Review Article

A Virus-like Particle Vaccine Against Infectious Bursal Disease Virus: Potential Uses and Applications

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Abstract | Infectious bursal disease (IBD) virus-like particles (VLPs) comprising only viral capsid proteins mimic the naïve configuration of authentic IBD virus particles. The VLPs show intrinsic immunogenicity and a high safety profile. Thus, VLPs are considered one of the most promising approaches to vaccine development and are an alternative to inactivated IBD vaccines. In addition, VLP technology has many applications. This paper reviews the potential of VLPs as an alternative to IBD vaccines, along with their specific applications in the field.

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Introduction

Infectious bursal disease (IBD) is a viral disease of poultry that has marked economic repercussions for the industry due to severe immunosuppression and high fatality rates. Infectious bursal disease virus (IBDV) is the causative agent of the disease, which causes aggressive destruction of immature B lymphocytes in the lymphoid organs, in particular the bursa of Fabricus (BF), in young chickens. Thus, IBDV infection leads to immune dysfunction, which in turn results in increased susceptibility to secondary infections and a reduced response to subsequent vaccination against other pathogens. Severe clinical manifestations and high mortality often occur in chickens infected with virulent IBDV between 3 and 6 weeks of age. Chickens infected at less than 3 weeks of age generally suffer severe immunosuppression without any clinical signs (Eterradossi and Saif, 2013).

IBDV belongs to the genus *Avibirnavirus* within the family *Birnaviridae*. It possesses a genome comprising two segments of double-stranded RNA (A and B). Segment A contains two overlapping open reading frames (ORFs): the smaller is named A2 and the larger A1. ORF A2 encodes a nonstructural protein (VP5), while ORF A1 encodes a precursor polyprotein (PP) that is self-cleaved by the viral encoded protease VP4 to yield three individual VPs: pVP2 (precursor VP2), VP3, and VP4. pVP2 is further cleaved to yield mature VP2. VP2 and VP3 induce protective immunity in chickens. Genome segment B encodes VP1, which has RNA-dependent RNA polymerase activity (Jackwood, 2013).

Most countries worldwide have suffered IBD outbreaks in young chickens due to virulent IBDVs or antigenic variants thereof. Vaccinating poultry against IBDV is common practice to prevent economic losses.

The vaccination strategy for containing the spread of IBDV is based on live attenuated and/or inactivated, or recombinant, vaccines. Traditionally, young chickens born in highly immunized parent flocks receive anti-IBDV from the mother. This passive immunity protects them from virulent IBDV infection during the first few weeks after hatching (Müller et al., 2012).

Recently, various protein expression systems, including *E. coli*, yeast, adenovirus, fowl pox virus, baculovirus, vaccinia virus, herpesvirus, and even plants, have been used to produce recombinant subunit vaccines based on IBDV VP2 (Müller et al., 2012). Another strategy for developing novel vaccines is to create virus-like particles (VLPs) comprising recombinant viral structural proteins but lacking the viral genome. VLPs mimic the naïve configuration of IBDV (Lee et al., 2015).

This review discusses the current knowledge about VLP vaccine development, as well as their potential application as alternatives to the commercial IBDV vaccines used by the poultry industry.

Current VLP strategies

Of all the IBDV structural proteins, VP2 and VP3 are important components of the viral capsid. The capsid is formed via self-assembly of both of these proteins in infected cells. Thus, expression of recombinant VLP proteins is based on the self-assembly of both VP2 and VP3 proteins upon expression in different vectors.

The first approach to VLP production uses recombinant baculovirus (Chevalier et al., 2002; Kibenge et al., 1999; Vakharia et al., 1993) or vaccinia virus (Fernández-Arias et al., 1998) to inducibly express the full-length or partial genome segment A of IBDV. This strategy is based on the fact that the PP is proteolytically processed to yield the pVP2, VP3, and VP4 structural proteins, with subsequent processing of pVP2 to yield mature VP2. The VP2, VP3, and VP4 proteins then self-assemble into icosahedral VLPs. In some cases, the structural proteins self-assemble to form VLPs without further processing of pVP2 to VP2 (Martinez-Torrecuadrada et al., 2003). Further studies demonstrated that expression of the wild-type polyprotein led to the formation of type I tubes rather than VLPs unless the five C-terminal residues were removed from VP3 (Chevalier et al., 2004).

Lee et al. (2015) developed a novel VLP strategy based on bi-directional co-expression of the PP and the viral protease, VP4, by a recombinant baculovirus carrying both segment A and the VP4 gene; the idea was to increase the efficiency of VLP assembly. Western blot analysis revealed that the yields of VP2 and VP3 were greater when VP4 was co-expressed with PP. Therefore, additional expression of VP4 appears to improve the yield of both VP2 and VP3 by increasing proteolytic activity. In particular, high expression of recombinant VP3, which is crucial for VLP formation, appears to have a positive impact on VLP formation. This in turn leads to increasingly efficient VLP formation.

Another type of VLP technology involves co-expression of two separate structural proteins, pVP2 (or VP2) and VP3, by co-infection of insect cells with two different recombinant baculoviruses (Jackwood et al., 2013; Martinez-Torrecuadrada et al., 2003; Ona et al., 2004). Although co-expression of the two separate structural proteins (pVP2 and VP3) in infected cells was successful, precise adjustment of the multiplicity of infection for both viruses was required for efficient VLP assembly (Hu et al., 2001). Further studies on co-expression of pVP2 (or VP2) and VP3 by a single recombinant baculovirus generated using a dual expression vector are needed.

VLPs are potential alternative IBDV vaccines

The major neutralizing sites within IBDV are in VP2; however, the sites are mainly conformation-dependent. The antigenic structure of the recombinant VP2 protein tends to vary according to the expression system employed; this has a marked effect on the generation of protective immunity in vaccinated chickens (Martinez-Torrecuadrada et al., 2003). Subunit vaccines based on recombinant VP2 alone fail to match the levels of protection induced by immunization with an inactivated IBDV vaccine (Fernández-Arias et al., 1998).

Recently, Lee et al. (2015) and Jackwood (2013) demonstrated that VLPs elicit a strong humoral immune response and provide satisfactory protection against IBDV in specific pathogen-free chickens. In particular, the protective immunity induced by VLP vaccines administered in the Montanide ISA70 adjuvant was comparable with that induced by a commercial inactivated vaccine administered in an oil-emulsion (Lee et al., 2015), indicating that the former may

be a potential alternative to oil-emulsion inactivated IBDV vaccines. If so, VLPs may be a major alternative to conventional IBDV vaccines.

Recombinant VLP vaccines have several advantages over conventional IBDV vaccines in terms of preparation. Currently, oil-emulsion inactivated IBDV vaccines are manufactured using large amounts of IBDV extracted from BF tissues from chickens infected with virulent strains of IBDV. This is why BF-derived IBDV antigens are thought to be more immunogenic than IBDV antigens derived from chicken eggs. However, preparation of IBDV vaccines requires handling of highly contagious virus and the inactivation of infectious viral antigens using bio-hazardous chemicals such as binary ethyleneimine. Importantly, this process is both time-consuming and labor intensive because it requires the use of large numbers of IBDV-free chickens and must be carried out in bio-secure animal facilities. This has a negative impact on both animal welfare and vaccine production costs. VLP vaccines overcome these limitations because they are noninfectious and can be readily prepared in cell culture facilities available within most laboratories.

One important point regarding the efficacy of IBD vaccines is the yield of vaccine antigen produced during preparation. The yield of VLP antigens required to provide protection against IBD is unclear. VLP antigens can be mono-valent or incorporated into a multivalent poultry vaccine, especially for use in breeder flocks. Jackwood (2013) demonstrated that vaccination with 0.1 ml of insect cell culture containing VLPs provided complete protection against IBDV. Lee et al. (2015) demonstrated that co-expression of PP and VP4 by a single recombinant baculovirus in insect cell cultures generated enough VLPs to construct a multivalent poultry vaccine. Nevertheless, further studies should compare the cost-effectiveness of VLP production with that of inactivated IBDV vaccines.

In the field, the efficacy of passive immunity in young chicks depends on the titer and uniformity of maternal-derived antibodies (MDAs), the success of IBDV vaccination, or exposure to wild-type virus. In particular, MDA uniformity is critical for the development of an optimal live attenuated IBDV-based vaccination program in young chicks. In the field, many flocks are subjected to inefficient vaccination programs due to poor MDA uniformity (e.g., a coefficient of variation >80%). Lee et al. (2015) reported that, within a flock, VLP vaccines induce more uniform antibody titers than a commercial inactivated IBDV vaccine. Thus, chicken within breeder flocks vaccinated with a VLP vaccine will endow their offspring with more uniform MDA titers, which would in turn facilitate optimal timing of IBD vaccination in young flocks.

IBD VLPs have various applications in the field

In the field, VLP technology can be applied in several ways to protect against IBDV. A current field virus can be used as the source of genetic material from which VLPs can be derived; thus, the antigenic structure of VLPs more likely resembles that of vvIBDV or IBDV antigenic variant than that of the current vaccine strain. Thus, VLP vaccine technology might lead to the development of tailor-made IBD vaccines that are homologous with field viruses responsible for IBD outbreaks (Lee et al., 2015). Vaccination of Beeder flocks with such VLPs could provide progeny chicks with effective protection via MDAs.

Modification of traditional VLP production methods means that a multivalent VLP vaccine containing VLPs derived from several types of IBDV can be generated. Jackwood (2013) developed a multivalent VLP vaccine comprising several VLPs; VLPs were produced in insect cells co-infected with a mixture of two different recombinant baculoviruses (containing both classic and variant pVP2 genes) and a recombinant baculovirus expressing VP3. Chickens vaccinated with the multivalent VLPs were protected from virulent variant and classical strains of IBDV. This indicates that multivalent VLP vaccines might be an effective tool for preventing IBD in regions in which classical virulent and antigenic variants are co-circulating.

IBD VLPs can also serve as marker vaccines to control IBD in conjunction with a diagnostic test that enables serological differentiation between vaccinated and infected animals (DIVA). Because IBD is endemic in most countries, VLP marker vaccines should be used prophylactically on farms or in regions that are free from IBD. Importantly, since currently available diagnostic tests are based on whole viral antigens or on the VP2 antigen, they cannot differentiate the VLP vaccine from field viruses. Thus, it is essential that we develop diagnostic tests that can make this differentiation if VLP vaccines are to be used as marker vaccines.

Lastly, chimeric IBD VLPs can be generated by inserting foreign protective epitopes into viral structural proteins. VLPs generally induce both B cell- and T cell-mediated immune responses (Müller et al., 2012), which induce protective immunity against foreign pathogens via the presentation of foreign epitopes, or via an adjuvant effect (Liu et al., 2012). Such chimeric IBD VLPs have been developed against influenza virus (Pascal et al., 2016) and human papillomavirus type 16 (Martin Caballero et al., 2012).

In conclusion, VLPs mimicking naïve IBDV particles are potential alternatives to IBDV vaccines and have various applications in the field. However, VLP technology has some limitations that need to be overcome by improving the yield of VLPs and developing a suitable DIVA diagnostic assay.

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