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Is host heparanase required for the rapid spread of heparan sulfate binding viruses?

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ABSTRACT

Vaccinia virus (VACV), like many other viruses, binds to cell surface heparan sulfate (HS) prior to infecting cells. Since HS is ubiquitously expressed extracellularly, it seemed likely that VACV-HS interaction may impede virus spread, with host heparanase, the only known mammalian endoglycosidase that can degrade HS, potentially overcoming this problem. In support of this hypothesis, we found that, compared to wild type, mice deficient in heparanase showed a 1–3 days delay in the spread of VACV to distant organs, such as ovaries, following intranasal inoculation, or to ovaries and spleen following intramuscular inoculation. These delays in spread occurred despite heparanase deficiency having no effect on VACV replication at inoculation sites. Subsequent in vitro studies revealed that heparanase treatment released VACV from HS expressing, but not HS deficient, infected cell monolayers. Collectively these data suggest that VACV relies on host heparanase to degrade HS in order to spread to distant sites.

1. Introduction

Numerous studies have shown that many viruses use cell surface glycosaminoglycans (GAGs), particularly heparan sulfate (HS), as an initial co-receptor to aid their attachment to host cells (Kamhi et al., 2013). Herpes simplex virus-1 (HSV-1) and vaccinia virus (VACV) are examples of such viruses that interact with HS during infection (Khanna et al., 2017). However, since HS is ubiquitously expressed on cell surfaces and in the extracellular matrix (ECM) (Casu and Lindahl, 2001; Coombe and Kett, 2005), this raises an intriguing paradox, namely, how do HS-binding viruses spread from sites of infection to other organs when they are surrounded by copious amounts of ECM and cell surface HS? An analogous paradox exists in the case of sialic acid binding influenza virus where influenza overcomes this problem by encoding in its genome the glycosidase, neuraminidase, which cleaves sialic acid groups from host cells, thereby enabling progeny virus to spread (Bouvier and Lowen, 2010). Could HS–binding viruses have similar mechanisms in place to facilitate their spread?

Heparanase, encoded by the HPSE-1 gene, is an endo-β-glucuronidase that regulates HS function by degrading HS in the ECM and basement membrane (BM), a prerequisite for metastatic tumour cells and leukocytes to invade tissues (Parish et al., 2001). Since heparanase is the only known mammalian endoglycosidase with HS degrading activity, it is possible that invading HS-binding viruses could rely on host derived heparanase activity to aid their spread. In fact, recent findings by Agelidis et al. (2017) and Hadigal et al. (2015) support this concept by showing that intra-corneal spread of herpes simplex virus-1 (HSV-1), a HS-binding virus, is host heparanase dependent (Agelidis et al., 2017; Hadigal et al., 2015). Furthermore, HSV-1 infection resulted in *upregulation of host-encoded heparanase* in infected cells, which was found to not only facilitate the release of progeny virus but also to promote disease pathogenesis by decreasing type I interferon signalling, thereby, increasing NF-κB signalling (Agelidis et al., 2017). These studies clearly demonstrated a key role for heparanase in the localized spread of HS-binding viruses. We attempted to build on these findings by evaluating the role of heparanase in the global spread of HS-binding viruses using

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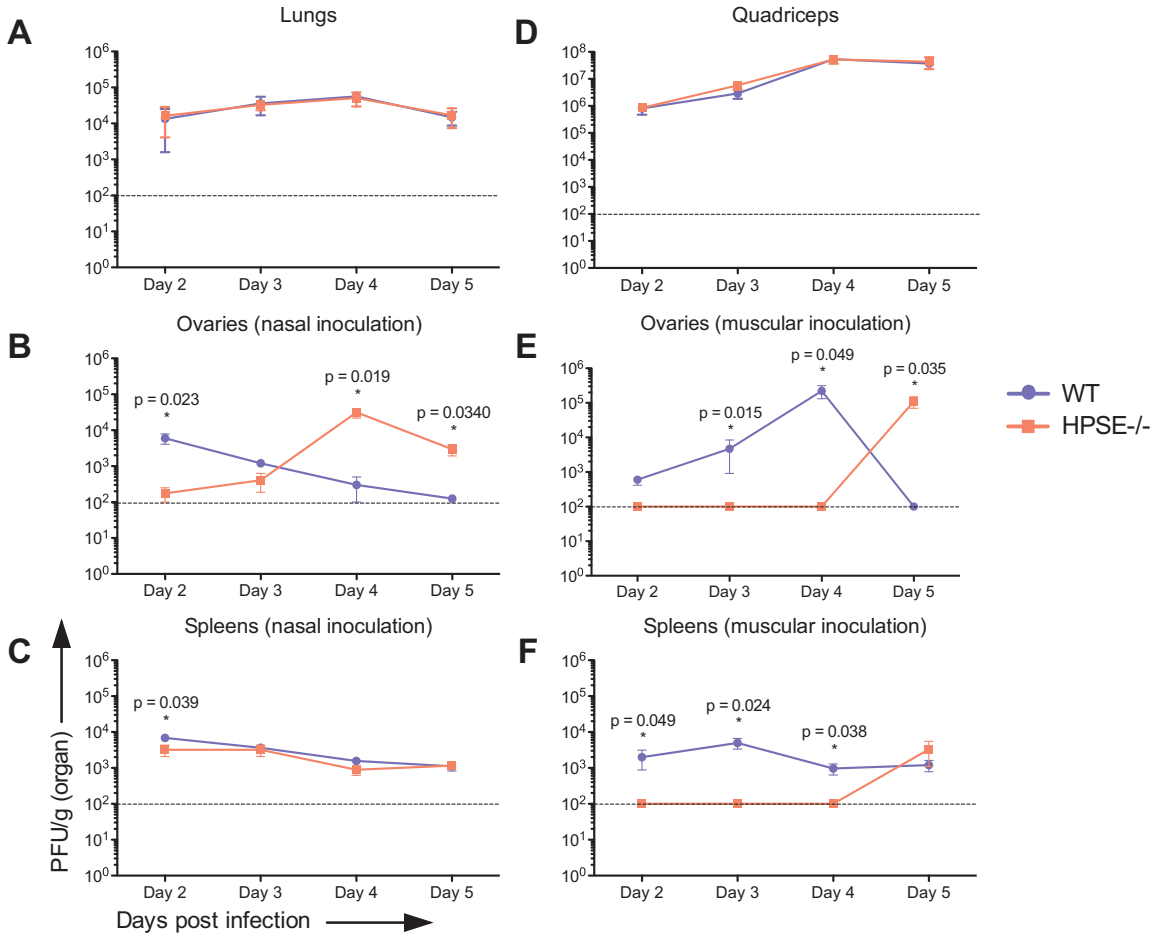

Fig. 1. Heparanase aids the spread of the Western Reserve (W*R*) strain of VACV from primary inoculation sites to secondary sites of infection. WT and heparanase deficient (HPSE-/-) mice were inoculated on day 0 with 10^5 PFU of VACV W*R* strain via either the nasal (lung) or the muscular (quadriceps) route. Over a 5-day period following inoculation VACV titers were measured by plaque assays in WT and HPSE-/- mice in the two primary inoculation sites, namely (A) lungs following nasal inoculation and (D) quadriceps following muscular inoculation and in two secondary infection sites, the ovaries and the spleen, following nasal (B and C) or muscular (E and F) inoculation. Data are presented as mean VACV PFU/g of each organ ± SEM (n = 4) and are representative of at least two independent experiments, with statistical analysis performed using unpaired Student's *t*-test.

VACV as a model virus. We hypothesized that VACV spread to distant organs from a 'restrictive' inoculation site would be hampered in the absence of host heparanase.

2. Results

2.1. Heparanase deficiency restricts long range VACV spread

To test our hypothesis, both wild type (WT) and heparanase knockout (HPSE-/-) C57BL/6 mice (Zcharia et al., 2009) were inoculated with a high dose of the Western Reserve (W*R*) strain of VACV both intranasally and intramuscularly (into quadriceps). Nasal and muscular routes of VACV inoculation served as 'restrictive' primary infection sites with differing propensities to spread to other organs, allowing tracking of the ability of VACV particles to migrate and infect distant organs. Also, since VACV is known to exhibit a strong tropism for ovarian tissue, ovaries served as an excellent secondary organ, along with spleen which is the most readily infected lymphoid organ, to evaluate spread of VACV to distant sites (Zhao et al., 2011; Rahbar et al., 2009).

Lung virus titers in nasal inoculated mice (Fig. 1A) and quadricep virus titers in muscle inoculated mice (Fig. 1D) revealed that VACV is able to establish an infection equally well in both WT and HPSE-/- mice at all time points measured, i.e., days 2–5 post inoculation. In contrast to the primary inoculation sites, HPSE deficiency had a profound effect on the spread of VACV to distant organs, particularly to the ovaries. Thus, in the case of intramuscular inoculation, despite moderate to high virus titers being observed in the ovaries (Fig. 1E) and spleen (Fig. 1F) of most WT animals there was ***no detectable VACV*** in the ovaries and spleen of all the HPSE-/- mice at days 2, 3 and 4 post inoculation. By day 5, however, comparable VACV titers were found in both WT and HPSE-/- spleen (Fig. 1F), but in ovaries, clearance of virus was observed in WT mice, whereas high virus titers were detected in HPSE-/- mice (Fig. 1E).

Differences in VACV titers in the ovaries of nasally inoculated WT and HPSE-/- mice were also observed, similar to intramuscularly inoculated animals (Fig. 1B), although not to the same extent. Thus, VACV was detected in the ovarian tissue of WT mice from day 2 onwards, with virus titers decreasing dramatically in WT mice by day 4 and there being no detectable virus on day 5 (Fig. 1B). Conversely, HPSE-/- mice had barely detectable VACV in their ovaries at days 2 and 3 post intranasal inoculation (Fig. 1B). However, by day 4 post inoculation there was ~100-fold increase in ovarian VACV titers, with the titers starting to decline by day 5. Surprisingly, the nasal delivery of VACV did not result in any significant difference in the splenic virus titers in both WT and HSPE-/- mice at all time points (Fig. 1C).

2.2. HS-bound VACV can be liberated by heparanase treatment in vitro

Our studies show that heparanase deficiency hampers the ability of

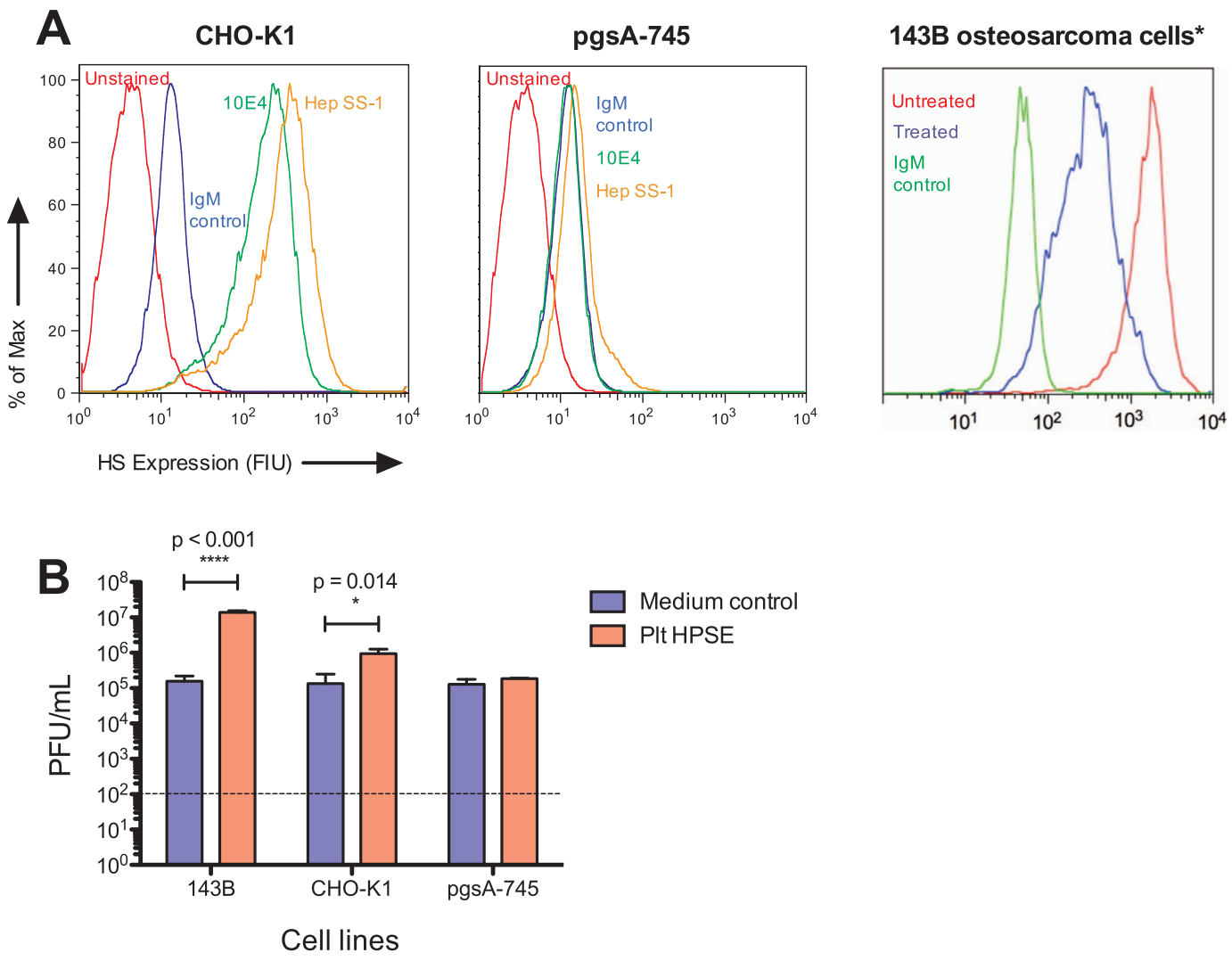

Fig. 2. Heparanase treatment of VACV infected cell monolayers *in vitro* results in the release of infectious VACV particles. (A) The cell surface expression of HS by CHO-K1 and pgsA-745 cells as determined by immunofluorescence flow cytometry using two HS-specific mAbs (clone 10E4 and Hep SS-1). The cell surface expression of HS by 143B osteosarcoma cells was determined using mAb 10E4, with the plot presented here been reproduced from an earlier publication (Khanna et al., 2017). **(B)** Monolayers of 143B osteosarcoma, CHO-K1 and GAG-deficient CHO-K1 (pgsA-745) cells were infected with the *WR* strain of VACV at a m.o.i. of 1 for 12 h. Infected monolayers were then treated for 1 h at 37 °C with medium alone or with human platelet derived heparanase (Plt HPSE) in medium to digest HS and liberate any HS bound VACV particles. The supernatants of the untreated and heparanase treated monolayers were then subjected to plaque assays on fresh monolayers of 143B osteosarcoma cells to quantify the number of infectious VACV particles released following heparanase treatment. Data presented as mean VACV PFU/mL ± SEM (n = 3) from each treatment, with statistical analysis performed using unpaired Student's *t*-test. For more experimental details refer to the Supplemental methods file associated with this manuscript.

VACV to rapidly spread to distant organs from primary infection sites, our prediction being that heparanase aids VACV spread by releasing infectious VACV particles bound to ECM HS. To address this, we used Chinese hamster ovary (CHO-K1) and GAG-deficient CHO-K1 (pgsA-745) cells in a series of in vitro assays. The GAG-deficient pgsA-745 cells, derived from the CHO-K1 parent cell line, lack the xylosyltransferase that mediates the first sugar transfer reaction in GAG formation, with this mutation leading to disruption of the synthesis of not only HS but also chondroitin sulfate (CS) by the pgsA-745 cells (Esko et al., 1985).

We first stained for cell surface HS expression by both CHO-K1 and pgsA-745 cell monolayers (Fig. 2A). Both the HS-specific mAbs used in this assay stain for cell surface HS in CHO-K1 cells. Lack of HS-specific staining in pgsA-745 cells show the lack of HS expression by these cells (Fig. 2A). We have previously shown that 143B osteosarcoma cell line, which is routinely used to propagate VACV, also has a very high levels of cell surface HS expression (Khanna et al., 2017). We also showed that treatment of 143B osteosarcoma cells with heparanase cleaves cell surface HS, resulting in a reduction in the subsequent cell surface HS staining (Khanna et al., 2017). Next, to determine whether HS-bound VACV can be liberated by heparanase treatment, confluent monolayers of 143B osteosarcoma, CHO-K1 and pgsA-745 cells were infected with the *WR* strain of VACV for 12 h, subjected to heparanase treatment for 1 h, and the release of VACV into the culture supernatant measured by standard virus plaque assays. 143B osteosarcoma cells served as a control cell line as they have normal HS expression and are commonly used for VACV plaque assays. Data in Fig. 2B clearly shows that heparanase treatment of VACV infected 143B osteosarcoma cells resulted in an ~100-fold increase in the release of infectious VACV particles compared to the medium control (Fig. 2B). Heparanase treatment of infected CHO-K1 cells also resulted in release of VACV but at a 10-fold lower level than that observed with the 143B osteosarcoma cells. This difference is probably due to the CHO-K1 cells being non-permissive for VACV infections as a result of the host range (*hr*) gene being disrupted

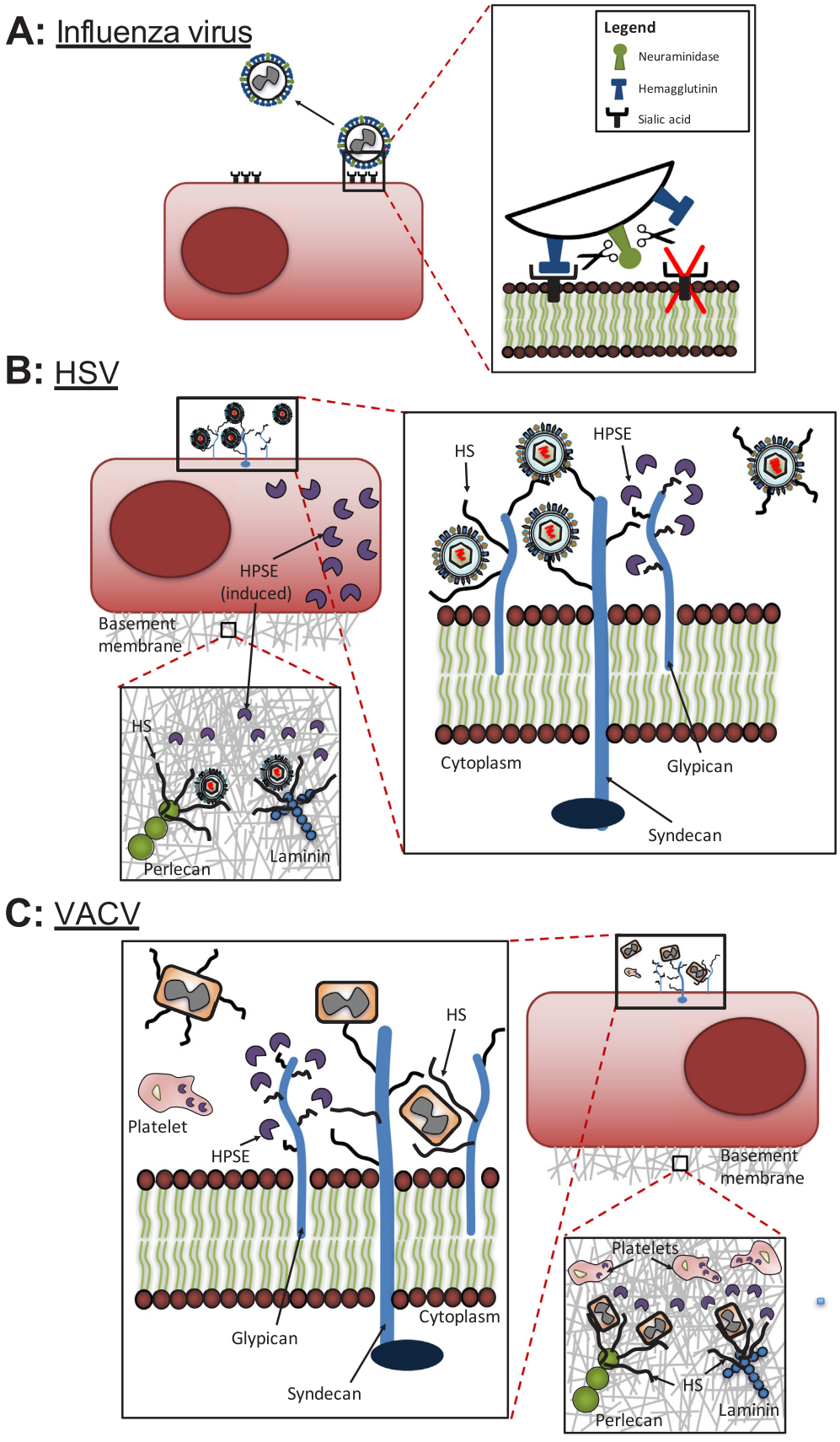

Fig. 3. Schematic representation of the role of neuraminidase in influenza virus spread and the role of heparanase in HSV-1 and VACV spread. (A) Neuraminidase, a major antigen expressed on the surface of influenza virus, cleaves the linkage between the sialic acids and the viral hemagglutinin, thereby, promoting virus release. Heparan sulfate (HS) binding viruses like (B) HSV-1 and (C) VACV rely on heparanase (HPSE) mediated cleavage of HS in order to spread. However, unlike influenza, HSV-1 and VACV do not encode for such enzymatic activity. (B) HSV-1 has been shown to induce the expression of HPSE in the infected cells, release of which cleaves HS in the extracellular matrix (ECM) and on cell surfaces and allows localized spread of HSV-1. In contrast, (C) VACV being highly cytopathic, likely attracts platelets at the site of infection, which in turn release pre-formed stored HPSE to promote inflammation in response to vascular injury. HPSE at the infection site results in the release of VACV from the ECM carrying HS fragments, which can potentially block the virus from further interactions with ECM HS. This allows for an easier long-range spread of VACV.

in the WR strain of VACV used in this study (Ramsey-Ewing and Moss, 1995; Franke et al., 1985; Drillien et al., 1978), although VACV is able to infect CHO-K1 cells and initiate the virus replication cycle. Therefore, it is likely that heparanase treatment of CHO-K1 cells only released virus particles in the initial inoculum that bound to the CHO-K1 ECM, whereas VACV was able to infect and form progeny virus in

the 143B osteosarcoma cells over the 12 h incubation period, resulting in the higher titer of VACV released by heparanase treatment from these cells. Importantly, heparanase treatment of GAG-deficient pgsA-745 cells resulted in VACV release identical to that seen in the medium control (Fig. 2B). This result demonstrates that the VACV released from the different cell lines by heparanase treatment was HS bound.

3. Discussion

The findings reported in this paper strongly suggest that heparanase plays a vital role in enabling the escape of VACV from sites of primary infection. Enzymatic degradation of carbohydrates to aid virus spread is not a novel concept. Influenza virus serves as a classic example where, in order to infect cells, the virus hemagglutinin recognizes cell surface sialic acid but this results in sialic acid expressed by infected cells inhibiting the release of progeny virus. Influenza virus overcomes this problem by encoding in its genome the glycosidase, neuraminidase, which cleaves sialic acid groups from host cells (Fig. 3A), thereby enabling progeny virus to spread (Bouvier and Lowen, 2010; Gamblin and Skehel, 2010). However, there is no evidence that HSV-1 and VACV encode heparanases that can liberate HS bound viruses from the ECM. Thus, this implies that the heparanase must be host derived. In the case of HSV-1, the virus induces the expression of host heparanase in infected cells (Fig. 3B), an ingenious mechanism for overcoming this problem. In contrast, we have no in vitro evidence that VACV is able to induce host heparanase expression in VACV infected cells (Fig. 3C), although we cannot rule out the possibility that in vivo VACV upregulates heparanase in specific cell populations. It seems more likely, however, that in order to spread to distant organs VACV takes advantage of pre-existing sources of host heparanase. In this regard platelets are unique, containing large amounts of pre-formed heparanase that is rapidly released following activation by vascular injury and a plethora of other stimuli (Freeman and Parish, 1998). Since VACV is highly cytopathic it will eventually induce local vascular injury, platelet activation and heparanase release. Indeed, recent studies indicate that activated platelets are also highly migratory and, hence, able to exert effects distant from their site of activation (Gaertner et al., 2017). Clearly this is a research area that warrants further investigation.

An intriguing feature of mammalian heparanase is that it is the only known endoglycosidase encoded by the mammalian genome that can cleave HS-chains, endoglycosidases being enzymes that cleave carbohydrate chains internally, distinct from exoglycosidases that degrade carbohydrate chains from their termini. In fact, heparanase acts like a restriction enzyme, cleaving HS-chains at specific sites to yield HS fragments ranging from ~4 – 5 kDa in size (Freeman and Parish, 1998). Thus, heparanase can liberate HS-binding viruses from ECMs (Fig. 2B), the released virus likely to still carry HS fragments that can potentially block the virus from further interaction with ECM HS (Fig. 3B and C).

Interestingly, unlike HSV-1, the absence of heparanase does not affect the ability of VACV to establish a local infection (Fig. 1A and D), this finding being attributed to the ability of VACV to directly infect neighbouring cells via an actin-dependent 'punching' mechanism that propels virus particles directly into adjacent cells (Smith and Law, 2004). In fact, we have previously shown that the MV form of VACV is able to infect neighbouring cells in a HS-independent manner, whereas EV, which is responsible for long-range dissemination, relies heavily on HS-interactions, with a likely dependency on heparanase (Khanna et al., 2017). Consistent with our previous studies, our results demonstrate that heparanase deficiency results in a delayed VACV spread, which further results in a concomitant delay in VACV clearance by the host immune system.

As several pathogens, including VACV, commonly exploit the mucosal surfaces of lungs to initiate and establish an infection, they have easy access to high numbers of circulating alveolar macrophages, Natural Killer cells and dendritic cells present in the bronchoalveolar lavage fluid (Abboud et al., 2015). Such cells could transport virions either directly into the circulation or to the draining lymph nodes, and eventually to the spleen, a process that would function relatively equally well in both WT and HPSE-/- mice. This would explain why nasal delivery of VACV did not result in differences in splenic virus titers in both WT and HPSE-/- mice. Of course, such an escape route would not be available to VACV inoculated intramuscularly.

In conclusion, studies with HSV-1 and VACV indicate that targeting heparanase could result in novel therapies for treating HS-dependent viral infections. Heparanase-blocking drugs are already being studied in several Phase I and Phase II clinical trials for various cancers (Liu et al., 2009; Ritchie et al., 2011; Dredge et al., 2011; Zhou et al., 2011; Rivara et al., 2016) and, thus, such drugs could easily be tested to determine their effectiveness in the treatment of HS-binding virus infections, just as neuraminidase inhibitors have been developed for controlling influenza (McKimm-Breschkin, 2013).

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Conflict of interest

We have no conflicts of interest to disclose.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.virol.2019.01.001.

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