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Protective immunity against influenza H5N1 virus challenge in mice by intranasal co-administration of baculovirus surface-displayed HA and recombinant CTB as an adjuvant

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ABSTRACT

The increasing number of recent outbreaks of HPAI H5N1 in birds and humans brings out an urgent need to develop potent H5N1 vaccine regimens. Here we present a study on the intranasal vaccination of recombinant baculovirus surface-displayed hemagglutinin (BacHA) or inactivated whole H5N1 viral (IWV) vaccine with a recombinant cholera toxin B subunit (rCTB) as a mucosal adjuvant in a BALB/c mouse model. Two groups of mice were vaccinated with different doses (HA titer of log 2^4 or log 2^8) of either HA surface-displayed baculovirus or inactivated whole viral vaccine virus adjuvanted with different doses (2 μg or 10 μg) of rCTB. The vaccinations were repeated after 28 days. HA specific serum IgG and mucosal IgA antibodies were quantified by indirect ELISA, and serum neutralizing antibody titer were estimated by hemagglutination inhibition (HI) assay and virus neutralization titer assay. Functional protective efficacy of the vaccine was assessed by host challenge against HPAI H5N1 strains. The results revealed that mice co-administered with log 2^8 HA titer of BacHA vaccine and adjuvanted with 10 μg of rCTB had a significantly enhanced serum IgG and mucosal IgA immune response and serum microneutralization titer compared with mice administered with unadjuvanted log 2^4 or log 2^8 HA titer of BacHA alone. Also vaccination with 10 μg of rCTB and log 2^8 HA titer of BacHA elicited higher HA specific serum and mucosal antibody levels and serum HI titer than vaccination with log 2^8 HA titer of inactivated H5N1 virus adjuvanted with the same dose of rCTB. The host challenge study also showed that 10 μg rCTB combined with log 2^8 HA titer of BacHA provided 100% protection against 10MLD_{50} of homologous and heterologous H5N1 strains. The study shows that the combination of rH5 HA expressed on baculovirus surface and rCTB mucosal adjuvant form an effective mucosal vaccine against H5N1 infection. This baculovirus surface-displayed vaccine is more efficacious than inactivated H5N1 influenza vaccine when administered by intranasal route and has no biosafety concerns associated with isolation, purification and production of the latter vaccine.

Introduction

Highly pathogenic avian influenza A virus, H5N1 strains are currently causing major morbidity and mortality in bird populations worldwide, especially throughout the Southeast Asia (Fielding et al., 2007). These outbreaks of H5N1 in poultry and an increasing number of cases in humans as well, are a cause for concern. Epidemiological predictions describe this situation as the pre-pandemic stage in the spread and adaptation of the H5N1 virus to the humans. This brings out an urgent need to develop potent vaccines against circulating influenza H5N1 strains (Kreijtz et al., 2007). The protective efficacies of currently licensed H5N1 influenza split and inactivated egg-derived whole virus H5N1 vaccines are low against heterologous virus infection and also show inadequate immunogenicity (Treanor et al., 2007). Development of a potential vaccine against the circulating HPAI influenza viruses during periods of epidemic poses a number of challenges, such as the need for biosafety containment facilities (Quan et al., 2007), thorough inactivation and evaluation of its immunogenicity. The process is risky, and is usually associated with low yields of virus in embryonated eggs used for virus propagation (Nicholson et al., 2001). As an alternative to conventional egg-based and mammalian cell culture produced influenza vaccine approaches, the production of recombinant hemagglutinin subunit vaccine is expeditious, safe, high-yielding and low in cost (Safdar et al., 2006). Earlier, Treanor

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et al. (2001) reported that intramuscular immunizations of baculovirus derived rHA subunit vaccine are safe and immunogenic in humans. Further, the efficacies of rHA based vaccines are comparable with traditional egg-based vaccines (Wang et al., 2003). Most of the previous studies have attempted to investigate HA subunit derived from recombinant baculovirus infected insect cells as a vaccine for influenza virus. However, the low solubility of the hydrophobic HA protein increases the difficulty of purification (Treanor et al., 2001). The expression of HA on the surface of baculovirus is an approach to circumvent the difficult purification of HA expressed in insect cells by other baculoviral strategies. Recombinant baculovirus surface-displaying hemagglutinin system has advantages such as high stability and purity than vaccine prepared from recombinant baculovirus infected insect cells. The surface displaying of HA protein on baculovirus will enable the rapid production of pre-pandemic and pandemic vaccines with minimal technical infrastructure at any location in the world obviating the need for riskful inactivation of virulent viruses and meticulous protein purification processes. Moreover, it has been previously reported that wild-type baculovirus itself enhances the innate immune responses and provides sufficient protection against influenza infection (Abe et al., 2003). The wild-type AcNPV (*Autographa californica* nuclear polyhedrosis virus) baculovirus stimulates the innate immune response through TLR9 and the viral gp64 mediated fusion is necessary for this stimulation (Abe et al., 2003). The HA surface-displaying baculovirus used in this study is also capable of transducing the HA protein into the cells of vaccinated animals. This baculovirus has the HA engineered under a WSSV ie1 promoter which facilitates a higher level of expression of HA both in the insect cells and in the transduced mammalian cells (Lu et al., 2007).

Various strategies are used to enhance the post-vaccination immune response such as dosage, route of administration, adjuvants and selection of vaccine strains. Most of the influenza vaccines have been administered by intramuscular or subcutaneous injection, which have been shown to be ineffective for the generation of protective immunity at the mucosal surfaces. Vaccination via an intranasal mucosal route is an important approach in controlling mucosally acquired infection like influenza infection (Ogra et al., 2001). In addition the intranasal immunization procedure is simple, reliable, and cheap compared with subcutaneous or intramuscular injection (Maeyama et al., 2002). Further, mucosal immunizations of particulate antigens are known to induce a better immune response than soluble antigens, in both the mucosal and peripheral immune compartments. This is because of the bypassing of the NALT (Nasal mucosa associated lymphoid tissue) by the soluble antigens, thus not priming a strong local immune response (Wu et al., 1997; Sminia and Kraal, 1999). This makes the use of VLPs or baculovirus surface-displayed HA as rational options over soluble HA purified from expression systems.

Usually mucosal administration of antigen alone does not induce an effective and long lasting immunity, therefore a mucosal adjuvant that can sufficiently enhance the mucosal antigen-specific IgA antibody response needs to be used. A number of chemical agents have been tested to enhance the immunogenicity of mucosally administered antigens, but most of them induce an insufficient immune response (Ogra et al., 2001). In the present study, we evaluate the efficacy of the intranasal co-administration of recombinant baculovirus surface-displayed hemagglutinin (BacHA) genes of three different H5N1 influenza virus strains and recombinant cholera toxin B subunits (rCTB) as a mucosal adjuvant, in mice challenged with H5N1 virus infection.

Results

Determination of HA specific antibody levels by indirect ELISA

The HA specific serum IgG antibody responses across the different groups, by indirect ELISA, revealed that the group vaccinated with log

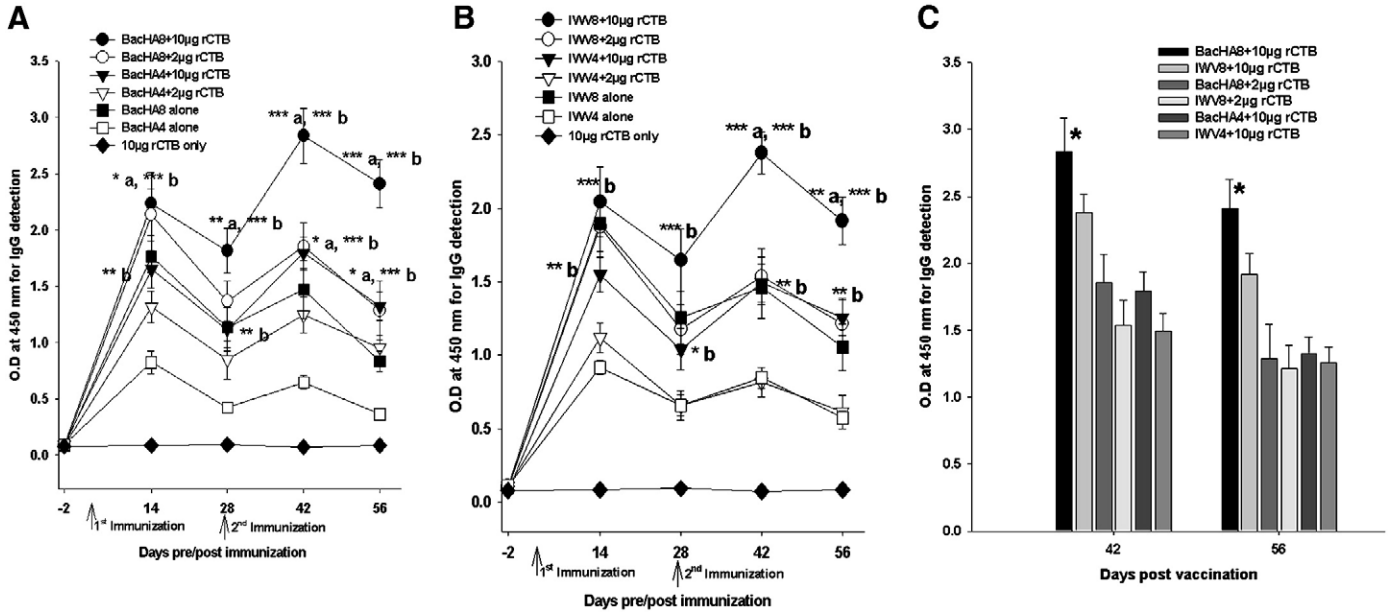

Fig. 1. (A) Serum (1:100) specific anti-HA IgG antibody response against rH5HA antigen in mice immunized i.n. with log 2^8 or log 2^4 HA titer of baculovirus expressed HA vaccine (BacHA 8 or BacHA 4) alone or combination with 2 or 10 µg of rCTB as adjuvant. Each point represents the arithmetic mean value ($n=6$)±SE. (a-when compared with log 2^8 HA titer of BacHA vaccine [BacHA 8] alone, b-when compared with log 2^4 HA titer of BacHA vaccine [BacHA 4] alone, *p–>p<0.05; **p–>p<0.02; ***p–>p<0.01). (B) Serum (1:100) specific anti-HA IgG antibody response against rHA antigen in mice immunized i.n. with log 2^8 or log 2^4 HA titer of H5N1 inactivated whole viral vaccine (IWV 8 or IWV 4) alone or combination with 2 or 10 µg of rCTB as adjuvant. Each point represents the arithmetic mean value ($n=6$)±SE. (a-when compared with log 2^8 HA titer of inactivated whole viral vaccine [IWV 8] alone, b-when compared with log 2^4 HA titer of inactivated whole viral vaccine [IWV 4] alone, *p–>p<0.05; **p–>p<0.02; ***p–>p<0.01). (C) Comparison of the serum (dilution 1:100) specific anti-HA IgG antibody response between log 2^8 HA titer of baculovirus expressed HA (BacHA) vaccine and H5N1 inactivated whole viral vaccine (IWV) with different concentration of rCTB as adjuvant. Each point represents the arithmetic mean value ($n=6$)±SE. (a-when compared with log 2^8 HA titer of baculovirus expressed HA (BacHA 8 and 10 µg of rCTB) (*p<0.05; ** p<0.02; *** p<0.01).