5	0

1) log $TCID_{50}$.
2) The protection rate was calculated by clinical sings.
3) No. of chickens isolated challenge virus / No. of tested chickens.

complex in some cases. This approach has made possible a safe and effective recombinant vaccine.

Genes of bacteria and virus, which are related to virulence, are made specific modification or deletion so that these agents are more stably attenuated. Thymidine kinase (TK) gene of herpesvirus are well known to virulence related gene and to be available gene to be deleted or inserted a foreign gene for the development of recombinant. The TK gene located at the unique long region is not an essential gene for the growth of viruses in tissue culture. The deletion of TK gene causes reduction of the virulence and the rate of the re-activation of viruses in the latent infectious states. Because of these characteristics, the TK gene has been used as the target gene for the development of recombinant in other alphaherpesviruses. ILT virus (ILTV) is prototype of herpesviruses in poultry diseases. Like as other alphaherpesviruses, TK gene deletion in ILTV causes the decreased the virulence of virus and produces the protection against challenge with virulent virus.

In S. gallinarum, strains with mutations in genes for the biosynthetic pathway of aromatic acids are attenuated because the growth factors *p*-aminobenzoic acid and dihydroxybenzoic acid are not available in sufficent quatities in the tissues. More recently, a *nuoG* mutation showing no NADH dehydrogenase I enzymatic activities, was shown to attenuated *S. gallinarum*.

2. Recombinant Vectors

The genes, which are not essential for growth of agent, can be genetically modified by the integration of foreign genes based on homologous DNA recombination techniques. Examples of this approach in case of herpesvirus (Marek's disease virus, herpesvirus of turkey, ILTV), adenovirus, fowlpox virus and bacteria such as intracellular microorganism.

Poxviruses are large double-stranded DNA viruses that replicated in the cytoplasm of infected cells. Their ability to tolerate large insertions of foreign DNA and their wide host range have allowed poxviruses to be developed as vectors for the expression of foreign genes for further analysis and for potential use as vaccines. Fowlpox virus has been used as a vector to develop poultry vaccines for protection against Newcastle disease, avian influenza, Marek's disease and infectious bursal disease.

Of these recombinant vaccines, the rabies vaccine, in which the rabies G protein is expressed in a vaccinia vector, has been widely used in the field to prevent the spread of rabies both in Europe and in the United States of America. A recombinant Newcastle disease virus vaccine, using fowlpox virus as the vector to express immunogenic proteins from the Newcastle disease virus, has been licensed as the first commercial recombinant vectored vaccine. Many other recombinant virus vaccines are still at the stage of laboratory or field-testing.

Live vaccine vectors are delivered at the mucosal surface, place in which the onset of infection takes place and the first defense line is laid. The use of bacterial vectors have advantages such as low batch preparations costs, facilitated technology transfer following development of the prototype, increased self-life and stability in the field respect to other formulations, easy administration and low delivery costs. The ability of intracellular bacteria to drive the CD4+ T-cell response toward a Th1 response makes them attractive candidates for vaccine vectors for those diseases in which cell-mediated immunity is more effective than humoral responses. However, live bacterial vectors have some problems. Problems associated with the use of live bacterial vectors are reversion to virulence, stability of the recombinant phenotype, horizontal gene transfer and pre-existing immunity which is produced by prior exposure to the bacterial vector.

3. Subunit Vaccines

The epitopes recognized by neutralizing antibodies are usefully found in just one or a few proteins present on the surface of pathogenic organism. Isolation of the genes encoding such epitope-carrying protein immunogens and their expression in heterologous hosts form the basis of development of recombinant subunit vaccines. The main advantage of using single

proteins displaying immunodominant epitopes as vaccines is the possibility of inducing protective immunity without having side effects and immune reactions caused by other parts of the pathogens. Potential challenges in the development of subunit vaccines are that they often are poorly immunogenic and have short *in vivo* half-life. In order to elicit a vigorous immune response, subunit vaccines often require multiple doses, as well as, the use of adjuvants.

The choice of expression system depends on many factors, including (i) the requirements for post-translational modification, (ii) the proteolytic stability of the target protein, (iii) whether the protein is secretable, (iv) the possibility of renaturation of a protein produced in a misfold form and (v) the acceptable costs for the final product. There are four major expression hosts that are commonly used to produce vaccine antigens; bacterial, yeast, insect and mammalian expression systems. In addition, transgenic plant expression systems have started to emerge, with the aim of utilizing the plant both for production of the subunit vaccine and for vaccine delivery via edible plant.

4. Peptide Vaccines

Chemically synthesized peptides are excellent tools in vaccine research since they are extensively used for definition T- and B-cell epitopes. However, synthetic peptides are also investigated as experimental vaccines. Peptides, identified as immunogenic epitopes, can elicit a strong immune response when delivered together with a carrier or an adjuvant, but immunized without carrier or adjuvant, they are generally not very immunogenic since they are rapidly cleared *in vivo*. A major drawback in the use of synthetic peptides is their limit in length. Synthetic peptides should normally be less than 50 amino acid residues so that they can be manufactured cost-efficiently. Since short synthetic peptides have a high degree of structural flexibility, and thus most probably would react with a wide spectrum B-cells upon immunization, they are not particularly suited as subunit vaccines in cases where the humoral part of the immune responses would be of importance for protection.

5. Nucleic Acid Vaccines

DNA vaccination has been become the fastest growing field in vaccine technology following at the beginning of the 1990's that plasmid DNA induces an immune response to the plasmid-encoding antigen. In contrast to recombinant vaccines, nucleic acid vaccines consist only of DNA (as plasmids) or RNA (mRNA), which is taken up by cells and translated into protein.

The quick acceptance of nucleic acid vaccines in experimental settings is due to the many advantages this strategy this strategy has over traditional vaccines. However, the efficacy of nucleic acid vaccines in many systems has not proven to be satisfactory, leading some to conclude that nucleic acid vaccines are not a viable alternative to conventional vaccines and will never replace them. Some studies, however, purport that DNA vaccines are more efficacious than some established vaccines. Indeed, DNA vaccines can circumvent many of the problems associated with recombinant vaccines, such as high costs of production, difficulties in purification, incorrect folding of antigen and poor induction of CD8+ T cells. DNA also has clear advantages over recombinant viruses, which are plaqued with the problems of pre-existing immunity, risk of insertion-mutation, loss of attenuation or spread of inadvertent infection.

1) DNA

DNA vaccines consists of plasmid DNA expression vectors of *E. coli* origin, which encode the antigen or antigens of interest under the control of strong viral promotors recognized by host. When the plasmid DNA is administered to an animal, the antigen is expressed in situ, leading to an antigen-specific immunity. DNA expression plasmids were delivered either by intramuscular injection in saline preparation, intravenous administration as liposome-DNA complexes, intranasally using a bacteria vector, by oral delivery of microencapsulated DNA, or by high velocity bombardment of DNA-coated particles.

Table 4. Genetically engineered vaccines and some of their characteristics

Vaccines	Typical characteristics
Protein immunogens	Define composition. Safe. Induces primarily humoral immunity, Need for adjuvants, Cost depends on production system.
Bacterial vectors	Attenuated pathogens. Possible oral vaccines. Humoral and cellular immunity. Surface display of antigenic determinants possible. A variety of delivery systems exist.
Viral vectors	Humoral and cellular immunity. Could be used for large or multiple immunogens. Risk for reversion into virulence through genetic recombination when using attenuated pathogens as vectors
Nucleic acids: DNA	Cost-efficient production. Stimulates cellular and humoral immune responses. Ineffi-cient transfection. Risk of integration into host genome not completely excluded. Possi-ble bacterial delivery. In vivo amplification systems available.
Nucleic acids : RNA	Unstable, No risk of integration into host genome. Do not have to enter the nucleus for translation. *In vivo* amplification systems available.

2) RNA

Genetic vaccination through the delivery of RNA has also been investigated , but to lesser extent than DNA vaccination. RNA expression is short-lived, and is thus less effective in inducing an immune response. The preparation and administration of RNA is trouble-some because of the low stability of the RNA. One advantage of the RNA strategy is that there is no risk of integration of the delivered gene into the host genome.

6. Edible Vaccines

It is essential to determine which system offers the most advantages for the production of the recombinant protein. The ideal expression system would be the one that produces the most safe, biologically active mater-ial at the lowest cost. The production of recombinant proteins in plants has many potential advantages for generating biopharmaceuticals relevant to clinical medicine. First, plant systems are more economic than industrial facilities using fermentation or bioreactor systems. Second, the purification requirement can be eliminated when the plant tissue containing the recombinant protein is used as a food (edible vac-cines). Third, plants can be directed to target proteins into intracellular compartments in which they are more stable, or even to express them directly in certain compartments. Fourth, the amount of recombinant product that can be produced approach industrial-scale levels. Last, risks arising from contamination with potential pathogens and toxins are eliminated or minimized.

VACCINE ADJUVANTS

One of the most critical components of a parenterally administered vaccine that can affect its efficacy and method of delivery is the adjuvant. An adjuvant acts as depots they increase the ability of low amounts of antigen to induce a protective immune response. Adjuvants work by a variety of mechanisms, including enhancing deposition and persistence of antigen, recruiting inflammatory cells, and altering the balance between Th1 and Th2 type responses. Several distinct classes of adjuvants have been used in the field of veterinary vaccines. Many compounds are described as having adjuvant-like activity. However, from a practical point of view such compounds need to at least act as a depot in order to be an effective delivery sys-tem for a commercial vaccine. The most prevalent adjuvants found in licensed veterinary vaccines are aluminum salts and oil emulsions. In addition, many compounds and methods have been researched to

induce effective immune response; immunostimuratory complexes (ISCOMS), microparticles or microspheres (aliphatic polyester microspheres, loposomes, alginate microparticles) and other (avridine, calcitrol, cholera toxin, cytokines).

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