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Immunoadjuvant activities of a recombinant chicken IL-12 in chickens vaccinated with Newcastle disease virus recombinant HN protein

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ABSTRACT

Recombinant fowlpox virus (rFPV/HN) expressing Newcastle disease virus (NDV) HN gene and rFPV/HN/chIL-12 co-expressing chicken IL-12 (chIL-12) and HN (rHN/chIL-12) genes have been characterized. rHN/chIL-12 or rchIL-12, expressed by our previous construct rFPV/chIL-12, co-administered with rHN was assessed for adjuvant activities of chIL-12. Chickens were vaccinated with various amounts of rHN/chIL-12 mixed with mineral oil (MO), intramuscularly. Levels of hemagglutination-inhibition (HI) antibody production depended on the concentration of the injected rHN or rHN/chIL-12. The lower HI antibody titers were obtained in chicken groups rHN/chIL-12/7-rHN/chIL-12/9, receiving 60 ng rHN/8 ng chIL-12 with MO, 30 ng rHN/4 ng chIL-12 with MO or 15 ng rHN/2 ng chIL-12 with MO, respectively, compared to those in chicken groups rHN/7-rHN/9, receiving rHN with MO alone. However, chickens in group rHN/chIL-12/7 or rHN/chIL-12/8 and rHN with MO alone showed the same effective protection. Chicken group rHN/chIL-12/9 was even more protective than that in group rHN/9. When rchIL-12 was co-injected with 15 ng rHN plus MO, chickens produced low levels of HI antibody titers; while higher levels of IFN-γ production and an effective protection rate (83%) were obtained. On the other hand, low levels of IFN-γ production and low protection response (50%) were obtained in chickens injected with rHN with MO alone. Taken together, when the concentration of rHN decreased to certain levels, rchIL-12 reduced HI antibody production. The increase in the induction of IFN-γ production might suggest the enhancement of the cell-mediated immunity which conferred the protection from the NDV challenge.

1. Introduction

Mammalian interleukin-12 (IL-12) is a well-characterized heterodimeric cytokine that is mainly produced by macrophages, dendritic cells and Langerhan cells (Kang et al., 1996; Macatonia et al., 1995; Skeen et al., 1996). IL-12 has regulatory effects on the immune system through its ability to stimulate natural killer and T-helper-1 (Th-1) cells to induce interferon (IFN-γ) production (Kobayashi et al., 1989; Xu et al., 1996). These effects, in turn, activate many functions including the enhancement of serum IgG2a antibody responses against a variety of antigens (Germann et al., 1995; Gracie and Bradley, 1996). Because of the promotion of the Th1 cell differentiation and IFN-γ production, IL-12 also involves in T-cell mediated immunity (Leonard et al., 1997; Rodriguez-Galan et al., 2009). Thus, IL-12 has shown significant promise as a vaccine adjuvant (Metzger, 2009). IL-12 is composed of two glycosylated

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disulfide-linked proteins, the α and β chains (Kobayashi et al., 1989). Both peptide chains must be expressed together to produce the biologically active IL-12 heterodimeric molecule (Ma and Trinchieri, 2001).

Sequences of chicken IL-12 (chIL-12) gene have been cloned (Degen et al., 2004; Balu and Kaiser, 2003). Functionally active chIL-12 was also characterized as a heterodimeric cytokine (Degen et al., 2004). Both mammalian IL-12 and chIL-12 have been expressed in eukaryotic systems, such as baculovirus and COS cells for characterization (Degen et al., 2004). Recently, chIL-12 synthesis in *Escherichia coli* (Thomas et al., 2008) and in plant (Medrano et al., 2010) expression system has been reported and they were biologically active as it induced the proliferation of chicken splenic lymphocytes, similar to that observed with COS cell derived chIL-12 (Degen et al., 2004). However, little is known about the in vivo activity. In our laboratory, we have prepared rchIL-12 from a fowlpox virus (FPV) recombinant (rFPV/chIL-12). This rchIL-12 induced the synthesis of IFN-γ in cultured primary chicken splenocytes. IFN-γ production was also detected in serum samples and in cultured primary splenocytes from chickens inoculated with rFPV/chIL-12 by wing-web puncture (wwp) or injected, intraperitoneally (ip), with rFPV/chIL-12-infected cell lysates (Su et al., 2010). The results demonstrated that rchIL-12 was biologically active both in vitro and in vivo.

Vaccine adjuvants are essential to enhance the host's immune response to antigens, particularly, to subunits of pathogen. In addition to traditional adjuvants, cytokines which are host-derived immunostimulators, have been described as immunopotentiators of vaccine, when co-administered either as heterologous expression product or following delivery by a viral vector (Hilton et al., 2002a,b; Villinger, 2003). Co-administered chicken IFN-γ (chIFN-γ) to chickens with antigen has been shown to enhance a prolonged secondary antibody responses that persisted at higher levels and for longer periods compared to antigen injected alone (Lowenthal et al., 1998). Treatments with chIFN-γ (Lillehoj and Choi, 1998; Lowenthal et al., 2000) or plasmids encoding chIFN-γ (Ding et al., 2004) resulted in protection from infection with *Eimeria* and reduced weight loss associated with this disease. However, when chickens were vaccinated with inactivated Salmonella enteritidis with co-administration of chIFN-γ, it enhanced protection against Salmonella enteritidis challenge without acceleration of antibody production (Takehara et al., 2003). Immunoadjuvant activities of chIL-1β were examined using tetanus toxoid as an antigen, when administered as recombinant protein, chIL-1β increased antibody response (Schijns et al., 2000). Moreover, co-administration of chIL-1β, chIFN-α and chIFN-γ showed an additive effect on the antibody response to the tetanus toxoid antigens. It has been shown that chIL-2 treatment resulted in an increase in the proportion of both $CD4^+$ and $CD8^+$ peripheral blood T cells within 48 h compared to control chickens, suggesting that it may be able to augment cell-mediated immune responses when co-administered with vaccine (Hilton et al., 2002a,b). Bursal lesion protection of a DNA vaccine against infectious bursal disease virus was enhanced by co-administration of a plasmid encoding chIL-2 (Li et al., 2004). ChIL-18 expressed in *E. coli* significantly enhanced antibody responses against Newcastle disease virus (NDV) and tetanus toxoid in vaccination of chickens (Degen et al., 2005). Recently, vaccination with chIL-18 expressed by *E. coli* or by the injected plasmids encoding chIL-18 was proved to be able to enhance both antibody and cell-mediated immune responses to NDV in chickens (Hung et al., 2010). The major activities of mammalian IL-6 not only involve acute-phase protein responses but also been implicated in the development of Th2 type responses (Van Snick, 1990). Cloning and characterization of chIL-6 have been described (Schneider et al., 2001). It has been shown that elevated antibody against *E. coli* K88 proteins induced by co-administering with plasmids encoding chIL-6 persisted longer than when induced by K88 proteins alone (Cho et al., 2004).

Since we have developed the techniques for the production of rchIL-12 using the FPV-vectored recombinant (Su et al., 2010), in the present study we attempted to extend studies to further examine the in vivo adjuvant activity of rchIL-12. To reach this purpose, recombinant hemagglutinin-neuraminidase (rHN) of NDV was produced by a recombinant virus rFPV/HN or co-expressed with chIL-12 by rFPV/HN/chIL-12 that was constructed using similar methods to rFPV/chIL-12 construction and used as the antigen for testing adjuvant activity of rchIL-12.

2. Materials and methods

2.1. Cell and virus

A virulent NDV (TW/2000-3) was a local isolate (Shien et al., 2002). The NDV was propagated in allantoic cavity of specific pathogenic free (SPF) chicken embryonated egg and used as the challenge virus at a dose of 10^4 minimal lethal dose (MLD). The NDV was also used for HN gene amplification. Parent FPV has been adapted to grow in DF1 cells (a chicken embryo fibroblast cell type, ATCC NO.: CRL-12203™) (Su et al., 2010) and was used for the construction of rFPV in this study. Parent FPV and cell-adapted NDV have minimum titers of 5×10^6 $TCID_{50}$/ml and 1×10^6 $TCID_{50}$/ml, respectively, as calculated (Reed and Muench, 1938). FPV recombinant virus, rFPV/chIL-12, containing chicken IL-12 (both α and β chains) and green fluorescent protein (GFP) genes has been characterized (Su et al., 2010). The GFP gene product was used as the selection marker for rFPV/chIL-12 purification. Chicken IL-12 and GFP genes were cloned into FPV non-essential gene F11L. The DF1 cells and rFPV/chIL-12 were used as an expression system for preparing rchIL-12. The concentration of rchIL-12 in cell lysates prepared from DF1 cells infected with rFPV/chIL-12 at 3 days postinoculation was determined to be 20,000 ± 2100 ng/ml. The rFPV/chIL-12 was also used to construct rFPV/HN/chIL-12 recombinant for the co-expression of NDV HN and chIL-12.

2.2. Chickens

All white Leghorn SPF chickens used in this study were purchased from the Animal Health Research Institute, Council of Agriculture, Taiwan at two weeks of age. All