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The potent adjuvant effects of chicken β-defensin-1 when genetically fused with infectious bursal disease virus VP2 gene

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ABSTRACT

Defensins are fundamental components of innate immune response. Current data favor that defensins play vital roles on both innate and adaptive immune responses. The aim of the present study was to investigate whether the chicken β-defensin-1 (also named avian β-defensin-1, AvBD1) has the potent adjuvant effects on DNA vaccine encoding IBDV VP2 gene, when genetically fused with VP2 gene. The recombinant vectors pcDNA3.1(+)-VP2 and pcDNA3.1(+)-AvBD1-VP2 were constructed as the DNA vaccines. Four groups of 14-day-old chickens were intramuscularly injected with PBS buffer, empty vector pcDNA3.1(+), recombinant pcDNA3.1(+)-VP2 and pcDNA3.1(+)-AvBD1-VP2. Results showed that VP2-specific antibody levels significantly increased following two recombinant DNA vaccine administrations ($p < 0.05$), compared with the group of PBS and empty vector. The antibody level of group immunized with pcDNA3.1(+)-AvBD1-VP2 was significantly higher than that of group immunized with pcDNA3.1(+)-VP2 after second vaccination ($p < 0.05$). The percentages of CD3+, CD4+ and CD8+ T-cell subtypes between groups of pcDNA3.1(+)-VP2 and pcDNA3.1(+)-AvBD1-VP2 obtained significantly different ($p < 0.05$), the latter was higher, at 7 days post-booster. The protection from IBD challenged by immunized chickens with DNA vaccines encoding IBDV VP2 gene alone was lower than that by immunized IBDV VP2 gene together with AvBD1 gene. The results indicated that AvBD1 has an adjuvant effects on improvement the IBDV VP2-DNA vaccine effectiveness.

1. Introduction

Defensins are small cationic antimicrobial peptides that constitute a major part of the innate immunity against pathogens and have been widely found in mammals, insects, fish, and birds (Ganz and Lehrer, 1998; Linde et al., 2008). The best known property of defensins is their ability against microorganisms, including bacteria, fungi, protozoa and enveloped viruses (Hancock and Lehrer, 1998; Diamand and Bevins, 1998; Linde et al., 2008). In addition to their direct antimicrobial activities, immunomodulatory properties have also been documented. Human neutrophil peptides (HNP)-1 and HNP-2 have been shown to be potent chemotactic for murine and human T-cells, monocytes, immature dendritic cells, and polymorphonuclear leukocytes (Territo et al., 1989; Chertov et al., 1996; Yang et al., 2000; Grigat et al., 2007). Yang et al. (1999) reported that human β-defensins (hBDs) induce the migration of both human resting memory T-cells and immature dendritic cells by interacting with CC chemokine receptor 6 and proposed that β-defensins may bridge innate and adaptive immunity of the host. HBDs have also been shown

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Table 1
Primers used for amplification of AvBD1 and IBDV VP2 genes.

Name of primers	Primer sequences	RE site introduced
AvBD1-P1[a]	5′-CGAAGCTT*AAACC***ATG**GGAAGGAAGTCAGATTG-3′	HindIII
AvBD1-P2[b]	5′-TTGGATCCGCCCCATATTCTTTTGC-3′	BamHI
VP2-P1[c]	5′-ATGAATTC **T**ACGAACCTGCAAGAT-3′	EcoRI
VP2-P2[d]	5′-ATAAGCTT *AAACC***ATG**ACGAACCTGCAAGAT-3′	HindIII
VP2-P3[e]	5′-ATCTCGAG **TCATCA**CCTTAGTGCCCGAATTAT-3′	XhoI

[a] The 5′ end of primer AvBD1-P1 contains HindIII restriction site (underlined), a Kozak translation initiation sequence (slanted) and an ATG start codon (bold).
[b] The 5′ end of primer AvBD1-P2 contains BamHI restriction site (underlined), deleted the stop codon.
[c] The 5′ end of primer VP2-P1 contains EcoRI restriction site (underlined), a base T added (bold).
[d] The primer VP2-P2 contains HindIII restriction site (underlined), a Kozak translation initiation sequence (slanted) and an ATG start codon (bold).
[e] The primer VP2-P3 5′ end contains XhoI restriction site (underlined), the stop codon TCA (bold).

to be chemotactic for monocytes and macrophages, and mast cells (Yang et al., 2002; Niyonasba et al., 2002, 2004; Soruri et al., 2007). In addition to direct chemotactic activity, defensins can exert indirect chemotactic activity by stimulating chemokine and cytokine secretion from a variety of cell types. For example, HNPs have been reported to possess immune-modulating properties via induction of adhesion and co-stimulatory molecules, activation of transcription factors and to increase the production of IL-8, then linking innate to acquired immunity (Vaschetto et al., 2007). HBD-3 can induce expression of co-stimulatory molecules CD80, CD86 and CD40 by interaction with TLRs 1 and 2 on monocytes and dendritic cells resulting in MyD88 signaling, leading to IL-1 receptor-associated kinase-1 phosphorylation (Funderburg et al., 2007). HBD-2 stimulates keratinocyte migration, proliferation and cytokine or chemokine production and initiates the process of tissue repair (Niyonasba et al., 2007). Moreover, several studies have demonstrated that defensins can act as adjuvants to enhance antigen-specific immunity in vivo (Lillard et al., 1999; Tani et al., 2000; Biragyn et al., 2001, 2002a).

To date, 14 different chicken β-defensin (AvBD1–14) genes have been found on chromosome 3 3q3.5–q3.7 of the chicken genome (Xiao et al., 2004; Lynn et al., 2004). The antimicrobial activities of AvBDs have also been extensively investigated (Dijk et al., 2008), but very little information is currently available regarding the immunomodulatory properties of these peptides. Thus, researches on this field should been carried out. Here, we describe for the first time a genetic DNA vaccine encoding infectious bursal disease virus (IBDV) VP2 protein fused with chicken AvBD1 mature peptide, elicited anti-VP2 antibodies with higher titers, demonstrating the potent adjuvant property of AvBD1. To our knowledge, there has been no report of using chicken AvBD1 as an adjuvant to design DNA vaccines against avian disease infection.

2. Materials and methods

2.1. Materials

Recombinant plasmids pET32-AvBD1, pET32-VP2, eukaryotic expression vector pcDNA3.1(+) (Invitrogen, Shanghai, China), *Escherichia coli* BL21 and DH5a strains (Novagen, Madison, USA) were preserved in Poultry Disease Laboratory in South China Agricultural University (SCAU). The rVP2 protein expressed in *E. coli* was purified and stored in Poultry Disease Laboratory of SCAU by Xiaomei Yang. HRP-labeling chicken anti-rabbit IgG antibody and FITC-labeling mouse anti-chicken CD3+, CD4+, and CD8+ monoclonal antibodies were bought from Southern Biotech Co., Ltd. (Bormingham, USA). The Taq-plus high fidelity PCR kit, pMD18-T and all restriction endonucleases used were purchased from TaKaRa Biotechnology (Dalian, China). Endo-free Plasmid Kit and DNA Agarose Gel Purification Kit were supplied by TIANGEN Biotech Co., Ltd. (Shanghai, China). Broiler chickens were supplied by Institute of Zootechnics Science (Guangdong Province, China).

2.2. Primers design and synthesis

According to cDNA sequence of the AvBD1 (accession no. AF033335) and IBDV VP2 (accession no. AF051838), specific primer pairs were designed for PCR, using the biological software DNAstar (listed in Table 1). Primer pairs AvBD1-P1/AvBD1-P2 are used for amplifying mature segment AvBD1, and the stop codon of AvBD1 gene was omitted when primers were designed. The expected size is 140 bp. Primer pairs VP2-P1/VP2-P3 are used for amplifying VP2 segment, the expected size is 1372 bp. An addition of base T in primer VP2-P1 is to make the ORF of AvBD1-VP2 fusion gene cassette proper. Primer pairs VP2-P2/VP2-P3 are used for amplifying VP2 entire coding region segment; the expected size is 1378 bp. Primers were synthesized by Invitrogen Biotech Co., Ltd. (Shanghai, China).

2.3. Construction of pcDNA3.1(+)-AvBD1-VP2 and pcDNA3.1(+)-VP2 vector

The complete sequence of AvBD1 gene comprises three segments: the signal peptide segment, prosequence segment and mature segment. Only mature fragment of AvBD1 gene was amplified from plasmid pET32-AvBD1 by PCR. After recovery and purification, the PCR products were ligated into plasmid pMD18-T to yield the recombinant plasmid pMD18-AvBD1. The AvBD1 segments were digested by restriction endonucleases HindIII and BamHI from pMD18-AvBD1, and ligated into the plasmid pcDNA3.1(+) digested by the same enzymes to construct the recombinant plasmids pcDNA3.1(+)-AvBD1. To con-