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Equine herpesvirus 1 (EHV1) infection of equine mesenchymal stem cells induces a pUL56-dependent downregulation of several cell surface markers

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Introduction

Equine herpesvirus (EHV1) is an ubiquitous alphaherpesvirus that will infect most horses during their lifetime, sometimes resulting in serious clinical symptoms. EHV1 is able to infect different cell types and tissues. This prompted us to investigate whether mesenchymal stem cells (MSC), capable to differentiate into a plethora of cell types, are susceptible to EHV1 infection and if so, whether infection has consequences on the MSC cell marker phenotype. Recently it was found that the majority of EHV1-infected cells in the blood are positive for CD172a (SIRPα) (SHPS1). Interestingly, CD172a is also expressed on human mesenchymal stem cells (MSC) (Vogel et al., 2003), but expression of CD172a on equine MSC has never been studied so far. Hence, our aim was to evaluate the expression of CD172a on equine MSC, to study whether these cells are susceptible to EHV1 infection, and what the consequences of such infection are with regard to the expression of MSC cell surface markers.

Results

1. Equine peripheral blood-derived mesenchymal stem cells (MSC) are CD172a positive and readily susceptible to EHV1 infection in vitro

Equine MSC showed on flowcytometry an identical cell surface immunophenotype as described previously: positive for CD29, CD44, CD90, CD105 and MHCI; negative for CD45, CD79a, MHCII (Figure 1). In addition, equine MSC are found to be CD172a positive. We next performed single-step growth curves for vAb4-infected MSC and NBL6 cells. EHV1 intracellular and extracellular virus titers in MSC were virtually identical to those in NBL6 cells, with the exception of a higher intracellular titer at 48 hours post infection (hpi) in NBL6 compared to MSC infected cells (Figure 2).

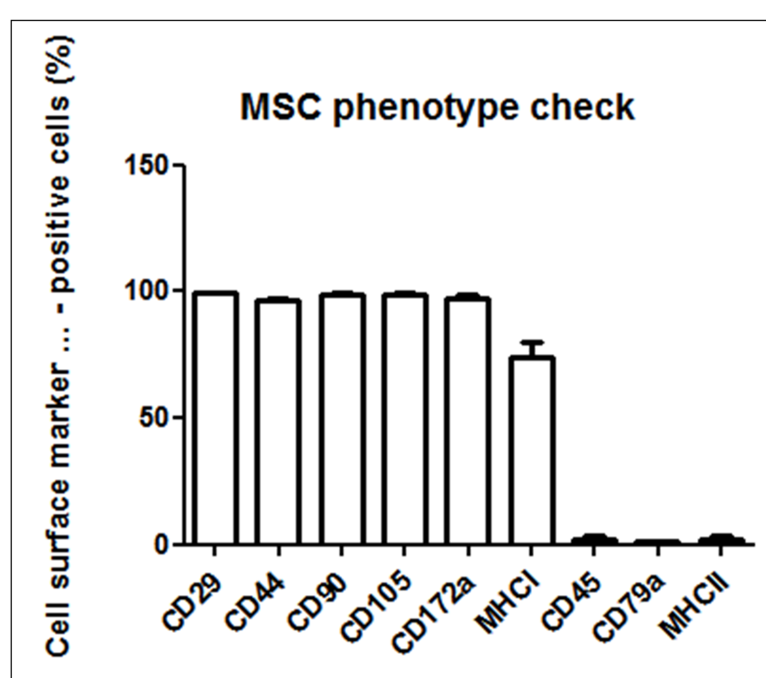

Figure 1: Flowcytometric immunophenotypical profile (incl. CD172a expression) of equine peripheral blood derived mesenchymal stem cells (MSC) (n ≥ 3; mean +- SEM).

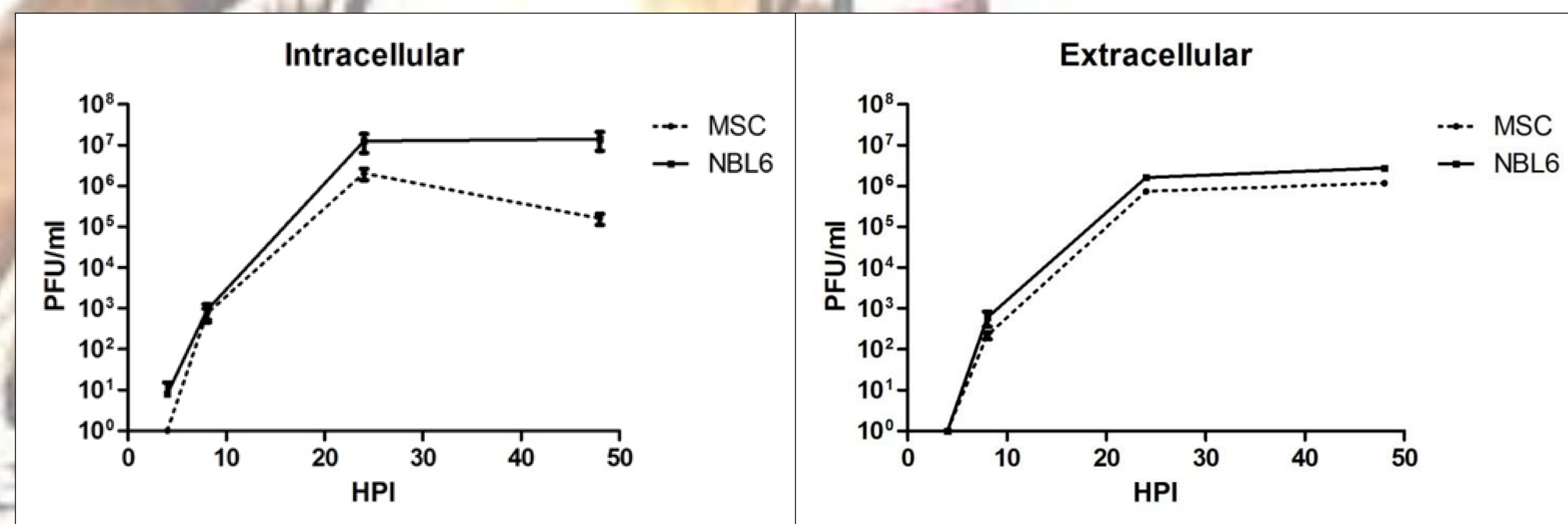

Figure 2: Single step growth kinetics of EHV1 vAb4 in equine MSC and NBL6 cells. Supernatant (extracellular) and cellular (intracellular) fractions were collected at the indicated time points and virus titers were determined by standard plaque assays (n ≥ 3; mean +- SEM).

2. Substantial alterations in the phenotypic cell surface profile of equine MSC upon EHV1 infection.

EHV1-infected MSC, displayed a substantial alteration in their cell surface marker profile. MHCI, CD29 and CD105 were significantly downregulated (Figure 3). CD44 showed a slight increase in cell surface expression, which was not significant, and the expression of CD90 and CD172a was virtually identical in non-infected compared to EHV1-infected MSC (Figure 3). The cell surface markers, used for immunophenotyping of MSC that are absent on these, i.e. CD45, CD79a, and MHCII remained negative on MSC at 16 hpi (data not shown).

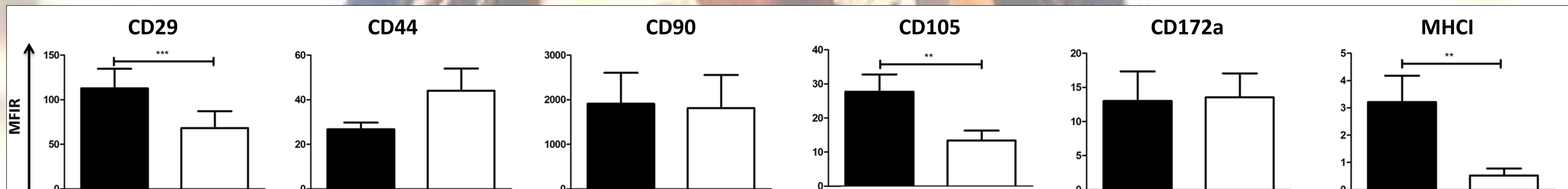

Figure 3: Equine peripheral blood derived mesenchymal stem cells were mock-infected (black shading) or infected with Ab4G (no shading), the egfp-expressing parental vAb4, and expression of several cell surface markers was analyzed at 16 hpi. The mean fluorescence intensity is normalized to the isotype control (MFIR) and expressed as the mean +- SEM (n ≥ 3). Students T-tests (**=P<0,01; ***=P<0,001).

3. Selective downregulation of cell surface molecules in EHV1-infected MSC depends on pUL56.

Downregulation of MHCI has been reported earlier in NBL6 cells and was shown to start as early as 2 hpi (Ma, Feineis et al. 2012). A time course study in equine MSC showed that downregulation of MHCI, CD29 and CD105 started as early as 4 hpi (Figure 4). Ma et al. (2012) identified the viral protein pUL56 as responsible for the MHCI downregulation in NBL6 cells. Expression of pUL56 in MSC coincided with cell surface marker downregulation (Figure 4). Consequently, we investigated whether pUL56 is involved in downregulation of cell surface markers in EHV1-infected MSC. To study this, MSC were infected with wild type Ab4G or its isogenic mutant virus Ab4GΔ1, which lacks the ORF1 gene encoding pUL56. Interestingly, despite similar infection rates, only wild type Ab4G but not UL56null Ab4GΔ1 induced downregulation of the three different cell surface markers in EHV1-infected MSC (Figure 5).

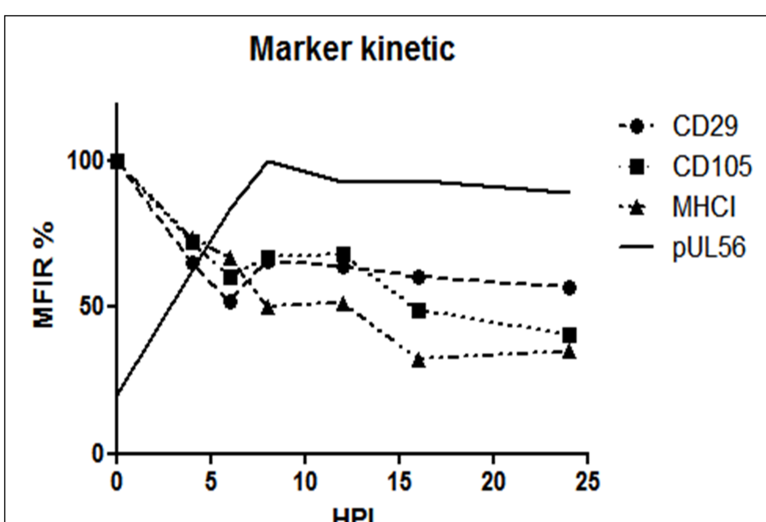

Figure 4: Kinetics of viral pUL56 protein expression and cell surface marker downregulation in Ab4G-infected MSC at indicated time points. The mean fluorescence intensity is normalized to the isotype control (MFIR) and expressed as the mean +- SEM (n ≥ 2). Cell surface marker and pUL56 MFIR at indicated time points is respectively normalized to time point 0 hpi and 8 hpi (MFIR%).

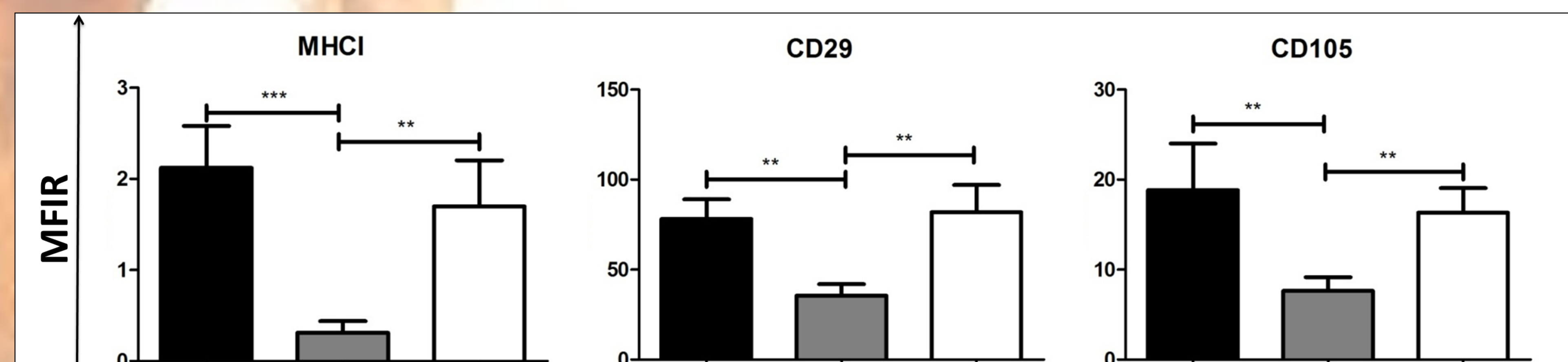

Figure 5: MSC were mock-infected (black shading), infected with Ab4G (grey shading) or infected with the mutant virus Ab4GΔ1 lacking the pUL56 encoding ORF1 gene. The mean fluorescence intensity is normalized to the isotype control (MFIR) and expressed as the mean +- SEM (n ≥ 3). One-way repeated measures ANOVA (**=P<0,01; ***=P<0,001).

Conclusion

In the current report, we show that equine MSC are indeed CD172a positive and susceptible to EHV1 infection. Interestingly, during infection of these MSC not only MHCI, but also CD29 and CD105 are downregulated in a pUL56-dependent manner. As a particular selection of immunophenotypical proteins on the cell surface was downregulated, it would be interesting to dissect how this selection is achieved. Also, based on the presence of MSC in different tissues and blood, it would be interesting to further assess the potential role of MSC in pathogenesis.

Acknowledgements

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