Antiviral Research 116 (2015) 27–33

Contents lists available at ScienceDirect

Antiviral Research

journal homepage: www.elsevier.com/locate/antiviral

Antiserum from mice vaccinated with modified vaccinia Ankara virus expressing African horse sickness virus (AHSV) VP2 provides protection when it is administered 48 h before, or 48 h after challenge

Eva Calvo-Pinilla [a], Francisco de la Poza [b], Simon Gubbins [a], Peter Paul Clement Mertens [a], Javier Ortego [b], Javier Castillo-Olivares [a,*]

[a] The Pirbright Institute, Pirbright, Woking, Surrey, United Kingdom
[b] Centro de Investigación en Sanidad Animal, CISA-INIA, Valdeolmos, Madrid, Spain

ARTICLE INFO

Article history:
Received 11 November 2014
Revised 13 January 2015
Accepted 22 January 2015
Available online 30 January 2015

Keywords:
African horse sickness
AHSV
MVA-VP2
Protection
Humoral immunity
Passive immunisation

ABSTRACT

Previous studies show that a recombinant modified vaccinia Ankara (MVA) virus expressing VP2 of AHSV serotype 4 (MVA-VP2) induced virus neutralising antibodies in horses and protected interferon alpha receptor gene knock-out mice (IFNAR −/−) against challenge. Follow up experiments indicated that passive transfer of antiserum, from MVA-VP2 immune donors to recipient mice 1 h before challenge, conferred complete clinical protection and significantly reduced viraemia.

These studies have been extended to determine the protective effect of MVA-VP2 vaccine-induced antiserum, when administered 48 h before, or 48 h after challenge. In addition, passive transfer of splenocytes was undertaken to assess if they confer any degree of immunity to immunologically naïve recipient mice. Thus, antisera and splenocytes were collected from groups of mice that had been vaccinated with MVA-VP2, or wild type MVA (MVA-wt), for passive immunisation of recipient mice. The latter were subsequently challenged with AHSV-4 (together with appropriate vaccinated or unvaccinated control animals) and protection was assessed by comparing clinical signs, lethality and viraemia between treated and control groups. All antiserum recipients showed high protection against disease (100% survival rates even in mice that were immunised 48 h after challenge) and statistically significant reduction or viraemia in comparison with the control groups. The mouse group receiving splenocytes from MVA-VP2 vaccinates, showed only a 40% survival rate, with a small reduction in viraemia, compared to those mice that had received splenocytes from MVA-wt vaccinates. These results confirm the primarily humoral nature of protective immunity conferred by MVA-VP2 vaccination and show the potential of administering MVA-VP2 specific antiserum as an emergency treatment for AHSV.

1. Introduction

African horse sickness (AHS) is an arthropod-borne viral disease of solipeds transmitted by haematophagous insects of the genus *Culicoides*, the horse being the most severely affected species. Mortality rates of AHSV outbreaks in immunologically naïve populations may exceed 90% (Mellor and Hamblin, 2004).

All of the different isolates, strains and serotypes of African horse sickness virus (AHSV) are classified within the species *African horse sickness virus,* genus *Orbivirus*, family *Reoviridae*. AHSV is closely related to bluetongue virus, which causes bluetongue disease in ruminants. The orbiviruses are characterised by a genome composed of ten linear segments of dsRNA, with one copy of each of the ten segments packaged within each virus particle. The spherical, non-enveloped capsid of AHSV is approximately 70 nm in diameter, is composed of three concentric protein layers (Roy et al., 1994). The outer-capsid layer is formed by two major structural proteins, VP2 and VP5 (encoded by genome-segments 2 and 6 respectively), and is primarily involved in cell attachment and cell entry. VP2 is the most variable antigen of AHSV and is responsible for serotype definition (Burrage et al., 1993).

AHSV infection in horses most often results in severe clinical disease and death, although animals that do survive, exhibit a solid, life-long but serotype-specific immunity. The humoral nature of AHS immunity has been associated with virus neutralising antibodies (VNAb) in both horses and mice using colostrum and

* Corresponding author at: The Pirbright Institute, GU 24 0NF Surrey, United Kingdom. Tel.: +44 (0)1483 231081.
E-mail address: javier.castillo-olivares@pirbright.ac.uk (J. Castillo-Olivares).

http://dx.doi.org/10.1016/j.antiviral.2015.01.009
0166-3542/

monoclonal antibodies respectively (Blackburn and Swanepoel, 1988; Burrage et al., 1993; Crafford et al., 2013). The main target of VNAb is AHSV VP2 (Burrage et al., 1993) and several studies have mapped neutralising epitopes to the amino terminal half of the VP2 protein (Bentley et al., 2000; Martinez-Torrecuadrada and Casal, 1995; Martinez-Torrecuadrada et al., 2001).

Although less important in this respect than VP2, AHSV-VP5 appears to be both directly involved in the formation of virus neutralising epitopes and indirectly involved by influencing the conformation of VP2 (Martinez-Torrecuadrada and Casal, 1995; Martinez-Torrecuadrada et al., 1996). Unsurprisingly, experimental vaccines based on the outer-capsid proteins VP2 and/or VP5, were successful (Castillo-Olivares et al., 2011; Chiam et al., 2009; Romito et al., 1999; Roy and Sutton, 1998; Scanlen et al., 2002; Stone-Marschat et al., 1996).

Recently, cell-mediated immune responses have been detected in horses following inoculation with live attenuated AHSV vaccines (Pretorius et al., 2012), or recombinant Canarypox virus expressing AHSV VP2 and VP5 (El Garch et al., 2012). More recently, cell-mediated immune responses have also been observed in interferon alpha receptor gene knock-out mice (IFNAR −/−) after vaccination with single MVA recombinant viruses expressing AHSV VP2 or NS1 (de la Poza et al., 2013). Since cellular immunity has been associated with protection against BTV, it is generally assumed that the same applies to AHSV.

After showing that MVA expressing AHSV VP2 (MVA-VP2) was protective against AHSV infection in mice (Castillo-Olivares et al., 2011) and horses (Alberca et al., 2014), the mechanisms of immunity underlying the protective effect of MVA-VP2 vaccination were investigated. Passive transfer of AHSV neutralising antiserum, from MVA-VP2 vaccinated mice to immunologically naïve recipients, was shown to be highly protective against a lethal AHSV challenge (Calvo-Pinilla et al., 2014).

In this paper, follow-up studies investigating the relative contribution of humoral and cell-mediated immunity to the protection conferred by MVA-VP2 vaccination, using passive immunisation of naïve recipient IFNAR −/− mice with splenocytes or antiserum from MVA-VP2 vaccinated mice, are described.

2. Materials and methods

2.1. Viruses and cells

Chicken embryo fibroblast (DF-1) (ATCC, Cat. No. CRL-12203) and Vero cells (ATCC, Cat. No. CCL-81) were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 2 mM glutamine, penicillin (100 units/ml), streptomycin (100 μg/ml) and 10% foetal calf serum (FCS). AHSV-4 (Madrid/87) (AHSV-4) was grown in Vero cells and MVA viruses grown in DF-1 cells. Virus stocks were generated by infection of confluent cells using a multiplicity of infection (MOI) of 0.1. At 48 h post-infection, or when a total cytopathic effect (CPE) was visible, the cells and supernatants were harvested and centrifuged. The virus was released from the cells by three freeze and thaw cycles and then titrated by plaque assay. The MVA-VP2 virus used in this study has been previously described (Chiam et al., 2009).

2.2. Mice

IFN α/β Ro/o IFNAR −/− mice on an A129 background were purchased from B&K Universal Ltd (UK). Eight-week old mice were used throughout. Mice were maintained under pathogen-free conditions and allowed to acclimatise to the biosafety level 3 (BSL3) animal facilities at the Centro de Investigacion en Sanidad Animal, INIA, Madrid (INIA-CISA), for 1 week before use. All experiments with live animals were performed under the guidelines of the European Community (86/609) and were approved by the ethical review committee of INIA-CISA (CEEA 2010-034).

2.3. Murine immunisations with MVA-VP2 and MVA-wt and preparation of donor antisera and splenocytes

Groups of mice were vaccinated (10^7 pfu/mouse) with either MVA-VP2 (Group 1a and Group 1b) or MVA-wt (Group 2) and used as donors of serum and splenocytes as shown in Table 1. A third dose was given on day 35 to 5 mice from Group 1a (mouse 1.6–mouse 1.10). Mice from Group 1b and Group 2 were euthanised on day 35. Mice from Group 1a were euthanized on day 50. Splenocytes and blood samples were collected at termination and subsequently used for passive immunisation. The blood was used to prepare donor antisera.

2.4. Adoptive transfer of splenocytes

Pools of spleen cells, obtained from mice from Group 1b (MVA-VP2) and Group 2 (MVA-wt), were transferred via intravenous injection (2×10^7 cells/mouse) to immunologically naïve recipients Groups 3 and 4 (Table 2a). Transfers were performed 24 h before infection with AHSV-4 (10^6 pfu/mouse).

2.5. Passive immunisation with donor antiserum

Donor antisera S1a, S1b and S2 were prepared by pooling individual serum samples from Groups 1a, 1b and 2 respectively (Table 2b). The donor antisera were inactivated (30 min at 56 °C) and used for intra-peritoneal injection of 4 Groups of 10-week-old recipient IFNAR −/− mice: (a) Group 5, received 400 μl of undiluted S1a antiserum administered 1 h before challenge; (b) Group 6 received 100 μl of diluted S1b antiserum, administered 1 h before challenge; (c) Group 7 received 100 μl of S1b 48 h before challenge and (d) Group 8 received 100 μl of S1b antiserum 48 after challenge.

Passively immunised mice were challenged with 10^6 pfu of AHSV-4 administered subcutaneously. MVA-wt (Group 9) and MVA-VP2 (Group 10) vaccinated control mice were infected at the same time as the recipients. Clinical signs and viraemia were evaluated in all the animals following challenge.

2.6. Evaluation of clinical signs after challenge with AHSV-4

Following challenge, animals were monitored twice daily and more regularly (at least three times per day) at the onset of any signs of morbidity, including: changes in behaviour and activity,

Table 1
Vaccination and preparation of splenocytes and antiserum donors.

Groups	Day 0	Day 21	Day 35	Day 50
1a (5 mice)	MVA-VP2 10^7 pfu/mouse	MVA-VP2 10^7 pfu/mouse	MVA-VP2 10^7 pfu/mouse	Collection of serum (S1a)
1b (5 mice)	MVA-VP2 10^7 pfu/mouse	MVA-VP2 10^7 pfu/mouse	Collection of serum (S1b) and spleen (L1)	
2 (5 mice)	MVA-wt 10^7 pfu/mouse	MVA-wt 10^7 pfu/mouse	Collection of serum (S2) and spleen (L2)	