Preparation of ChIL-2 and IBDV VP2 Fusion Protein by Baculovirus Expression System

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This study aims to produce an effective subunit vaccine against infectious bursal disease virus (IBDV). The genes of chicken interleukin-2 (ChIL-2) and IBDV viral protein 2 (VP2) were amplified and fused by splice overlap extension-polymerase chain reaction (SOE-PCR). The fusion gene was digested by EcoR I/Kpn I and inserted into pBacPAK8 vector, resulting in recombinant transfer plasmid pBacPakVP2-IL2. The recombinant plasmid was transfected into Sf-9 cells accompanied with hybrid nuclear polyhedrosis virus (HyNPV) genome DNA and lipofectin. Plaque-purification indicated that we had got the recombinant Hy-VP2-IL2. Fusion protein VP2-IL2 was expressed effectively both in insect cells and bombyx mori. The expression of fusion protein was confirmed by ELISA, SDS-PAGE and Western blotting assay, respectively. This efficient system allows us to meet the need for inexpensive vaccines required by the poultry industry. Cellular & Molecular Immunology. 2005;2(3):231-235.

Key Words: IBDV, ChIL-2, fusion gene, fusion protein

Introduction

Infectious bursal disease virus (IBDV) causes a severe disease of young chickens, which targets the bursa of Fabricius (1). IBDV destroys immature B cells in the bursa, and results in severe immunosuppression of the humoral immune system (2). Chickens around world are constantly exposed to IBDV, which leads to billions dollars losses in the poultry industry. The genome of IBDV consists of two segments of dsRNA. Segment B encodes viral protein 1 (VP1, 90 kD), the viral polymerase, segment A encodes a polyprotein that is cleaved into viral proteins VP2 (47 kD), VP4 (28 kD) and VP3 (32 kD). Segment A also encodes putative VP5, a 17 kD protein present in IBDV-infected cells (3). VP2 is the major host protective antigen and elicits neutralizing antibodies and protection (4, 5), thus VP2 was chosen to be vaccine candidate against IBDV infection. The VP2 gene has been expressed by Escherichia coli, Saccharomyces cerevisiae, fowl poxvirus, herpesvirus and baculovirus. The vaccinations with these expression products have exhibited variable levels of protection (6, 7).

However, traditional inactivated or attenuated vaccines could not provide enough protection against these antigenic variants and were very virulent (vv) IBDV (8). Recent studies focused on the importance of T cells in IBDV pathogenesis have shown that cell-mediated immunity may be more important in IBDV infections than previously thought (9).

Interleukin-2 (IL-2), initially known as T-cell growth factor, is a powerful immuno-regulatory lymphokine which produced by lectin- or antigen-activated T cells (10). When eukaryotic expression plasmid (pCI-IL-2) or recombinant chicken IL-2 (rChIL-2) expressed in E. coli was co-administered with the H5 subtype inactivated avian influenza virus (AIV) vaccines, both can enhance the efficiency of H5 vaccine (11). ChIL-2 could enhance protective immunity against avian pathogens, which would introduce a new weapon into the control of infectious diseases of poultry.

In this study, we constructed fusion transfer vector containing the IBDV VP2 and ChIL-2 genes in tandem with six histamines. The purpose of the present study was to express the fusion protein in bombyx mori. After purification and identification, the newly developed fusion protein may offer an available vaccine candidate which is efficient, convenience and cheap for poultry industry.

Materials and Methods

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**Construction of recombinant transfer vectors**

The **Construction of recombinant transfer vectors**

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**Viral infection**

The recombinant baculovirus **Viral infection**

The **Viral infection**

**Production of fusion protein in silkworm larvae**

Silkworm larvae in early fifth-instar were used for infection by recombinant viruses. Before inoculation, the larvae were placed on ice for 5-10 min. Twenty microliters of the viral solution (2 × 10^6 pfu/ml) was injected subcutaneously into the body of the larvae. Half an hour after the injection, the larvae were fed with mulberry leaves and reared at 23-25°C.

**Immunofluorescence microscopy analysis**

The method for direct observation through negative staining was performed as previously described (15). Infection cells were washed twice with phosphate buffered saline (PBS), fixed with 3.7% paraformaldehyde for 10 min at room temperature and washed three times with PBS. Cells were permeabilised with 0.1% Triton in PBS for 5 min at room temperature, washed with PBS and blocked with 1% BSA in PBS for 30 min at room temperature. Slides were incubated with IBDV polyclonal antibody and chicken interleukin-2 polyclonal antibody respectively for 1 h in a moist chamber at room temperature. Anti-rabbit FITC-conjugated antibody (Promega, USA) was diluted 1:100. Images were captured with the reflected Light Fluorescence Microscope (Olympus, Japan).

**SDS-PAGE and Western blotting analysis**

The sample was mixed with loading buffer, then boiled for 5 min, centrifuged for 1 min and 20 μl of clear supernatant was loaded on to a 12.5% slab gel. Following electrophoresis, the gel was either stained by Coomassie brilliant blue, or used for Western blotting analysis by transferring onto PVDF membrane (Amersham, USA). The PVDF membrane was then soaked in blocking solution containing 5% skim milk and 0.05% Tween-20 in PBS overnight at 4°C. After washing with PBS containing 0.05% Tween-20, the membrane was incubated with IBDV polyclonal serum as primary antibodies. Following washing, the membrane was incubated with an HRP-conjugated goat anti-rabbit IgG and colour-developed in DAB substrate. After washing, PVDF membrane was examined with IL-2 polyclonal serum, the same secondary antibody and substrate.

**Results**

**Plasmid construction**

IBDV VP2 and ChIL-2 fusion gene was acquired with SOE-PCR (Figure 1A), which was appropriately 1,900 bp. The fusion gene products were cloned into the vector pBacPak8 resulting pBacPakVP2-IL2, which was verified by restriction endonuclease digestion (Figure 1B) and DNA sequencing (data not shown).

**Production the recombinant baculovirus**

The pBacPak8 vector is a baculovirus transfer vector designed for protein production in insect cells or larvae. The expression vector recombinant with wild type viral DNA yields recombinants, which are polyhedrin negative, produces foreign gene products. After three rounds of plaque-purification, the recombinant baculovirus Hy-VP2-IL2 was successfully constructed.
Sf-9 cells infected by wild type baculovirus had no fluorescence when they reacted with IBDV polyclonal serum (Figure 2A) or primary antibody or ChIL-2 polyclonal serum (Figure 2C). Sf-9 cells were infected by recombinant baculovirus shown brilliant fluorescence in reacting with IBDV polyclonal serum and ChIL-2 polyclonal serum (Figure 2D). Fusion protein expressed in the Sf-9 cells had the dual immunity to either IBDV antibody or ChIL-2 antibody.

SDS-PAGE and Western blotting
Harvesting the superstratum of Sf-9 cells which were infected by recombinant baculovirus Hy-VP2-IL2. The newly moulted fifth-instar silkworm larvae were used for infection and expression. Haemolymph were collected to measure the fusion protein expression peak by ELISA everyday post-infection. The results shown that peak arrived on the 4th day post-infection (date not shown). The silkworm larvae haemolymph was collected on the same day. The haemolymph was identified by SDS-PAGE and stained by Coomassie Brilliant Blue. Western blotting showed that VP2-IL2 fusion protein had expressed successfully in insect larvae haemolymph. As shown in Figure 3, a dominant band of approximately 60 kD, which is corresponds to the molecular weight of fusion protein, was observed in the Hy-VP2-IL2 infected samples.

Discussion
When infectious bursal disease appeared in chickens in 1962, the disease ignited as “Gumboro disease” after the geographic location of the first recorded outbreaks. From then on IBDV is a constant threat in the poultry industry worldwide (16). In order to argument the protection against IBDV infection, many methods were tried. DNA vaccines were prepared using immune stimulating complexes (ISCOM) as adjuvant, which could improve the antibody response of DNA vaccine (17). Traditional vaccines of IBDV have focused on stimulating the B cell reaction. However, T cell mediated immunoreactions play more important roles than ever thought (9). Plasmid DNA expressing VP2 of IBDV together with ChIL-2 induced better protection than vaccination with VP2 plasmid alone (18). In this paper, we designed the
VP2-IL2 fusion protein linked by (Gly, Ser)$_4$ and chose the HyNPV-silk worm system to express them. The data presented above showed that high levels of expression of fusion protein in a functionally active state could be achieved in silkworm larvae.

The main reason that we chose VP2 and ChIL-2 genes fused and expressed in baculovirus system were below: (1) IL-2 is a lymphokine that is responsible for the proliferation and differentiation of native T cells as well as for the activation of cytolytic T cells and natural killer cells; (2) the short half-life of IL-2 in circulation is expected to be prolonged by fusion techniques; (3) fusion protein can make up the limitation of traditional IBDV vaccines in T-cell mediated immunoreactions. Expression of the fusion protein can ensure that both the antigen and the vaccine are delivered to the same antigen presenting cell (APC) so allowing the direct activation of the APC; (4) the silkworm has become an ideal multi-cellular eukaryotic model system for basic research. At the same time we have chosen the HyNPV instead of traditional BmNPV or AcNPV, because HyNPV owns both characters of BmNPV and AcNPV. That is to say, recombinant baculovirus Hy-VP2-IL2 can grow in Sf-9 cells (or Sf21 cells) and BmN cells, even the silkworm larvae. This should be the new convenience method when using the baculovirus-insect cells or larvae system.

Cytokines are natural modulators of the immune system and offer the potential chance of further improving the protective immune response of conventional vaccines against avian pathogens of economic importance to the poultry industry. But many questions still remained. For example, how much partial protection against IBDV can be achieved by using the fusion protein as vaccination? How long is the half-life of the fusion protein in prolonged circulation? How to balance the dosage of fusion protein between high immunity efficiency and low clinical side-effects?

In conclusion, we succeeded in preparing recombinant VP2-IL2 fusion protein from haemolymph of silkworm B. mori larvae infected with Hy-VP2-IL2. The method for producing the fusion protein VP2-IL2 will be suitable for large-scale preparation of vaccination antigen against IBDV and may be applicable to produce other recombinant protein. The use of the fusion protein generated in larvae is safe, effective and inexpensive. These findings may offer a potential vaccine candidate for controlling infectious bursal disease in poultry.

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References


