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Short communication

Role of the UL45 protein in herpes simplex virus entry via low pH-dependent endocytosis and its relationship to the conformation and function of glycoprotein B

Stephen J. Dollery, Kristin D. Lane, Mark G. Delboy, Devin G. Roller, Anthony V. Nicola*

Department of Microbiology and Immunology, Virginia Commonwealth University, School of Medicine, Richmond, VA 23298-0678, United States

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ABSTRACT

Herpesviruses commandeer distinct cellular pathways to enter target cells. The mechanism by which herpes simplex virus (HSV) selects a pH-dependent, endocytic route or a pH-independent route remains to be elucidated. We investigated the role of the non-glycosylated viral envelope protein UL45 in HSV entry via endocytosis. UL45 plays a role in mediating cell–cell fusion and has been proposed to functionally interact with gB to regulate membrane fusion. Thus, we also probed the impact of UL45 on the structure and function of gB present in virions. A UL45 deletion virus successfully entered cells via low pH, endocytic pathway with wild type kinetics. In the absence or presence of UL45, the antigenic conformation of virion gB appeared unaltered. Antibodies to gB neutralized infection of the UL45-deletion virus and wild type virus to a similar extent, regardless of whether the target cells supported low pH endocytic or non-endocytic entry routes. Lastly, HSV virions were inactivated by low pH regardless of the presence of UL45. The results, together with previous studies, suggest that UL45 plays distinct roles in cell–cell fusion and virus–cell fusion during acid-dependent entry.

Herpes simplex virions can use pH-dependent endocytosis to initiate infection of host cells (Nicola et al., 2003). Other human herpesviruses have also been shown to utilize low pH, endocytic pathways for entry (Akula et al., 2003; Finnen et al., 2006; Ryckman et al., 2006). HSV may use an acid-dependent pathway to enter mucosal epithelial cells at the portal of entry in the human host (Nicola et al., 2005). Infection of neurons is thought to occur via a pH-independent mechanism (Lycke et al., 1988; Nicola et al., 2005; Stiles et al., 2008). Direct, acid-independent penetration at the cell surface is the entry pathway in Vero cells (Wittels and Spear, 1991), which are a commonly used cell type to study HSV biology. The utilization of multiple cellular pathways to enter different physiologically relevant cell types is an emerging theme among herpesviruses (Frampton et al., 2007; Miller and Hutt-Fletcher, 1992; Nicola et al., 2005, 2003; Raghu et al., 2009; Ryckman et al., 2006; Van de Walle et al., 2008).

How HSV entry via a particular cell pathway is determined is not well-understood. Both virus determinants (Delboy et al., 2006; Roller et al., 2008) and host factors such as cell receptors (Arii et al., 2009; Delboy et al., 2006; Gianni et al., 2004; Milne et al., 2005; Roller et al., 2008; Stiles et al., 2008) influence the route taken. Entry by an endocytic mechanism may also be distinguished by the involvement of host kinases (Cheshenko et al., 2003, 2005; Clement et al., 2006; Hoppe et al., 2006; Nicola et al., 2005; Nicola and Straus, 2004; Petermann et al., 2009). For the human herpesviruses EBV (Wang et al., 1998) and HCMV (Wang and Shenk, 2005), distinct viral envelope protein complexes determine target cell tropism and entry pathway. For HSV, glycoproteins gB, gD, and gH–gL are required for entry via pH-independent (Spear, 1993) and pH-dependent (Nicola and Straus, 2004) pathways. We theorize that one or more specific HSV envelope proteins direct the virus to the low pH endosomal route. Several HSV envelope proteins are non-essential for replication on Vero cells. With the exception of gC (Nicola and Straus, 2004), these proteins have not been evaluated for potential involvement in entry via endocytosis.

The HSV UL45 membrane protein lacks consensus sites for addition of N-linked carbohydrates. The UL45 gene is expressed late and encodes a 172 residue, ~18 kDa type II membrane protein that is dispensable for growth in Vero cells (Cockrell and Muggeridge, 1998; Visalli and Brandt, 1991, 1993). The UL45 protein is required for cell–cell fusion induced by a syncytial variant of HSV. Specifically, syncytium formation due to a Y854K mutation in the cytoplasmic tail of gB requires wild type UL45 (Haanes et al., 1994). Thus, UL45 may mediate fusion events during HSV infection

* Corresponding author at: 1101 E. Marshall St., Room 6-038, P.O. Box 980678, Richmond, VA 23298-0678, United States. Tel.: +1 804 828 9730; fax: +1 804 828 9946.
E-mail address: anicola@vcu.edu (A.V. Nicola).

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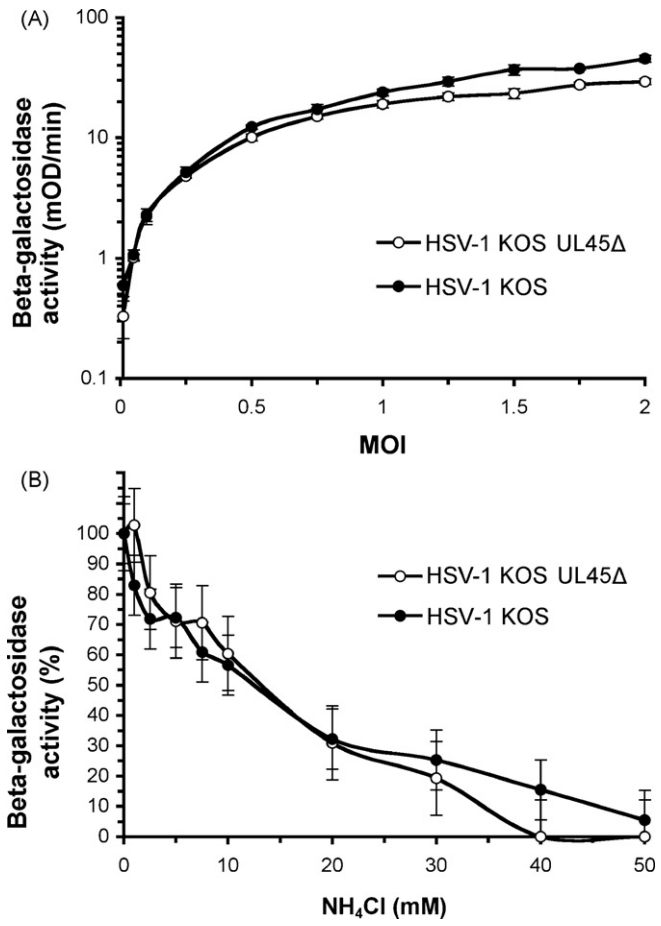

Fig. 1. (A) Entry of HSV-1 KOS UL45Δ into CHO-nectin-1 cells. HSV-1 KOS or KOS UL45Δ was added to CHO-nectin-1 cells at MOIs ranging from 0 to 2. Beta-galactosidase activity of cell lysates was quantified at 6 h post-infection as an indication of viral entry. Entry of the two viruses was not statistically different ($p = 0.40$, Student's *t*-test). (B) Effect of ammonium chloride on the cell entry of HSV-1 KOS UL45Δ. CHO-nectin-1 cells were treated with indicated concentrations of ammonium chloride for 30 min. HSV-1 KOS or KOS UL45Δ (MOI of 1) was added to cells for 6 h in the continued presence of agent. Entry was measured as the percent of beta-galactosidase activity of cell lysates relative to that obtained in the absence of lysosomotropic agent. Data are means of quadruplicate determinations with standard deviation. Entry was not statistically different in the presence of NH_4Cl ($p = 0.94$, Student's *t*-test).

through a functional interaction with gB. gB is conserved among all herpesviruses and plays an essential role in HSV fusion and entry (Heldwein and Krummenacher, 2008). Recently, pH-dependent conformational changes were detected in HSV gB (Dollery et al., 2010). We investigated a potential role for UL45 in HSV entry by pH-dependent endocytosis and examined its influence on the structure and function of gB in virions.

CHO cells that express the cellular receptor nectin-1 are a model system to study low pH, endocytic entry of HSV. We measured the ability of a UL45-null mutant, HSV-1 KOS UL45Δ (Visalli and Brandt, 1991) (provided by C. Brandt) to enter these cells. HSV-induced expression of beta-galactosidase is frequently used as a reporter of successful viral entry. CHO-nectin-1 cells are CHO-K1 cells stably transformed to express nectin-1 and contain the *Escherichia coli* lacZ gene under the control of the HSV ICP4 promoter (Geraghty et al., 1998) (provided by G. Cohen and R. Eisenberg). The level of entry of HSV-1 UL45Δ into CHO-nectin-1 cells was very similar to wild type KOS at a range of MOIs (Fig. 1(A)), suggesting that UL45 is non-essential for efficient entry into these cells.

Different strains of HSV can travel distinct entry routes in the same cell type (Delboy et al., 2006; Roller et al., 2008). For example, the ANG path strain of HSV enters CHO-nectin-2 cells via a pH-independent pathway that is distinct from the pH-dependent entry of the rid1 strain (Delboy et al., 2006). To assess the contribution of UL45 to the selection of entry route, we tested the effect of a lysosomotropic agent on entry into CHO-nectin-1 cells. Ammonium chloride is a weak base that buffers the pH of acidic cellular compartments and characteristically inhibits entry of viruses that require a low pH for entry, such as HSV (Nicola et al., 2003). Entry of HSV-1 UL45Δ was inhibited by ammonium chloride in a concentration-dependent manner (Fig. 1(B)). All together, the data suggest that the absence of UL45 bestows no apparent defect in the ability of HSV to engage the low pH, endosomal entry route.

To address whether the absence of UL45 alters the conformation of gB, we characterized the reactivity of UL45Δ virions with mouse monoclonal antibodies (MAbs) to gB. Twofold dilutions of $\sim 5 \times 10^5$ PFU wild type or UL45Δ virions were blotted directly to nitrocellulose membranes. Blots were probed with MAbs designated SS10, SS106 and SS144 (Bender et al., 2007, 2005; Heldwein et al., 2006) (provided by R. Eisenberg and G. Cohen) and H126, H1817, and H1359 (Virusys). All six antibodies tested displayed equivalent reactivity with gB from both viruses (Table 1). The ectodomain of gB is an elongated, rod-like structure comprised of several folded domains (Heldwein et al., 2006). The MAbs evaluated detect distinct epitopes across the gB molecule (Table 1) (Bender et al., 2007), suggesting that in the absence of UL45, gB is not globally altered. This contrasts with virions containing highly fusogenic forms of gB. Virion gB from such fusion-from-without strains of HSV has a pronounced reduction in reactivity with MAbs to Domain I, the fusion domain of gB (Roller et al., 2008).

Glycoprotein-specific antibodies that neutralize virus infection in the absence of complement target glycoprotein function. To investigate the influence of UL45 on the function of gB in virus entry, MAbs specific for gB were tested for their ability to neutralize UL45-null HSV. Since CHO-nectin-1 cells do not support plaque formation, Vero and HaCaT cells were employed. Table 1 shows that HSV-1 UL45Δ was sensitive to neutralization by MAbs H126, SS10, SS106 and SS144. In contrast, neutralization by H1359 was not detected. Each antibody tested neutralized wild type KOS to a similar extent. Thus, by these measures, the absence of UL45 does not appear to alter the conformation or function of gB.

Vero cells support HSV entry by pH-independent fusion with the plasma membrane, and HaCaT cells support a pH-dependent, endocytic mechanism (Nicola et al., 2005). All of the MAbs to gB tested neutralized UL45Δ to a similar extent whether assayed on Vero or HaCaT cells (Table 1). Within the limits of these experiments, no difference in the role of UL45 in these two entry pathways was detected. Although UL45 influences cell–cell fusion (syncytium

Table 1
Effect of deletion of UL45 from HSV-1 KOS on the reactivity and neutralization activity of gB-specific antibodies.

MAb	Notes	Reactivity[a]		Neutralization[b]			
				Vero		HaCaT	
		wt	UL45Δ	wt	UL45Δ	wt	UL45Δ
H126	Domain I	+	+	+	+	+	+
H1359	Domain III	+	+	–	–	–	–
H1817	Domain VI	+	+	nd	nd	nd	nd
SS10	Domain IV	+	+	+	+	+	+
SS106	Domain V	+	+	+	+	+	+
SS144	Domain V	+	+	+	+	+	+
αUL45	Polyclonal to UL45p[c]	+	–	nd	nd	nd	nd

wt, wild type; nd, not determined.
[a] Measured by dot blot or Western blot.
[b] +, >50% plaque reduction at MAb dilution <0.002; –, <50% plaque reduction.
[c] Visalli and Brandt (1993) provided by C. Brandt.