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Inoculation of mares and very young foals with EHV-1 glycoproteins D and B reduces virus shedding following respiratory challenge with EHV-1

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Abstract

We have previously demonstrated that intramuscular inoculation of EHV-1 glycoprotein D (gD) and glycoprotein B (gB) produced by a recombinant baculovirus and formulated with the adjuvant Iscomatrix™ elicited virus-neutralizing antibody and gD- and gB-specific ELISA antibody in adult horses. In this study, 14 mares and their very young foals were inoculated with a combination of baculovirus-expressed EHV-1 gD and EHV-1 gB (EHV-1 gDBr) and challenged with a respiratory strain of EHV-1. Following experimental challenge, inoculated mares and foals shed virus in nasal secretions on significantly fewer occasions compared to uninoculated mares and foals. Uninoculated foals born from inoculated mares were no more protected against experimental challenge than uninoculated foals born from uninoculated mares. The results suggest that it is indeed possible to induce partial protection in very young foals through vaccination, and while the inoculation did not prevent infection, it did reduce the frequency of viral shedding with the potential to thereby reduce the risk and prevalence of infection in a herd situation.

Keywords: Equine herpesvirus 1; Vaccination; Glycoprotein D; Glycoprotein B

1. Introduction

Equine herpesvirus 1 (EHV-1) is a major cause of epidemic abortion, perinatal mortality, respiratory disease and occasionally neurological disease (Crabb and Studdert, 1995). Natural infection with EHV-1 and the antigenically cross-reactive EHV-4 does not provide a sufficiently high level of long term immunity to consistently protect against EHV disease and horses may experience reactivation of disease throughout their lives (Baxi et al., 1995; Borchers et al., 1999). Prior to the introduction of routine vaccination with a whole virus EHV-1/EHV-4 vaccine (Duvaxyn™) in Australia in 1997, studies of foals on Thoroughbred stud farms in the Hunter Valley of New South Wales found serological evidence of infection of foals as young as 30 days of age with a maximum incidence of EHV-1 infection at approximately 3 months of age

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(Gilkerson et al., 1997). The epidemiological data indicated that foals were experiencing EHV-1 infection both before and after weaning, with the most likely source of infection prior to weaning being reactivation of latent infection in mares with subsequent spread to susceptible mares and foals. These studies were consistent with a continuing cycle of endemic EHV-1 infection (Gilkerson et al., 1999), with mares, unweaned foals and recent weanings as the source of virus for each new crop of foals. Subsequent studies supported a similar cycle for EHV-4 (Foote et al., 2004). Current vaccination against EHV-1 is through multiple doses of inactivated whole virus preparations. Several of these have been shown to reduce the severity of respiratory disease and/or the risk of abortion, including Duvaxyn™ (Heldens et al., 2001), an inactivated EHV-1/EHV-4 vaccine. However, following the introduction of Duvaxyn™ to Australia in 1997, a further series of epidemiological studies on the same stud farms that were used in the previous study showed that the cycle of infection was continuing in mares and foals (Foote et al., 2003). Foals are born with a moderately competent lymphoid immune system (Perryman et al., 1980) although immunologically naive at birth (Lunn, 1997a). Since there is no transplacental transfer of immunoglobulins across the epitheliochorial placenta, foals must absorb passively transferred maternal immunoglobulins from colostrum (Jeffcott, 1974). The passive transfer of colostrum has been shown to be important in minimizing infectious disease (McGuire et al., 1975; Raidal, 1996). However, current protocols where mares are vaccinated do not prevent infection of young foals with EHV-1 or EHV-4, with virus detected as early as 11 days of age (Foote et al., 2004). It has been suggested that a factor that significantly affects de novo immune responses after birth is the suppressive effect of passively transferred maternal antibodies (Van Maanen et al., 1992). Therefore, vaccination of foals less than 5 months of age is not currently recommended for commercially available vaccines (Wilson and Rossdale, 1998). Nonetheless if foals are not protected against EHV-1 or EHV-4 infection at a very young age they become latently-infected carriers and therefore a potential source for outbreaks of EHV-1 and EHV-4 disease throughout their lives. Envelope glycoproteins of EHV-1 have been investigated as possible vaccine candidates mainly using murine models of EHV-1 respiratory disease (Tewari et al., 1994; Osterrieder et al., 1995; Stokes et al., 1997; Kukreja et al., 1998a; Packiarajah et al., 1998; Zhang et al., 1998) and EHV-1 abortion (Walker et al., 2000). Inoculation of mice with EHV-1 glycoprotein D (gD) expressed by a recombinant baculovirus (Love et al., 1993) led to rapid clearance of challenge EHV-1 from target organs in the respiratory tract in association with virus-neutralizing antibody, as well as delayed-type hypersensitivity and proliferative lymphocyte responses (Tewari et al., 1994). Similar results in mice were later obtained with other EHV-1 gD constructs (Stokes et al., 1997; Zhang et al., 1998). Protective effects were also generated by inoculation of mice with gD in a DNA expression vector (Ruitenberg et al., 1999) or with DNA boosted by recombinant gD as protein (Ruitenberg et al., 2000b). The strong protective response elicited by EHV-1 gD is likely to be due at least in part to gD possessing a strong complement-independent neutralizing epitope(s) (Flowers and O'Callaghan, 1992).

Protective effects in mice have been reported for various baculovirus-expressed EHV-1 gB constructs (Osterrieder et al., 1995; Packiarajah et al., 1998). Upon challenge with EHV-1, gB-inoculated mice had reduced clinical signs, rapid clearance of virus from the lungs and reduction in herpesvirus-induced pathology. Low levels of neutralizing antibody combined with the level of protection in mice suggested that cell-mediated immunity to gB may have been contributing significantly to the protection conferred. In a murine model of EHV-1 abortion (Kukreja et al., 1998b), pregnant mice inoculated with baculovirus expressed EHV-1 gB demonstrated body weight gain post-challenge infection and as opposed to control mice, experienced no maternal deaths and had a significantly higher litter survival rate.

There have been few reports detailing the testing of recombinant glycoproteins in the horse. A reduction in virus shedding following experimental EHV-1 challenge was observed in horses inoculated with a canarypoxvirus expressing glycoproteins B, C and D of EHV-1 Kentucky D strain (Audonnet et al., 1999) and over 50% of horses inoculated with EHV-1 gD DNA developed increased ELISA antibody and EHV-1 neutralizing antibodies (Ruitenberg et al., 2000a). Previously we tested antibody responses to EHV-1 gD (Foote et al., 2005) and EHV-1 gB (unpublished) produced in insect cells by recombinant baculoviruses, and delivered with the ISCOM-related