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In ovo DNA immunisation followed by a recombinant fowlpox boost is fully protective to challenge with virulent IBDV

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Abstract

The aim of this study was to investigate the potential use of DNA vaccination delivered in ovo for protecting against challenge with infectious bursal disease virus (IBDV). Using a plasmid expressing the β-galactosidase gene, DNA was successfully delivered to the embryo after in ovo injection and localises to the proventriculus and thymus. The coding sequence for the immunogenic IBDV protein, VP2, was cloned into pCI-neo, creating pCI-Vp2. Complete protection against IBDV was obtained by priming in ovo with pCI-Vp2, followed by boosting with the fowlpox recombinant, fpIBD1, also expressing the VP2 gene. This complete protection was not evident with either of the experimental vaccines on their own. An antibody response was not detected after the prime-boost vaccination, even after chicks had been challenged with IBDV, implying that the DNA prime delivered in ovo stimulated a protective cellular immune response.

Keywords: DNA vaccine; In ovo vaccination; IBDV

1. Introduction

Infectious bursal disease (IBD) is an economically important disease of chickens and is manifested by chronic immunosuppression, morbidity and, in some cases, mortality [1]. Infectious bursal disease virus (IBDV), the causative agent [2–4], predominantly targets B cells residing in the bursa of Fabricius [5,6], resulting in bursal atrophy, oedema and abrogation of antibody responses. Significantly, IBD-induced immunosuppression increases the occurrence of disease caused by opportunistic pathogens and prevents young chickens from responding optimally to vaccines. IBDV is a member of the Birnaviridae [2–4] whose genome consists of bi-segmented double-stranded RNA enclosed within a non-enveloped protein capsid [3]. The genome encodes five proteins, of which VP2 and VP3 are the major structural proteins forming the outer and inner part of the protein capsid, respectively [7,8]. VP2 is considered the host-protective antigen as it contains those antigenic regions responsible for the induction of neutralising antibodies [9].

IBDV is highly infectious and resistant to environmental exposure [10]. Vaccination is essential to protect chickens from outbreaks of infection and is especially important during the first weeks after hatching. Before the onset of laying, hens are usually vaccinated with inactivated oil-emulsified IBD vaccines to induce high titres of antibodies that can be transferred to the egg [11]. Maternal antibodies are essential for protection but these rapidly decline and, within a few days of hatching, chicks are vaccinated with live IBD vaccine. In the late 1980s, vaccination failures were reported in different parts of the world, due to the emergence of very virulent strains of IBDV in Europe and Asia, and antigenic variants in the USA [12,13]. In Europe, very virulent strains were able to overcome high levels of maternally derived immunity and cause a substantial increase in mortality [13,14]. To combat this, more virulent strains of IBDV were introduced as vaccines but these risk causing immunosuppression and/or

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clinical disease in chicks that are poorly protected by maternally derived antibody and genetically susceptible [15,16]. Novel approaches to vaccine development may be beneficial and one possibility is the use of recombinant DNA technology to construct vaccines that avoid the side-effects of the virulent vaccines and are not affected by maternal antibodies.

In ovo vaccination at 18 days of incubation (DI) is a relatively new method for successfully vaccinating chick embryos. More than 80% of commercial broilers are vaccinated using this method in the USA, principally against Marek's disease. In addition to promoting early immunity, automated in ovo vaccination delivers a more uniform dose of vaccine to the egg and induces early resistance to field challenge [17] than manual vaccination of the neonate. At 18 DI, the current age at which automated in ovo vaccination is carried out, manual or automated injection usually deposits the inoculum into the amniotic fluid, which is then imbibed by the embryo [18–22].

DNA vaccination was first demonstrated in the early 1990s, where the in vivo expression of an encoded gene was shown to stimulate an antibody response after gene-gun vaccination in mice [23]. Since then, many studies have illustrated the advantages of DNA vaccination, which include simplicity of production, ease of manipulation and biological and chemical stability. In addition, DNA vaccines can stimulate both cellular and antibody immune responses and induce protection against challenge with infectious agents. DNA vaccination against IBDV has been previously reported with varying degrees of success [24–31]. To our knowledge, protection against IBDV challenge after in ovo administration of a DNA vaccine has not been demonstrated. A DNA plasmid containing the genes coding for IBDV VP2, VP3 and VP4 was administered via the in ovo route and expression observed in the embryonic liver and muscle [32]. Encouragingly, in ovo vaccination with a DNA vaccine against infectious bronchitis virus stimulated complete protection when followed by a live viral boost [33]. However, a single vaccination with DNA delivered via the in ovo route may not be feasible due to the immaturity of the chick's immune system. A novel heterologous 'prime-boost' vaccination regime has recently been shown to be highly effective in improving the efficacy of DNA vaccines in some mammalian species [34,35]. This strategy involves priming with a DNA vaccine and then boosting with a recombinant virus vaccine, usually a poxvirus, both expressing the same antigen gene.

fpIBD1 is an experimental recombinant vaccine, which expresses amino acids 1–532 of the segment A polyprotein from IBDV (strain F52/70) under the control of a vaccinia virus promoter within the FP9 strain of fowlpox [36]. The inserted sequence includes the whole of VP2 and part of VP4. Vaccination with fpIBD1 protects against mortality resulting from challenge with virulent (F52/70) and very virulent (CS89) strains of IBDV [36]. However this protection is only partial for, after challenge, bursal lymphocyte depletion was still evident, as indicated by bursal involution, histopathology and reduced antibody response to other antigens [36,37]. The extent of the partial protection mediated by fpIBD1 is dependent on the severity of virus challenge and the genotype of the chick [38].

In this present study, a DNA vaccine encoding the VP2 gene from IBDV strain F52/70 was administered to chicks in ovo, followed by a boost with fpIBD1 at 1 week of age. Protection against IBDV challenge was then examined.

2. Materials and methods

2.1. Chickens

Embryos were obtained from a disease-free flock of Rhode Island Red (RIR) chickens maintained at the Institute for Animal Health (IAH), Compton, UK. The parent hens were shown to be free of antibodies to a number of pathogens including IBDV, chicken anaemia virus and Marek's disease virus. Therefore it was considered that their progeny were specified-pathogen free (SPF) and lacked maternal antibodies to IBDV. The embryos were incubated according to standard procedures and chicks were reared in clean conditions before being transferred to filtered-air positive pressure rooms and separated into experimental groups. Infected and non-infected chickens were maintained in separate rooms. Food and water were available ad libitum. All animal procedures followed the UK Home Office guidelines for animal welfare.

2.2. Viruses

Stocks of IBDV strain F52/70 [39] were produced by infecting 3-week-old chickens and isolating virus from the bursa of Fabricius 3 days later, as described earlier [38]. The titre of virus stock was kindly determined by Dr. Adriaan van Loon (Intervet BV, The Netherlands) [40]. The recombinant vaccine, fpIBD1, and its parent vector, FP9, were produced by tissue culture passage on chicken embryo fibroblasts (CEF) [38].

2.3. Plasmid construction

DNA was isolated from fpIBD1 using sodium dodecyl sulphate (SDS) and proteinase K. Forward and reverse primers for polymerase chain reaction (PCR) were based on the published F52/70 sequence [41] and were designed to amplify a fragment that included the start codon of Vp2 and the predicted proteolytic cleavage site of VP2/VP4 [42]. The primer sequences were as follows: forward primer 5′-CCGGAATTCCGGCGGGATCCCGATGACAAACCTGCAAGATCAAAC-3′ and reverse primer 5′-CCGGAATTCCGGCGGGATCCCGCTAAACTACGGGATTCTGGGGCA-3′. PCR was carried out using 50 ng fpIBD1 genomic DNA, 5 U *Taq* polymerase (Promega, Southampton, UK), 1 mM dNTP mix (Promega), 25 mM