Herpes simplex virus particles interact with chemokines and enhance cell migration

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Herpes simplex virus type 1 and type 2 (HSV-1 and HSV-2, respectively) are among the most prevalent human pathogens, causing a variety of diseases. HSV modulation of the chemokine network remains poorly understood. We have previously identified secreted glycoprotein G (SgG) as the first viral chemokine-binding protein that enhances chemokine function as a novel viral immunomodulatory mechanism. However, gG is also present at the viral envelope and its role in the virus particle remains unknown. Here we have addressed the chemokine-binding capacity of HSV particles and the functionality of such interaction in vitro. We adapted surface plasmon resonance assays and demonstrated the ability of HSV particles to bind a specific set of human chemokines with high affinity. Moreover, we identified gG as the envelope glycoprotein mediating such interaction, as shown by the lack of binding to a HSV-1 gG mutant. In contrast to HSV-1, HSV-2 gG is cleaved and the chemokine-binding domain is secreted (SgG2). However, we found that HSV-2 particles retain the ability to bind chemokines, potentially through SgG2 associated to the viral envelope or non-processed precursor protein. Moreover, we found that HSV particles increase cell migration independently of chemokine binding to envelope gG. This work provides insights into HSV manipulation of the host immune system.

Chemokines are chemotactic cytokines that coordinate the recruitment and activation of immune cells to the site of infection, and therefore are essential elements shaping the onset and the outcome of the infection (Baggiolini, 1998). Alterations in the chemokine network may be primarily responsible for diseases caused by excessive inflammatory responses and there is growing evidence showing that chemokines regulate multiple aspects of the nervous system during homeostasis and disease (Conrady et al., 2009; Karin, 2010; Li & Ransohoff, 2008; Turner et al., 2014). Although a comprehensive analysis of the role of chemokines expressed during HSV infection is lacking, several studies support the relevance of chemokines on HSV pathogenesis (Cornaby et al., 2015). Many chemokines are upregulated upon HSV infection and play a role in the tissues where viral replication and spread take place (McGrory et al., 2004; Molesworth-Kenyon et al., 2005; Rangel-Moreno et al., 2005; Thapa & Carr, 2008). HSV invasion of the nervous system is greatly influenced by the immune response at the primary site of infection. In fact, CXCL10−/−, CXCL9−/−, CXCR3−/− or CCR5−/− mice show a higher susceptibility to genital herpes and an earlier colonization of the central nervous system due to defects in the effector leukocyte mobilization to the infected mucosa (Thapa & Carr, 2008, 2009; Wickham et al., 2005). However, despite their relevance, the role of chemokines in HSV pathogenesis is still poorly understood. The importance of the chemokine network for...
herpesvirus infection is further highlighted by the evolution of a number of strategies to modulate cytokine and chemokine functions (Alcami, 2003). In particular, our previous work identified HSV-1 and HSV-2 glycoprotein G (gG1 and gG2, respectively) as the first viral chemokine-binding proteins (vCKBPs) that, in sharp contrast to the rest of CKBPs encoded by pathogens, enhance chemokine function (Viejo-Borbolla et al., 2012) through modulation of chemokine receptor trafficking and oligomerization (Martínez-Martín et al., 2015). gG1 is a structural protein present in the viral envelope and at the plasma membrane of the infected cell (Ackermann et al., 1986). On the contrary, HSV-2 gG is proteolytically processed giving rise to secreted gG2 (SgG2) (Roizman et al., 1984), which contains the N-terminal domain responsible for chemokine interaction (Viejo-Borbolla et al., 2012). Lack of gG1 leads to virus attenuation in HSV-1 mouse models of infection (Balan et al., 1994; Meignier et al., 1988; Weber et al., 1987); however the role of gG2 is currently unknown. Interestingly, gG encoded by non-human alphaherpesviruses acts as an inhibitory vCKBP in vitro and in vivo (Costes et al., 2005; Van de Walle et al., 2007; Viejo-Borbolla et al., 2010). Fidil herpesvirus-1 (FeHV-1) gG has been shown to mediate binding of CCL3 to the viral envelope (Costes et al., 2006). However, a full screening of chemokine interactions and a determination of the binding affinities could not be performed in that study.

In this work, we sought to study the chemokine-binding capacity of virion-anchored gG and analyse its function in vitro. In order to address these questions, we adapted surface plasmon resonance (SPR)-based assays, using a BIAcore biosensor, to the study of virion–chemokine interactions. This approach allowed us not only to determine whether virions interact with chemokines but also to measure the chemokine-binding specificity, the kinetics of purified HSV particles and the relevance of the glycosaminoglycan (GAG)–binding site of the chemokine in the interaction. We show that both HSV-1 and HSV-2 particles bind specific human chemokines with high affinity, and in the case of HSV-1, demonstrate that this interaction is mediated by gG on the viral envelope. Interestingly, HSV particles promoted cell migration independently of direct interaction with the chemokine, suggesting unrecognized mechanisms of cellular activation triggered by the virus. This work provides new insights into HSV modulation of the chemokine system.

RESULTS

HSV-1 particles bind chemokines through gG

We have previously reported the ability of SgG1 and SgG2, gG1 found at the plasma membrane and SgG2 present in the supernatant of infected cell cultures to bind chemokines (Viejo-Borbolla et al., 2012). Currently, there are no data regarding the ability of virion-associated HSV gG to bind chemokines. In order to address this question, we adapted SPR technology to evaluate the ability of whole HSV particles to interact with chemokines using a BIAcore biosensor. HSV stocks were prepared by Ficoll purification, inactivated by psoralen treatment and UV light irradiation, and the integrity and purity of the viral preparations was assessed by direct visualization of uranyl acetate negatively stained samples by electron microscopy (Fig. S1a, available in the online Supplementary Material). Purified HSV-1 particles were covalently immobilized through amine groups to the activated dextran matrix of sensor chips (Fig. S1b, c). Reproducible binding was achieved after several injections of a given concentration of chemokine, without significant changes in the baseline following successive cycles of binding and regeneration (not shown). The sensorgram in Fig. S1(d) shows the association and dissociation phases corresponding to increasing concentrations of CXCL12 injected over the wt HSV-1 chip, showing a dose-dependent binding to the viral surface. All commercially available human chemokines were screened for binding to both wt HSV-1 and a gG-deficient HSV-1 mutant (ΔgG HSV-1) where expression of gG was disrupted by the insertion of the β-galactosidase gene (Balan et al., 1994). We found that wt HSV-1 particles interacted with 11 out of 44 human chemokines screened (Fig. 1a, Table 1), with the same specificity previously described for soluble recombinant SgG1 (Viejo-Borbolla et al., 2012). By contrast, chemokine binding dramatically dropped or disappeared when the ΔgG HSV-1 was used (Fig. 1b, Table 1). The utilization of SPR allowed us to calculate the affinity constants for the interactions between HSV-1 and all targeted chemokines which interacted with high affinity in the nanomolar range (Table 1). On the contrary, the kinetic parameters corresponding to ΔgG HSV-1 could not be calculated due to the lack of high affinity interactions with chemokines (Table 1).

Chemokine binding to gG present in the viral envelope was further demonstrated by crosslinking assays using radioiodinated CXCL12. We detected chemokine bound to wt HSV-1 but not to ΔgG HSV-1 (Fig. 1c). Similar levels of viral proteins were detected in the virus using a polyclonal anti-HSV antibody (Fig. 1d). The lack of gG expression was confirmed by Western blotting using a monoclonal anti-gG1 antibody (Fig. 1e). In addition, binding of CXCL12α to Ficoll-purified HSV-1 particles was confirmed by electron microscopy. An anti-gG1 antibody detected gG in the wt HSV-1 envelope, while no staining was observed for the deletion mutant (Fig. 1f, top panels). A polyclonal antibody against glycoprotein B (gB) and glycoprotein D (gD) recognized both viruses (Fig. 1f, middle panels). Ficoll-purified virions were incubated with the chemokine, and the presence of CXCL12α bound to viral particles was visualized by immunogold labelling using an anti-CXCL12α antibody and quantified. CXCL12α binding to wt HSV-1 particles was fivefold higher than that detected with ΔgG HSV-1 (Fig. 1f, bottom panels), consistent with the SPR-binding results. In addition, we observed that pre-incubation of the chemokine with the soluble GAG heparin competitively inhibited in a dose-dependent manner the chemokine interaction with HSV-1 (Fig. 2), indicating that chemokines...
interact with the virion surface through their GAG-binding domain, as previously described for SgG1 (Viejo-Borbolla et al., 2012).

Altogether these experiments indicated that HSV-1 particles bind a specific set of human chemokines with high affinity. Furthermore, we identified gG1 present in

![Graph](http://jgv.microbiologyresearch.org)

**Fig. 1.** HSV-1 binds chemokines through gG present at the viral envelope. (a and b) Sensorgrams showing the interaction between the indicated human chemokines (injected at 100 nM) and wt HSV-1 (a) or gG-deficient mutant (ΔgG HSV-1) (b). The arrow indicates the end of the injection. Chemokines have been labelled according to the corresponding response units bound at the end of the injection, from top to bottom. All curves were analysed with the BIAevaluation software and represent the interaction of the chemokine after subtraction of the blank curve. (c) Crosslinking assays showing the interaction of wt and ΔgG HSV-1 with \(^{125}\text{I}\)-labelled hCXCL12. Sucrose-cushion-purified viruses were incubated with iodinated chemokine and crosslinked with bis(sulfosuccinimidyl)suberate. The samples were resolved by SDS-PAGE, fixed and visualized by autoradiography. Molecular masses are indicated in kilodaltons. The arrow shows the position of the gG1–chemokine complex. (d and e) Western blot showing the expression of viral proteins (d) and the presence or absence of HSV-1 gG (e) in the sucrose-purified viruses used in the crosslinking assay. (f) Analysis of purified virions (wt HSV-1, left; ΔgG HSV-1, right) by immunogold labelling using specific antibodies to detect gG1 (upper panels), gB/gD (middle panels) or CXCL12α (lower panels). In the latter, the virions were incubated with CXCL12α prior to staining. Viruses were visualized by electron microscopy. The average number of gold particles corresponding to CXCL12 per virion is indicated below the images. Some of the gold particles are marked by yellow arrows.
the viral envelope as the main HSV-1 glycoprotein conferring specific chemokine-binding ability to the virus.

**HSV-2 particles interact with chemokines with high affinity**

Contrary to gG1, gG2 is proteolytically processed giving rise to an N-terminal domain that is secreted to the extracellular medium, which we previously identified to interact with chemokines (Viejo-Borbolla et al., 2012). The nature of the cellular proteases involved in the cleavage, to what extent membrane-anchored gG2 is processed and whether all processed SgG2 is secreted or some remains bound to the particle have not been elucidated. We next addressed whether HSV-2 particles, which should lack the chemokine-binding domain present in SgG2, interacted with chemokines. Similarly to HSV-1, wt HSV-2 particles were covalently coupled to sensor chips. Unexpectedly, we found that HSV-2 interacted with chemokines, although to a lesser extent as compared to HSV-1 (Fig. 3a), with the same specificity and affinity previously shown for SgG2 (Viejo-Borbolla et al., 2012) (Table 1). The reduced binding of chemokines by HSV-2 particles may reflect a lower amount of SgG2 present at the surface of virions. An mAb directed against the secreted portion of gG2 detected SgG2 associated to the purified virus preparation used for coupling to the BIAcore chip (Fig. 3b, third lane), indicating that the cleaved N-terminal domain of gG2 is present in the virion, likely accounting for chemokine binding to HSV-2. This result also suggests that most of the gG2 present in the virus particle has been proteolytically cleaved since we did not detect a higher molecular size band of ≈120 kDa corresponding to the full-length, uncleaved gG2 (Balachandran & Hutt-Fletcher, 1985) (Fig. 3b, third lane).

**HSV synergizes with chemokines triggering enhanced cell migration**

We next addressed whether gG1 located at the surface of the viral envelope could modulate migration, as previously described for SgG1 (Viejo-Borbolla et al., 2012). Supernatants from mock- or HSV-infected cells were separated into a filtrate and a concentrated fraction, enriched for viral particles and the total number of viral particles present in wt and ΔgG HSV-1 concentrated supernatants was quantified by electron microscopy. MonoMac-1(MM-1) cell migration was measured towards a constant amount of wt and ΔgG HSV-1 that were added to the lower compartment of transwell plates, alone or in combination with increasing concentrations of CXCL12 or CCL2 (Fig. 4a, b, respectively).
Interestingly, we found that wt and ΔgG HSV-1 enhanced cell migration triggered by both chemokines, despite CCL2 showing no binding to HSV particles nor secreted HSV gG (Viejo-Borbolla et al., 2012) (Fig. 1, Table 1). However, there was a modest yet reproducible difference in the level of potentiation of CXCL12-mediated chemotaxis when comparing wt HSV-1 and the gG-deficient virus, consistent with a gG-mediated enhancement of migration when the chemokine analysed interacted with gG. A similar significant increase in the migration of CXCR5-expressing B cells towards CXCL13 was observed in the presence of wt HSV-1 (Fig. 4c), further suggesting a synergic effect of the HSV particles on chemokine-directed migration (Fig. 5). In addition, wt HSV-2 particles also potentiated migration towards CXCL12 (Fig. 4d).

**DISCUSSION**

We have adapted the SPR technology to systematically analyse the interaction of chemokines and whole HSV particles, showing for the first time the ability of these virions to bind chemokines, and furthermore we identified gG as the viral envelope glycoprotein mediating such interaction. These results demonstrate that native HSV gG present in its natural environment (the viral envelope) interacts with chemokines. Despite gG2 being described as a proteolytically cleaved protein, our results indicate that HSV-2 retains the ability of interacting with specific human chemokines. Interestingly, we found that both HSVs promoted chemotaxis independently of direct interaction with the chemokine, further supporting a role for modulation of cell migration during HSV infection.

The chemokines that interact with HSV are expressed in tissues where the virus replicates and spreads. HSV-associated recruitment and activation of immune cells to the site of infection may have important consequences for the onset of infection and the dissemination of HSV within the host. First, the ability of HSV to enhance cell migration could attract cells whose infection could increase viral loads and facilitate virus spread. In this regard, HSV infection of human keratinocytes induces migration of non-infected keratinocytes towards the infected plaque through a non-characterized mechanism (Abaítau et al., 2013). Second, enhancement of chemokine receptor function could facilitate the early steps of infection, since the activation of mitogen-activated protein kinase and NFκB pathways induced by chemokine signalling is known to be required for HSV replication (McLean & Bachenheimer, 1999). Third, by interfering with the function of a specific set of chemokines, HSV could shift the immune response towards one beneficial for viral replication. In line with this hypothesis, it has been previously shown that a peptide from SgG2 has proinflammatory properties that increase the recruitment of monocytes and neutrophils that in turn secrete reactive oxygen species, which inhibit cytotoxicity and accelerate apoptosis of NK cells (Bellner et al., 2005). This property has not been demonstrated for full-length SgG2. Future studies should certainly address whether HSV particles are covered with chemokines in vivo, as well as the relevance of this interaction during the viral infection.

Biosensors have been already used to analyse the interaction between viruses and different ligands (Casasnovas & Springer, 1995; Lea et al., 1998; Vega et al., 2011). However,
to our knowledge, such procedure has never been utilized to measure the interaction of host soluble factors with an enveloped DNA virus as complex as HSV. Further, we identified gG as the protein responsible for such interaction, showing that HSV gG acts as a vCKBP also in the context of the viral particle. gG is the least conserved glycoprotein between HSV-1 and HSV-2 and, interestingly, despite the distinct sequence and topology of HSV-1 and HSV-2 gG, both viruses have retained the ability to bind and modulate the same set of chemokines, a fact that evidences the importance of the chemokine system for HSV biology. It will be important to elucidate the structural basis for such conserved binding to chemokines by the divergent HSV gGs. The only vCKBP previously found to bind chemokines in its virion anchored form is the FeHV-1 gG homologue, which has been shown to inhibit chemokine activity (Costes et al., 2006). Our study, however, is the first to describe the complete chemokine-binding profile of viral particles, to determine the affinities of the interactions, and their functional relevance in vitro. Interestingly, the involvement of chemokine binding during an infection by FeHV-1 or HSV may be completely different given the opposite function of both vCKBPs (Costes et al., 2005; Viejo-Borbolla et al., 2012). Three independent reports have shown that lack of gG expression in HSV-1 leads to different degrees of virus attenuation (Balan et al., 1994; Meignier et al., 1988; Weber et al., 1987). In addition, gG1 has been implicated in the entry, but not in the initial attachment, to polarized epithelial cells (Tran et al., 2000), and we have recently demonstrated that SgG2 modulates receptor trafficking and disposition at the cell membrane (Martínez-Martín et al., 2015). Our former and current results suggest that the dysregulation of the chemokine network could in part explain the lower virulence of gG1 mutant viruses.

Membrane-anchored gG2, but not gG1, is proteolytically processed to release the N-terminal domain that binds...
chemokines. Interestingly, we found that HSV-2 particles also bind chemokines, probably through an interaction mediated by SgG2 that remained associated to the viral envelope. We have shown that SgG2 interacts with the plasma membrane through a high affinity interaction mediated by GAGs, a property that is relevant for the function of the protein (Martínez-Martín et al., 2015). Alternatively, it could be that some gG2 molecules are not fully processed and contain the N-terminal domain. However, the proportion of full-length gG2 is minor since it was not detected in the virion preparations with a specific antibody directed against the secreted domain of gG2 (Liljeqvist et al., 2002). Finally, we cannot rule out at present the possibility that the non-secreted portion of gG2 may bind chemokines, contributing also to the binding observed to the viral particles.

HSV interacts with chemokines with nanomolar affinities, with HSV-2 showing higher affinities than HSV-1, in agreement with our previous results for SgG2 and SgG1. No affinities could be calculated for the HSV-1 gG-deficient mutant, indicating that either none of the chemokines interacted with that virus or that they did so with very low affinity. We have demonstrated that gG1 present at the viral envelope and at the plasma membrane of infected cells is the only HSV glycoprotein that binds chemokines (Viejo-Borbolla et al., 2012). Although very unlikely, the presence of additional proteins with that capacity in the HSV-2 envelope has not been unequivocally discarded yet. It has been shown that HSV-1 gB exerts chemokine binding (Nakayama et al., 2006); however, the affinities calculated were 1000-fold lower than those we measured for SgG. The biological significance of the chemokine-binding activity of gB has not been demonstrated. The potential binding to chemokines mediated by gB did not contribute to the interactions we detected by our SPR approach, given the low chemokine concentrations we tested (10–500 nM as the highest concentration injected). The construction of an HSV-2 mutant virus devoid of gG2 will unequivocally address whether SgG2, similarly to gG1, is the only viral protein capable of interacting with chemokines.

Virion-mediated increase of cell migration in vitro has been previously described for HIV (Lin et al., 2000) and vaccinia virus (Valderrama et al., 2006). Contrary to these previous studies, HSV-mediated enhancement of migration was dependent on the presence of chemokines, and was induced upon cell stimulation with inactivated viruses during short periods of time (30–45 min), consistent with our previous results showing that cells respond faster to SgG/chemokine gradients compared to the chemokine alone (Viejo-Borbolla et al., 2012). wt HSV-1 promoted CXCL12-mediated cell migration further than its gG-deficient counterpart, suggesting a role for viral envelope gG in chemokine-driven cell migration. This was supported by the fact that no differences in migration enhancement between wt HSV-1 and ΔgG HSV-1 were observed with
CCL2, a chemokine not bound by HSV gG. Intriguingly, gG-deficient HSV particles also enhanced cell migration, regardless of the chemokine studied. We hypothesize that HSV particles activate chemokine-independent signalling pathways, possibly through engagement of unknown cellular receptor(s), which converge with chemokine-dependent signals to synergistically promote cell migration (Fig. 5). In this regard, diverse stimuli, including chemokines, have been shown to functionally cooperate through engagement of different surface receptors to regulate cell functions, such as migration (Gouwy et al., 2008; Kumar et al., 2006; Molon et al., 2005; Proudfoot & Uguccioni, 2016). Alternatively, virus binding to an uncharacterized molecule on the surface could trigger chemokine receptor reorganization on the plasma membrane, leading to increased cell activation and improved chemokine binding to its cognate GPCR. The characterization of the pathways responsible for such synergy between viruses and chemokines certainly deserves further investigation.

In summary, by using a combination of SPR, electron microscopy and crosslinking assays, we identified both HSV-1 and HSV-2 as the first human viruses that bind chemokines with high affinity. This interaction is mediated by viral envelope gG, acting as a vCKBP in the context of HSV infection. The characterization of the pathways responsible for such synergy between viruses and chemokines certainly deserves further investigation.

**METHODS**

**Antibodies.** The anti-gG1 monoclonal LP-10 antibody was kindly provided by Dr Helena Browne (Cambridge University, UK) (Richman et al., 1986) and the monoclonal anti-SgG2 antibody was a generous gift from Dr Jan-Ake Liljeqvist (Göteborg University, Sweden) (Liljeqvist et al., 2002). The anti-gB/gD antibody was kindly provided by Dr Enrique Tabares (Universidad Autonoma de Madrid, Spain) (Domingo et al., 2003). The anti-CXCL12a antibody was purchased from PeproTech.

**Chemokines.** Recombinant chemokines (hCCL1, hCCL2, hCCL3, hCCL3L1, hCCL4, hCCL4L1, hCCL5, hCCL7, hCCL8, hCCL11, hCCL13, hCCL14, hCCL15, hCCL16, hCCL17, hCCL18, hCCL19, hCCL20, hCCL21, hCCL22, hCCL23, hCCL24, hCCL25, hCCL26, hCCL27, hCCL28, hCXCL1, hCXCL2, hCXCL3, hCXCL4, hCXCL5, hCXCL6, hCXCL7, hCXCL8, hCXCL9, hCXCL10, hCXCL11, hCXCL12a, hCXCL12b, hCXCL13, hCXCL14, hCXCL16, hCXCL17 and hCXCL18) were obtained from PeproTech, with the exception of hCCL25 and hCCL13, which were from R&D Systems. Radiolabeled CXCL12 was obtained from PerkinElmer.

**Viruses and cells.** Wt HSV-1, strain SC-16, Agg HSV-1, and wt HSV-2 particles were purified by Ficoll gradient, as described previously (Rodger et al., 2001). In brief, viral particles present in the supernatants from infected cells were ultracentrifuged at 18 000 r.p.m. during 2 h using a SW28 rotor (Beckman Coulter). The pellet was layered onto a 15–30% Ficoll 400-DL gradient (Sigma), and fractions were subsequently collected and analysed by SDS-PAGE using a polyclonal anti-gB/gD antibody. Fractions enriched in viral particles were diluted in PBS and ultracentrifuged at 21 000 r.p.m. for 2 h in a SW28 rotor. The pellet was resuspended in PBS and kept at −80 °C. For comparative reasons, HSV particles were purified using a 15–30% sucrose cushion using standard procedures (Rodger et al., 2001). Prior to any further manipulation, viruses were inactivated by psoralen treatment and UV light irradiation (Davies et al., 2005). Protein concentration of the viral stocks was measured using a BCA Protein Assay kit (Pierce), and the purity and integrity of the viral preparations was assessed by electron microscopy visualization as described below.

**Purification of viruses.** Wt HSV-1, Agg HSV-1 and wt HSV-2 particles were purified by Ficoll gradient, as described previously (Rodger et al., 2001). In brief, viral particles present in the supernatants from infected cells were ultracentrifuged at 18 000 r.p.m. during 2 h using a SW28 rotor (Beckman Coulter). The pellet was layered onto a 15–30% Ficoll 400-DL gradient (Sigma), and fractions were subsequently collected and analysed by SDS-PAGE using a polyclonal anti-gB/gD antibody. Fractions enriched in viral particles were diluted in PBS and ultracentrifuged at 21 000 r.p.m. for 2 h in a SW28 rotor. The pellet was resuspended in PBS and kept at −80 °C. For comparative reasons, HSV particles were purified using a 15–30% sucrose cushion using standard procedures (Rodger et al., 2001). Prior to any further manipulation, viruses were inactivated by psoralen treatment and UV light irradiation (Davies et al., 2005). Protein concentration of the viral stocks was measured using a BCA Protein Assay kit (Pierce), and the purity and integrity of the viral preparations was assessed by electron microscopy visualization as described below.

**Negative staining and immuno-gold labelling.** Purified viruses were fixed with 2% glutaraldehyde and attached to copper grids, and subsequently stained with 2% uranyl acetate during 40 s. For immuno-gold staining, Ficoll-purified viruses were fixed with 0.1% glutaraldehyde and attached to nickel grids. The viruses were incubated with 500 nM CXCL12α over 15 min at room temperature prior to fixation when indicated. Samples were incubated with TBS buffer (50 mM Tris/HCl pH 7.5, 150 mM NaCl) containing 3% BSA and with the corresponding primary antibody during 1 h at room temperature, followed by incubation with 10 nm gold-conjugated protein A (Cell Microscopy Center) or with a 10 nm gold-conjugated anti-mouse immunoglobulin (British Biocell) when the primary antibody used was produced in mouse. Grids were negatively stained and examined on a JEM1010 electron microscope (JEOL) operating at 80 kV. Images were taken with a slow scan CCD camera (Bioscan).

**Covalent coupling of viruses to sensor chips.** Ficoll-purified viruses were diluted in acetic buffer pH 4 (1:1) prior to their covalent immobilization to the short carboxy-dextran matrix of F1 (CM3) sensor chips (BIACore AB) via amino groups. This mild acidification of viruses was found to be non-disruptive, as confirmed by subsequent cell infection assays (not shown). The immobilization was performed following the standard amino coupling chemistry technique according to manufacturer’s instructions. To improve the sensitivity of the SPR procedure, CM3 chips with shorter dextran chains were selected. Small quantities of viruses were coupled (2000–4000 response units) to avoid mass transport events and to generate low-density surfaces that would be suitable to measure the kinetic parameters of the possible virus–chemokine interaction. Briefly, the carboxyl groups of the chip surface were activated by injecting a 1:1 mixture of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (1 M) and hydroxyethylamine pH 8.5 at 5 µl min⁻¹. Following attachment in the Fc-1 flow cell, the remaining surface carboxyl groups were quenched with 35 µl of 1 M ethanolamine pH 8.5 at 5 µl min⁻¹. The Fc-1 flow was left uncoupled as a control for non-specific chemokine binding to the dextran surface.

**Determination of HSV-binding specificity by SPR using a BIAcore biosensor.** For screening purposes, chemokines were injected at a 100 nM concentration over the surface of a F1 (CM3) sensor chip containing viral particles in HBS-N buffer (10 mM HEPES, 150 mM NaCl pH 7.4) at a flow rate of 5 µl min⁻¹ and association and dissociation were monitored at 20 °C. HBS-N was also used as a running buffer. The chip surface was regenerated after each chemokine injection with two or three 30 µl pulses of HBS-N buffer pH 4.5–5.5. Regeneration conditions were optimized empirically by testing various reagents in order to remove bound protein while maintaining surface activity. For kinetic analysis, different concentrations of each chemokine were injected at a
flow rate of 30 µl min⁻¹ over a 2 min period and allowed to dissociate for 5 min. Chemokines that did not bind under screening conditions were considered negative and were not taken into further consideration for the study. Bulk refractive index changes were removed by subtracting the background corresponding to the reference flow cell, and the average response of a blank injection was subtracted from all analyte sensorgrams to remove surface plasmon resonance. The average kinetic data were globally fitted to a 1:1 Langmuir model.

Chemotaxis assays. Chemotaxis was measured essentially as described previously (Viejo-Borbolla et al., 2012). Chemokines were placed in the lower compartment of 96-well ChemoTx System plates (3 µm pore; Neuro Probe) typically with (5×10⁻⁵–5×10⁻⁹) particles or without viral particles in RPMI 1640 containing 1% FBS and MM-1 cells were placed in the upper chamber. After 30–45 min, the number of MM-1 cells in the lower chamber was determined using CellTiter 96 AQuosOne Solution Cell Proliferation assay (Promega) measuring absorbance at 492 nm. For the preparation of the virus stocks used in chemotaxis assays, Vero cells were infected and kept in Opti-MEM (Invitrogen) for 3 days, harvested and centrifuged (10000 g, 10 min) at room temperature. Then, bis(sulfosuccinimidyl)suberate (Pierce) dissolved in 5 mM sodium citrate pH 3 was added to the reaction at a final concentration of 5 mM and incubated for 30 min. The samples were separated by SDS-PAGE, fixed with H₂O₂ containing 20% methanol and 10% acetic acid for 30 min. Then, the gel was dried and exposed to Kodak films for different periods of time.

Chemokines and leukocyte traffic. Chemotaxis of viruses was used to perform the chemotaxis assays shown. One Solution Cell Proliferation assay (Promega) measuring absorbance at 492 nm. For the preparation of the virus stocks used in chemotaxis assays, Vero cells were infected and kept in Opti-MEM (Invitrogen) for 3 days, harvested and centrifuged (10000 g, 10 min) at room temperature. Then, bis(sulfosuccinimidyl)suberate (Pierce) dissolved in 5 mM sodium citrate pH 3 was added to the reaction at a final concentration of 5 mM and incubated for 30 min. The samples were separated by SDS-PAGE, fixed with H₂O₂ containing 20% methanol and 10% acetic acid for 30 min. Then, the gel was dried and exposed to Kodak films for different periods of time.

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Statistical analyses. Statistical significance (P-value) was calculated using the indicated tests with the software GraphPad Prism.

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