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ORIGINAL

Expression of the hemagglutinin HA1 subunit of the equine influenza virus using a baculovirus expression system

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Abstract
Equine influenza virus is a leading cause of respiratory disease in horses worldwide. Disease prevention is by vaccination with inactivated whole virus vaccines. Most current influenza vaccines are generated in embryonated hens' eggs. Virions are harvested from allantoic fluid and chemically inactivated. Although this system has served well over the years, the use of eggs as the substrate for vaccine production has several well-recognized disadvantages (cost, egg supply, waste disposal and yield in eggs). The aim of this study was to evaluate a baculovirus system as a potential method for producing recombinant equine influenza hemagglutinin to be used as a vaccine. The hemagglutinin ectodomain (HA1 subunit) was cloned and expressed using a baculovirus expression vector. The expression was determined by SDS-PAGE and immunoblotting. A high yield, 20 μg/ml of viral protein, was obtained from recombinant baculovirus-infected cells. The immune response in BALB/c mice was examined following rHA1 inoculation. Preliminary results show that recombinant hemagglutinin expressed from baculovirus elicits a strong antibody response in mice; therefore it could be used as an antigen for subunit vaccines and diagnostic tests.

Los dos primeros autores contribuyeron por igual en la realización del trabajo.
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PALABRAS CLAVE
Hemaglutinina;
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Vacunas

Expresión de la subunidad HA1 del virus de influenza equina utilizando un sistema de expresión en baculovirus

Resumen
El virus de la influenza equina es una de las principales causas de enfermedad respiratoria en caballos de todo el mundo. La prevención de la enfermedad es a través de la vacunación con vacunas a virus inactivado. La mayoría de las vacunas se producen en huevos embrionados, de los cuales los viriones son cosechados del líquido alantoideo e inactivados químicamente. Aunque este sistema ha servido bien durante años, el uso de huevos como sustrato para la producción de vacuna presenta varias desventajas bien reconocidas (costo, provisión de huevos, manejo de los residuos, rinde por huevo). El objetivo del presente trabajo fue evaluar preliminarmente un sistema de expresión en baculovirus como método de producción de hemoaglutinina recombinante (rHA) para ser utilizada como vacuna para la prevención de la influenza equina. Para ello el ectodominio de la hemaglutinina (la subunidad HA1) del virus de la influenza equina se expresó en células de insecto infectadas con un baculovirus recombinante. La expresión fue demostrada por SDS-PAGE e inmunoblotting. El método empleado fue capaz de producir gran cantidad de rHA1. En este estudio se obtuvieron 20 μg/ml (200 μg de HA1 purificada de $2,5x10^7$ células infectadas). La respuesta inmune fue evaluada mediante la inmunización de ratones BALB/c. Los resultados preliminares demostraron que la proteína recombinante expresada en baculovirus genera una fuerte respuesta inmune en ratones, por lo tanto podría ser utilizada como antígeno para la producción de una vacuna a subunidades y en pruebas diagnósticas.

Introduction

Equine Influenza is a disease that affects horses, donkeys and mules, which is currently considered one of the main causes of equine respiratory diseases around the world. Its causative agent is a virus belonging to the *Orthomyxoviridae* family. Conventional equine influenza vaccines contain inactivated whole viruses either as an aqueous suspension or combined with adjuvants such as aluminum hydroxide or aluminum phosphate. For vaccine production, the influenza virus is usually grown in the amniotic cavity of fertile hens' eggs or in a cell culture maintained in medium containing trypsin. Virions are harvested from egg allantoic fluid or culture medium and are inactivated or treated with detergent. A basic disadvantage these systems present is their variability and the heterogeneity of their cellular constituents[9,13]. Therefore, other methods for equine influenza vaccine production were developed. These include a canarypox-vectored vaccine (ProteqFlu, Merial Inc., USA), a subunit vaccine containing purified hemagglutinin and neuraminidase proteins adjuvated with ISCOMatrix (Equilis Prequenza), an attenuated live vaccine (Flu Avert™ I.N., Intervet) and many inactivated influenza vaccines, with the latter being the most widely used commercially. Vaccination against equine influenza has been recently reviewed[12]. During 2008, Protein Sciences Corporation reported the development of a human influenza vaccine, named FluBlok®, using the insect cell/baculovirus technology. This vaccine was shown to be safe and efficacious in clinical trials[3,4,15]. Insect cell systems have been widely used to produce recombinant proteins. The high yield obtained by baculovirus-infected insect cells makes them attractive tools for pharmaceutical protein production[6].

Hemagglutinin is the dominant glycoprotein on the surface of the influenza virus and a recognized key antigen in the host response to influenza virus in both natural infection and vaccination[17]. The protein, encoded by segment 4 of the viral genome, is initially translated as a preprotein (HA0) that is then split into two subunits (HA1 and HA2), which subsequently assemble to form a trimeric structure (HA1-HA2). In the spatial conformation that the mature hemagglutinin takes, only the HA1 subunit becomes exposed and it is, therefore, where most of its antigenic determinants are found[16].

The present study was aimed at evaluating a baculovirus expression system as a potential method for producing recombinant equine influenza hemagglutinin.

Materials and methods

Virus and cells

An Argentine equine influenza virus strain previously isolated in our laboratory, sharing high homology to strain A/equine/Argentina/1999 (H3N8) (more than 99%) and closely related to Floride clade 1 strains, was propagated in the allantoic cavity of 11 day-old embryonated hen's eggs.

Spodoptera frugiperda (Sf9 and Sf21) cells (Gibco, Grand Island, NY, USA), used for the transfection and propagation of recombinant viruses, were cultured at 27 °C in TC-100 media (US Biological, Swampscott, MA, USA) supplemented