Full protection against African horsesickness (AHS) in horses induced by baculovirus-derived AHS virus serotype 4 VP2, VP5 and VP7

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African horsesickness virus serotype 4 (AHSV-4) outer capsid protein VP2, or VP2 and VP5 plus inner capsid protein VP7, derived from single or dual recombinant baculovirus expression vectors were used in different combinations to immunize horses. When the proteins were purified by affinity chromatography, the combination of all three proteins induced low levels of neutralizing antibodies and conferred protection against virulent virus challenge. However, purified VP2 or VP2 and VP5 in the absence of VP7 failed to induce neutralizing antibodies and protection. Immunization with non-purified proteins enhanced the titres of neutralizing antibodies. Again, the combination of the three proteins was able to confer total protection to immunized horses, which showed absence of viraemia. The antigenicity of recombinant VP2 was analysed with a collection of 30 MAbs. Both purified and unpurified recombinant VP2 proteins showed different antigenic patterns in comparison to that of VP2 on virions. An immunization experiment with four more horses confirmed these results. The vaccine described here would not only prevent the disease, but would drastically reduce the propagation of the virus by vectors.

Introduction

African horsesickness (AHS) is a virus disease that causes morbidity in equids and a high mortality in horses (often exceeding 90%). The disease is caused by an arthropod-borne virus (African horsesickness virus; AHSV) within the genus *Orbivirus* of the family *Reoviridae* (Holmes, 1991). AHSV is transmitted to susceptible animals by biting midges (*Culicoides* spp.), which become infected by feeding on blood from sick animals containing high concentrations of infectious virus. The virus is mainly confined to sub-Saharan Africa, although severe epizootics have occurred on occasions in other parts of the world, including northern Africa, the Middle East and southern Europe (Lubroth, 1988; Rodríguez *et al.*, 1992; Mellor, 1993).

As in other gnat-transmitted orbiviruses, AHSV contains seven structural proteins (VP1–VP7), which are organized into two concentric protein capsids (Oellermann *et al.*, 1970), and a genome of 10 dsRNA segments (Bremer, 1976). The outer capsid consists of two major protein species, VP2 and VP5, of which VP2 (with a molecular mass of 124 kDa) is the major serotype-specific antigen (Bremer *et al.*, 1990) and the main target for the neutralizing response of the host (Ranz *et al.*, 1992; Burrage *et al.*, 1993; Martínez-Torrecuadrada *et al.*, 1994; Martínez-Torrecuadrada & Casal, 1995). The function of VP5 in protection remains unclear. The inner capsid is formed by two major proteins, VP3 and VP7, enclosing three other minor proteins, VP1, VP4 and VP6, that are closely associated with the virus genome (for review see Roy *et al.*, 1994*a*).

Like other virus diseases, vaccination and preventive measures are essential to protect horses against AHSV and to control the spread of the virus. Currently, both attenuated live virus vaccines and inactivated virus vaccines for AHSV are available commercially (Erasmus, 1978; Dubourget *et al.*, 1992; House *et al.*, 1994). Inactivated AHSV vaccine is generally preferred for two main reasons: (i) risks associated with the live vaccine can be avoided; and (ii) infected animals can be distinguished from vaccinated animals (Laviada *et al.*, 1995). However, like insufficient attenuation, incomplete inactivation of the virus is a threat to animal health. Recent developments in the use of baculovirus as a gene expression system have provided a powerful strategy for the development of a non-infectious, safe virus vaccine.

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