Inoculation with DNA encoding the glycoprotein gp2 reduces severity of equine herpesvirus 1 infection in a mouse respiratory model

Brief Report

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Summary. The envelope glycoprotein 2 (gp2) of equine herpesvirus 1 (EHV-1) has no known homologue in other herpesviruses with the exception of some equid alphaherpesviruses. In order to investigate the potential of gp2 as a vaccine antigen, expression vectors were constructed to encode full-length gp2, a truncated version lacking the membrane anchor, and the C-terminal region. Intramuscular inoculation of mice with these DNA constructs induced neutralizing antibody against EHV-1 and, following intranasal challenge with EHV-1, mice inoculated with any of the gp2 DNA constructs cleared virus more rapidly from their lungs than control mice. The rate of clearance was comparable to that for glycoprotein D DNA, indicating gp2 as a potential antigen for inclusion in a subunit vaccine.

Equine herpesvirus 1 (EHV-1) is an important respiratory pathogen of the horse with clinical and economic significance for the equine industry world-wide [1, 5]. Primary EHV-1 infection results in a localised respiratory infection that can rapidly progress to a systemic infection via cell-associated viraemia with the potential to cause abortion in pregnant mares. Furthermore, EHV-1 has also been associated with central neurological disease. EHV-1 is an alphaherpesvirus with most but not all genes showing positional and sequence homology to those of herpes simplex virus 1 (HSV-1) and Varicella zoster virus (VZV) [25]. The EHV-1

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genome has counterparts of all 76 genes in common with the antigenically cross-reacting equine herpesvirus 4 (EHV-4) [24], which is associated mainly with respiratory disease [1, 5].

Alphaherpesviruses encode eleven or more glycoproteins that have functions in virus attachment, entry into host cells, cell-to-cell spread and virus egress. Homologues of most of these glycoproteins are present in EHV-1 [25]. However, homologues of EHV-1 gene 71 encoding the envelope glycoprotein gp2 [22, 28] have been identified only in two other equid alphaherpesviruses, EHV-4 and asinine herpesvirus 3 (AHV-3) [4], with no known related gene in other herpesviruses of any subfamily. Gp2, also known as gp300 [31], appears to be abundant in EHV-1 virions and to be one of the most immunogenic viral antigens for the natural host [1, 4]. In vitro studies using an EHV-1 gene 71 deletion mutant demonstrated that while gp2 was not essential for virus growth in cell culture [21], it was involved in facilitating virus entry and egress [23]. In mice, this deletion mutant lacked full expression of virulence [11]. Strain-specific differences in the degree of impairment of virus release were evident, as shown for progeny virus of EHV-1 bacterial artificial chromosomes (BACs), where the BAC replicon replaced gene 71 [15]. This impairment was greatly increased in a double gp2/gM deletion mutant [16]. A transposon insertion in gene 71 in a BAC containing the genome of EHV-1 strain HVS25A, resulted similarly in a reduced yield of virus and impaired virus release (I. Napier, personal communication).

The sequenced EHV-1 gp2 open reading frame encodes a polypeptide of 797 amino acids [25] which is characterised by a serine-threonine rich N-terminal domain and a cysteine-rich C-terminal domain. During infection in cell culture, gp2 undergoes endoproteolytic cleavage at adjacent arginine residues 506 and 507 [29], resulting in separation of the highly O-glycosylated N-terminal region from a 42 kDa C-terminal cleavage product. Despite the C-terminal region of EHV-1 gp2 having 73% amino acid identity to that of EHV-4 gp2, it is antigenically distinct and can only be detected by antibodies induced by EHV-1 and not EHV-4 [10].

Herpesvirus envelope glycoproteins are major inducers of virus-specific immune responses and the ability of several EHV-1 glycoproteins to induce protective immune responses has been investigated in mice. Notably, partial protection against EHV-1 challenge was achieved in mice using recombinant baculovirus-expressed gD [20, 26] as well as with an E. coli-expressed gD polypeptide [32], making gD a preferred candidate for a subunit vaccine. EHV-1 gB [12, 13] and gC [19, 27] afforded some protection against experimental EHV-1 challenge, as did glycoproteins H and L when co-administered but not separately [9]. However, as yet there are no reports of the ability of gp2 to induce protective immune responses against EHV-1, although the gp2-specific monoclonal antibody 1G12 [2] has virus-neutralizing activity in the presence of complement (J. Wellington, unpublished results). One useful approach to examine the immune response to new antigens is by direct DNA inoculation, in which a plasmid DNA expression vector undergoes in vivo transfection, leading to endogenous expression of a specific antigen. This results in similar antigen presentation to that occurring during the course of a natural infection, with the potential to induce both humoral and cellular immune