The Veterinary Journal 210 (2016) 85–88

Contents lists available at ScienceDirect

The Veterinary Journal

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

Short Communication

Equine herpesvirus type 1 (EHV1) induces alterations in the immunophenotypic profile of equine monocyte-derived dendritic cells

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ARTICLE INFO

Article history:
Accepted 14 December 2015

Keywords:
Down-regulation
Equine herpesvirus 1
Monocyte-derived dendritic cells
pUL56

ABSTRACT

Equine herpesvirus 1 (EHV1) is an α-herpesvirus that can infect a variety of different cells in vitro and in vivo, including dendritic cells (DC) which are essential in the immune response against EHV1. Infection of equine monocyte-derived DC (MDDC) with EHV1 induced down-regulation of major histocompatibility complex I (MHCI), CD83, CD86, CD206, CD29 and CD172a, but not of CD11a/CD18 and MHCII. This down-regulation was not mediated by the virion host-shutoff (VHS) protein or pUL49.5. Interestingly, down-regulation of CD83 and CD86 was in part mediated by pUL56. Taken together, these data indicate that EHV1 employs different and still unresolved mechanisms to induce down-regulation of several functionally important cell surface proteins on equine DC.

The *Herpesviridae* family consists of large DNA viruses that encode viral proteins capable of antagonizing the immune system (Vossen et al., 2002; Van de Walle et al., 2009). Dendritic cells (DC) elicit cytotoxic T-lymphocyte (CTL) responses and maintain anti-viral immunity by presenting viral antigens on major histocompatibility complex (MHC). Infection of human DC with human α-herpesviruses herpes simplex virus 1 (HSV1) and varicella-zoster virus (VZV) impairs their capacity to support T-proliferation and induces selective down-regulation of cell surface markers associated with antigen presentation (MHCI, CD1a) and co-stimulation (CD40, CD80, CD83, CD86), but does not lower cell surface expression of MHCII and CD11c (Kruse et al., 2000; Mikloska et al., 2001; Morrow et al., 2003; Samady et al., 2003). Equine herpesvirus 1 (EHV1) is a ubiquitous α-herpesvirus that initially replicates in the upper respiratory epithelium and may cause clinical signs such as abortion or equine herpesvirus myeloencephalopathy due to viral replication-associated tissue damage (Allen and Bryans, 1986). Equine DC are susceptible to EHV1 infection which, for unknown reasons, can reduce the capacity of DC to support T-proliferation (Siedek et al., 1997, 1999; Van de Walle et al., 2009).

The aim of the present study was to evaluate if EHV1 infection results in down-regulation of equine DC cell surface markers, and if so, to explore the underlying mechanism(s). Equine monocyte-derived DC (MDDC) were generated as previously described and characterized by (1) morphology and (2) $MHCI^{high}$, $MHCII^{high}$, $CD11a/CD18^{high}$, $CD172a^{high}$, $CD206^{med\text{-}high}$, $CD86^{med\text{-}high}$ and $CD83^{low\text{-}med}$ flow cytometric immunophenotype (Hammond et al., 1999; Baghi et al., 2014) (Ethical Committee EC2010/147). Donor horses consisted of four mares (two Belgian Warmbloods aged 6 and 15 years old, one 6-year-old French Trotter and one 13-year-old cross breed), and one 5-year old Arab stallion.

Similar to human blood DC (de Andrés et al., 2012), equine MDDC were $CD29^{high}$, indicating that this β1 integrin can be used to immunophenotype equine DC. To investigate the impact of EHV1 infection on cell surface marker expression on equine MDDC, MDDC (cultured for 2 days with interleukin-4 and granulocyte macrophage-colony stimulating factor and detached 1 day later) were mock-inoculated or inoculated with the enhanced green fluorescent protein (eGFP) expressing EHV1 strain Ab4G at multiplicity of infection 10. After 16 h, cells were detached and incubated with isotype controls or mouse monoclonal antibodies specific for MHCI (PT85A), MHCII (CVS20), CD11a/CD18 (CVS9), CD29 (TDM29), CD83 (HB15e), CD86 (IT2.2), CD172a (DH59B) and CD206 (15-2). Subsequently, cells were labeled with Cy5 goat anti-mouse IgG antibody and incubated with the vitality dye 7-amino-actinomycin D. Cell surface expression was analyzed on live/eGFP+ cells by flow cytometry at 16 h post inoculation (hpi), as previously described (Hammond et al., 1999; Baghi et al., 2014; Claessen et al., 2015). The mean fluorescent

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http://dx.doi.org/10.1016/j.tvjl.2015.12.008
1090-0233/

intensity of at least four independent repeats from at least two different horses was normalized to the isotype control and expressed relative to the mock mean fluorescence intensity ratio (MFIR):

MFIR% = [(MFI virus/MFI isotype)/(MFI mock/MFI isotype)] × 100. Two-sided Student's *t* test for paired observations was performed and significant differences were set at $P < 0.01$.

EHV1-infected MDDC showed a significant decrease (% reduction) in MFI expression of CD83 (81%), MHCI (63%), CD29 (44%), CD86 (43%), CD206 (46%) and CD172a (26%). In contrast, EHV1 infection did not significantly affect cell surface levels of CD11a/CD18 or MHCII on DC in our study (Fig. 1). Overall, our results on down-regulation of cell surface markers (or absence thereof) on EHV1-infected MDDC

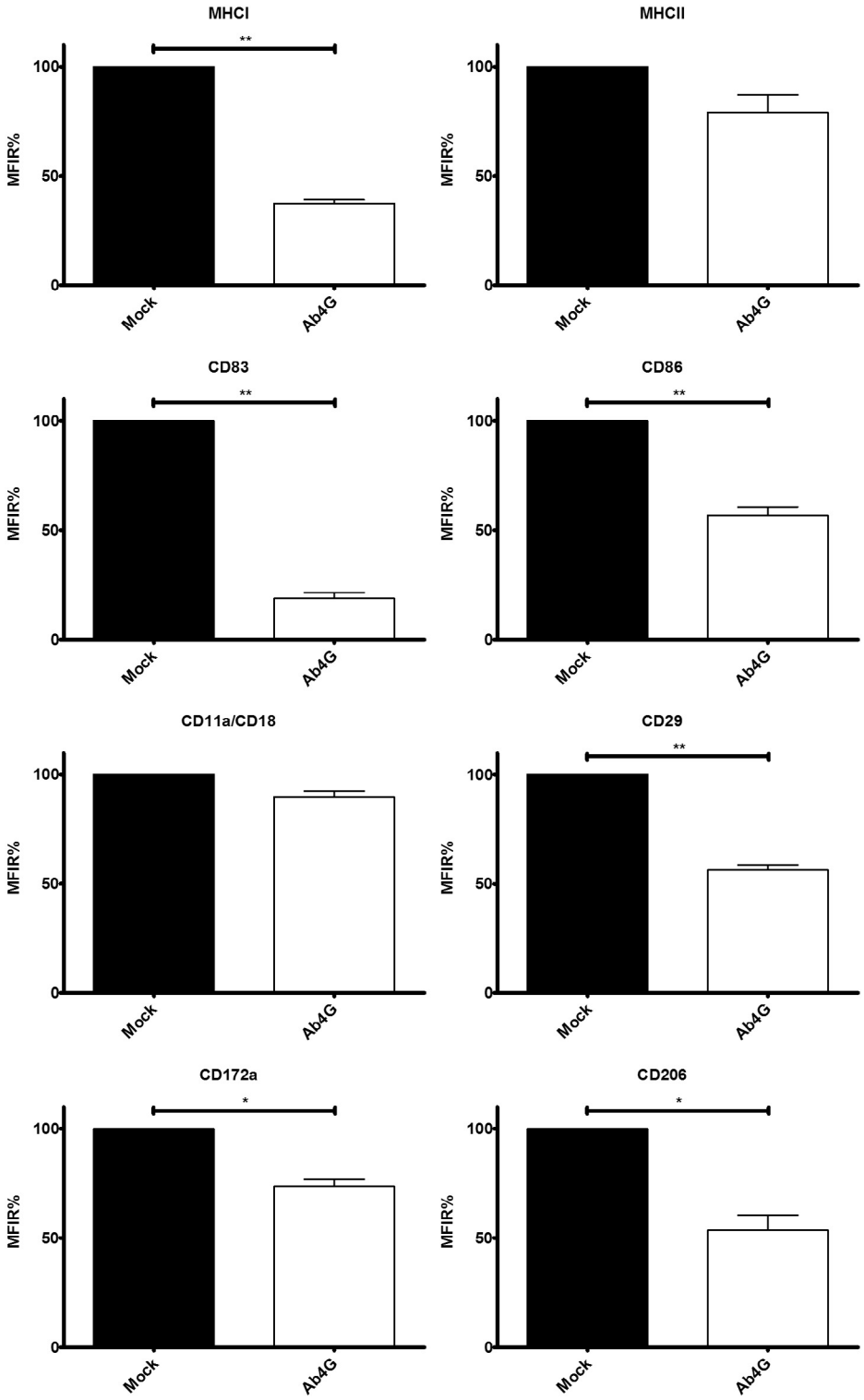

Fig. 1. Selective down-regulation of selected surface markers on EHV1-infected equine monocyte-derived dendritic cells (MDDC). The cell surface immunophenotype was analyzed on live/enhanced green fluorescent protein (eGFP+) cells by flow cytometry. Mean fluorescence intensity ratio, MFIR% = [(MFI virus/MFI isotype)/(MFI mock/MFI isotype)] × 100, with statistical significance $^{*}P < 0.01$; $^{**}P < 0.001$.