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Recombinant infectious bursal disease virus expressing Newcastle disease virus (NDV) neutralizing epitope confers partial protection against virulent NDV challenge in chickens

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ABSTRACT

In this study, the regions in the infectious bursal disease virus (IBDV) genome that are amenable to the introduction of a sequence encoding a virus-neutralizing epitope of Newcastle disease virus (NDV) hemagglutinin-neuraminidase (HN) protein were identified. By using the reverse genetics approach, insertions or substitutions of sequences encoding the NDV epitope were engineered in the exposed loops (P_{BC}, P_{HI} and $P_{AA'}$) of the VP2 capsid protein and the N terminus of the nonstructural VP5 protein as well as the pep7a and pep7b regions of the pVP2 precursor of a commonly used IBDV vaccine strain, Gt. Three recombinant IBDVs expressing the NDV epitopes were successfully rescued in the P_{BC}, pep7b and VP5 regions and the expressed epitope was recognized by anti-HN antibodies. Genetic analysis showed that the IBDV recombinants carrying the NDV epitopes were stable in cell cultures and in chickens. Animal studies demonstrated that the IBDV recombinants were innocuous in chickens. Vaccination with the recombinant viruses generated antibody responses against both IBDV and NDV, and provided 70–80% protection against IBDV and 50–60% protection against NDV. These results indicate that the recombinant IBDV has the potential to serve as a novel vaccine vector for other pathogens. In future studies, it is worth considering research to improve IBDV vector vaccine to get complete protection and safety of animals and humans.

1. Introduction

Infectious bursal disease virus (IBDV), a member of the genus *Avibirnavirus* in the family *Birnaviridae*, causes considerable economic losses in the poultry industry worldwide by inducing an acute and highly contagious disease in young chickens with high mortality (van den Berg, 2000). IBDV is a non-enveloped, icosahedral virus which has a polyploid genome containing two segments of double-stranded RNA. The smaller segment, B, encodes VP1, a 90-kDa viral RNA-dependent RNA polymerase (von Einem et al., 2004). The larger segment, A, contains two partially overlapping open reading frames (ORFs). The smaller ORF encodes VP5, a 17-kDa nonstructural protein. VP5 is dispensable for viral replication in vitro and in vivo (Yao et al., 1998), which makes it a prime candidate for the construction of marked vaccines carrying deletions. The larger ORF encodes a 110-kDa precursor polyprotein that is proteolytically cleaved by the viral protease VP4 (Birghan et al., 2000) to form the pVP2 precursor (48 kDa) as well as VP4 (28 kDa) and VP3 (32 kDa). Throughout virion maturation, pVP2 is further processed by several proteolytic cleavages at its C terminus for conversion into mature VP2 (41 kDa) and four structural peptides composed of 46, 7, 7 and 11 amino acids (pep46, pep7a, pep7b, and pep11), all associated with the virus particle (Chevalier et al., 2005; Da Costa et al., 2002). VP2 and VP3 are the major structural proteins, constituting 51% and 40% of the virion, respectively (van den Berg, 2000). The four peptides are located either at the external surface of the viral particle (pep7a, pep7b, and pep11) or underneath the surface at the fivefold or quasi sixfold axes (pep46) (Chevalier et al., 2005). It was showed that pep46 and pep11 are essential to viral assembly and play a key role in the viral cycle (Chevalier et al., 2005; Galloux et al., 2007). In contrast, pep7a and pep7b are not essential to viral assembly, and reverse genetics shows that they are dispensable for virus rescue (Chevalier et al., 2005; Da Costa et al., 2002).

The IBDV capsid consists of a single shell formed by 260 trimers of protein VP2 organized in a $T = 13$ icosahedral lattice (Coulibaly et al., 2005). The available structural data for VP2 (Coulibaly et al., 2005; Garriga et al., 2006; Lee et al., 2006) reveal that this protein is folded into three distinct domains named projection

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(P), shell (S), and base (B). Expression of VP2 by itself leads to dodecahedral subviral particles (SVP) containing 20 VP2 trimers (Castón et al., 2001) and exposing five loops of the P domain away from the surface of the SVP, in which loops P_{BC} (aa 219–224), P_{DE} (aa 250–254), P_{FG} (aa 283–287) and P_{HI} (aa 315–324) located at the head of domain P, while loop $P_{AA'}$ (aa 184–195) located near domain S (Coulibaly et al., 2005). Previous studies carried out on the virus cell tropism and virulence (Brandt et al., 2001; Qi et al., 2009) suggest that loops P_{DE} and P_{FG} containing residues 253 and 284 are involved in virus-cell receptor binding, and loops P_{BC} and P_{HI} are the most suitable sites for the insertion of foreign peptides (Rémond et al., 2009).

Recently, the potential of IBDV to serve as an antigen delivery system has been explored. It was shown that the immunodominant epitope of foot-and-mouth disease virus (FMDV) could be effectively inserted into the P_{BC} loop to produce IBDV SVPs with chimeric VP2 in insect cells (Rémond et al., 2009). The produced subviral particles reacted with FMDV monoclonal antibodies and elicited a neutralizing antibody response in immunized mice. More interestingly, Upadhyay et al. (2011) successfully recovered recombinant IBDVs expressing c-Myc and human hepatitis C virus (HCV) epitopes in the VP5 region of segment A. In spite of these promising results, the tolerable size and sites in the IBDV genome for the insertion have not been fully studied. Further exploration of the insertion sites used in previous studies and other sites with different antigenic epitopes would shed more light on the development of IBDV vectors.

Newcastle disease virus (NDV) is a member of the genus *Avulavirus* in the family *Paramyxoviridae*. NDV causes an economically important disease and has the potential to infect all species of birds worldwide, with the mortality and morbidity rates varying among species and strains of the virus (Alexander, 1997). The envelope of NDV contains two glycoproteins, the virus hemagglutinin-neuraminidase attachment protein, HN, and the fusion protein, F, which form spike-like protrusions on the outer surface of the virion. The HN and F proteins are important for virus infectivity and pathogenicity (Nagai et al., 1976). They produce virus neutralizing antibody responses and are the protective antigens (Boursnell et al., 1990; Cosset et al., 1991; Sun et al., 2008). Major antigenic determinants and epitopes that stimulate the production of virus-neutralizing antibodies have been determined for the HN (Chambers et al., 1988) and F proteins (Toyoda et al., 1988).

The present study explored the possibility of recovering recombinant IBDVs carrying NDV HN neutralizing epitopes with the aim of further studying the potential of IBDV vectors and developing a safe and efficient vaccine candidate against NDV. The insertion sites used in previous studies were further explored by inserting or substituting the NDV epitope in the P_{BC} and P_{HI} loops of the VP2 or the VP5 region. The previously unexplored regions, $P_{AA'}$ loop of VP2, pep7a and pep7b of pVP2 precursor, were also tested as potential insertion sites. Here, three recombinant IBDVs expressing the NDV epitopes were successfully recovered in the P_{BC}, pep7b and VP5 regions, and they elicited neutralizing antibody responses against both IBDV and NDV in immunized chickens. These results clearly suggest that it is possible to use IBDV as a novel vaccine vector for other pathogens and the recombinant IBDVs generated in this study have the potential for development into a bivalent vaccine candidate against virulent NDV as well as IBDV infection.

2. Materials and methods

2.1. Viruses, cells and plasmids

The very virulent IBDV (vvIBDV) strain Gx was isolated and identified as previously described (Wang et al., 2003). IBDV strain Gt was attenuated from vvIBDV Gx after blind passages in vivo (Wang et al., 2004). The NDV vaccine strain LaSota and the virulent strain F48E9 were originally received from the China Veterinary Culture Collection. The NDV live vaccine LaSota was obtained from a commercial manufacturer (Weike, Harbin, China). Primary chicken embryo fibroblast (CEF) cells were prepared from 10-day-old specific-pathogen-free (SPF) chicken embryos. DF1 (a CEF cell line) cells were kindly provided by Dr. Zhigao Bu, Harbin Veterinary Research Institute, the Chinese Academy of Agricultural Sciences, and cultured in Dulbecco's modified Eagle's medium (DMEM; Invitrogen, Carlsbad, CA). The eukaryotic expression vector pCAGGS (Niwa et al., 1991) was kindly provided by Dr. J. Miyazaki, University of Tokyo, Japan.

2.2. Animals

SPF white leghorn chickens were obtained from the Experimental Animal Center of Harbin Veterinary Research Institute, the Chinese Academy of Agricultural Sciences and housed in negative-pressure-filtered air isolators. Animal experiments were approved by the Animal Ethics Committee of the Institute and performed in a biosafety level 2 facility in accordance with animal ethics guidelines and approved protocols.

2.3. Construction of plasmids

Construction of the plasmids pCAGGmGtAHRT and pCAGGmGtBHRT containing segments A and B of IBDV Gt flanked by the hammerhead ribozyme (HamRz) sequence at their 5′ ends and hepatitis delta ribozyme (HdvRz) sequence at their 3′ ends, respectively, has been described previously (Qi et al., 2007). Plasmid pCAGGmGtAHRT was used as template to construct different plasmids of IBDV segment A containing foreign sequences. Plasmid pCAGGmGtBHRT was used to co-transfect DF1 cells with plasmids of segment A for virus rescue. Ten different constructs of segment A were created by inserting or substituting a sequence encoding a conserved neutralizing epitope (PDEQDYQIR, residues 345–353) of NDV HN protein in the loops of VP2, the peptide regions of pVP2 or the N terminus of VP5, as shown in Fig. 1. First, plasmids pIBDV-1 and pIBDV-2 were constructed by inserting the NDV epitope sequence into the P_{BC} loop of VP2 (between nt 793 and 794 of segment A) or replacing the nucleotides encoding the P_{BC} loop (nt 785–802 of segment A) with the epitope sequence, respectively. The epitope was also inserted into the P_{HI} loop (between nt 1087 and 1088) for pIBDV-3 or replaced the P_{HI} loop (nt 1073–1102) for pIBDV-4. Additionally, the $P_{AA'}$ loop of VP2 was inserted (between nt 697 and 698) or replaced (nt 686–712) with this epitope for pIBDV-5 and pIBDV-6. Second, the NDV epitope was inserted into the pep7a region of pVP2 (between nt 1600 and 1601) for pIBDV-7 or the pep7b region (between nt 1621 and 1622) for pIBDV-8. Third, the NDV epitope was inserted into the N terminus of VP5 (between nt 99 and 100) for pIBDV-9 or replaced the N terminus of VP5 (nt 100–126) for pIBDV-10.

All the manipulations were done by performing overlapping PCR using the respective primers (Table 1). To construct pIBDV-1, two PCR fragments (878 bp and 2582 bp) were amplified with primer pairs HamCla1F/IBDV-1R and IBDV-1F/HdvKpn1R, respectively. These fragments were combined and amplified into one fragment using the flanking primer pair HamCla1F/HdvKpn1R. The amplified fragment was digested with ClaI/KpnI and ligated into pCAGGS vector under control of the hybrid CMV enhancer/chicken β-actin promoter to yield plasmid pIBDV-1. Similarly, plasmid pIBDV-2 was generated using the primer pairs HamCla1F/IBDV-2R, IBDV-2F/HdvKpn1R and HamCla1F/HdvKpn1R. The construction of plasmids pIBDV-3–pIBDV-10 was performed similarly using the respective primers. All the plasmids were sequenced to confirm the desired sequence changes in segment A.