Timing of Galectin-1 Exposure Differentially Modulates Nipah Virus Entry and Syncytium Formation in Endothelial Cells

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ABSTRACT

Nipah virus (NiV) is a deadly emerging enveloped paramyxovirus that primarily targets human endothelial cells. Endothelial cells express the innate immune effector galectin-1 that we have previously shown can bind to specific N-glycans on the NiV envelope fusion glycoprotein (F). NiV-F mediates fusion of infected endothelial cells into syncytia, resulting in endothelial disruption and hemorrhage. Galectin-1 is an endogenous carbohydrate-binding protein that binds to specific glycans on NiV-F to reduce endothelial cell fusion, an effect that may reduce pathophysiologic sequelae of NiV infection. However, galectins play multiple roles in regulating host-pathogen interactions; for example, galectins can promote attachment of HIV to T cells and macrophages and attachment of HSV-1 to keratinocytes but can also inhibit influenza entry into airway epithelial cells. Using live Nipah virus, in the present study, we demonstrate that galectin-1 can enhance NiV attachment to and infection of primary human endothelial cells by bridging glycans on the viral envelope to host cell glycoproteins. In order to exhibit an enhancing effect, galectin-1 must be present during the initial phase of virus attachment; in contrast, addition of galectin-1 postinfection results in reduced production of progeny virus and syncytium formation. Thus, galectin-1 can have dual and opposing effects on NiV infection of human endothelial cells. While various roles for galectin family members in microbial-host interactions have been described, we report opposing effects of the same galectin family member on a specific virus, with the timing of exposure during the viral life cycle determining the outcome.

IMPORTANCE

Nipah virus is an emerging pathogen that targets endothelial cells lining blood vessels; the high mortality rate (up to 70%) in Nipah virus infections results from destruction of these cells and resulting catastrophic hemorrhage. Host factors that promote or prevent Nipah virus infection are not well understood. Endogenous human lectins, such as galectin-1, can function as pattern recognition receptors to reduce infection and initiate immune responses; however, lectins can also be exploited by microbes to enhance infection of host cells. We found that galectin-1, which is made by inflamed endothelial cells, can both promote Nipah virus infection of endothelial cells by “bridging” the virus to the cell, as well as reduce production of progeny virus and reduce endothelial cell fusion and damage, depending on timing of galectin-1 exposure. This is the first report of spatiotemporal opposing effects of a host lectin for a virus in one type of host cell.

Nipah virus (NiV) is an emerging zoonotic paramyxovirus that targets endothelial and neural cells. Infection with NiV can result in a severe encephalitic or respiratory syndrome with case fatality rates ranging from 40 to 100% in humans (1, 2). Although initial outbreaks involved transmission from bats to pigs and then from pigs to humans, more recent outbreaks have been shown to involve human-to-human virus transmission (2). There are currently no approved vaccines or antiviral agents targeting NiV for human cases, and quarantine has been the predominant measure to restrain the spread of the virus (3).

NiV preferentially infects microvasculature endothelial cells that express the entry receptor ephrinB2 (4). The NiV attachment protein NiV-G binds to ephrinB2 or ephrinB3 (5) on the host cell plasma membrane, triggering the NiV fusion protein, NiV-F, to execute fusion of the viral envelope with the host cell membrane. Infected microvascular endothelial cells produce NiV-F and NiV-G, which results in fusion of the cells into syncytia (6); this syncytium formation contributes to vascular

compromise and hemorrhage, two hallmarks of Nipah virus infection (7).

Endothelial cells respond to viral infection by producing inflammatory mediators, including cytokines, which regulate leu-

Received 10 September 2014 Accepted 3 October 2014

Accepted manuscript posted online 10 December 2014

Citation Garner OB, Yun T, Pernet O, Aguilar HC, Park A, Bowden TA, Freiberg AN, Lee B, Baum LG. 2015. Timing of galectin-1 exposure differentially modulates Nipah virus entry and syncytium formation in endothelial cells. *J Virol* 89:2520–2529. doi:10.1128/JVI.02435-14.

Editor: R. W. Doms

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doi:10.1128/JVI.02435-14

kocyte trafficking into adjacent tissues, and by presenting viral antigens (8). Among the inflammatory mediators produced by endothelial cells are the galectins, a family of mammalian lectins implicated in immune regulation. Vascular endothelial cells express several galectin family members, including galectin-1 and -9. For example, infection of vascular endothelial cells with Ebola virus results in endothelial cell activation (9) and increased expression of galectin-1 (10, 11). Infection of endothelial cells with Dengue virus induces expression of galectin-9 (12), as does binding of double-stranded RNA to Toll-like receptor 3 on endothelial cells (13).

Galectins play several roles in modulating host-pathogen interactions. Galectins can participate in innate immune recognition and clearance of pathogens; however, pathogens can also use galectins as attachment receptors. Galectins specifically recognize a subset of microbes based on binding to pathogen specific polysaccharides (14). For example, galectin-4 and galectin-8 bind to and directly kill human blood-group B expressing *Escherichia coli* (15), and galectin-3 binds to and directly kills *Candida albicans* (16) in the absence of immunoglobulin or complement. In contrast, *Pseudomonas aeruginosa* and *Trypanosoma cruzi* use galectin-3 to bind to and infect human cells, and galectin-1 on human cervical epithelial cells is an attachment factor for *Trichomonas vaginalis* (17–19). Similarly, galectin-1, which preferentially recognizes LacNAc (Gal β 1,4GlcNAc)-containing glycans, increases human immunodeficiency virus (HIV) infection of monocyte-derived macrophages through stabilization of virus attachment (20–22), and galectin-9 promotes HIV infection of T cells by regulating the cell surface redox status (23). Thus, different galectins can have pleiotropic and sometimes opposing roles in the etiology and pathophysiology of microbial infections. However, opposing effects of a single galectin on a specific virus have not been reported.

Using NiV-F/G-pseudotyped particles, our labs previously demonstrated that galectin-1 produced by endothelial cells can inhibit endothelial cell syncytium formation mediated by NiV-G and NiV-F (11, 24). In these studies, galectin-1 reduced NiV-F mediated fusion of endothelial cells by interfering with NiV-F maturation, reducing lateral mobility of NiV-F on the plasma membrane and inhibiting the conformational change in NiV-F triggered by receptor binding to NiV-G that is necessary for cell-cell fusion (11). Although galectin-1 produced by endothelial cells can clearly inhibit postinfection cell-cell fusion triggered by NiV-F, our prior studies did not address the effect of galectin-1 on NiV attachment and entry into host cells. In the present study, we found that galectin-1 can enhance live NiV attachment to and infection of endothelial cells by bridging glycans on the viral envelope to cell surface glycoproteins. While the presence of galectin-1 enhances NiV entry, galectin-1 also inhibits syncytium formation of infected cells. In both cases, a specific N-linked glycan site on NiV-F plays a critical role in mediating galectin-1's effects. Our results highlight the complex roles galectin-1 may play in viral pathogenesis.

MATERIALS AND METHODS

Cells, virus, and reagents. Vero cells (American Type Culture Collection) were maintained in MEM α (Invitrogen) with 10% fetal bovine serum (FBS) (HyClone) and 2 mM GlutaMAX in 5% CO₂ at 37°C. 293T cells were maintained in Dulbecco modified Eagle medium (DMEM; Invitrogen) with 10% FBS (HyClone) and 2 mM GlutaMAX. VeroCCL-81 cells

were maintained in DMEM (Gibco) with 10% FBS (HyClone). Human umbilical vein endothelial cells (HUVECs; BD Biosciences) were maintained in MDCB-131 complete media with fetal bovine serum and antibiotics (VEC Technologies, Inc.). HUVECs (Lonza) were maintained in endothelial basal medium-2 (EGM-2) with EGM-2 SingleQuots.

Recombinant human galectin-1 was expressed in *E. coli* and purified by affinity chromatography on lactosyl-Sepharose, as described previously (25). Galectin-1 has unpaired thiols in the carbohydrate recognition domain that can form disulfide bonds and inactivate the lectin, so galectin-1 is prepared and stored in phosphate-buffered saline (PBS) with dithiothreitol (DTT) and, in all assays, buffer controls are the stock PBS with 1.2 mM DTT at the appropriate dilution (26).

NiV-F and -G were pseudotyped onto a reporter vesicular stomatitis virus (VSV) expressing *Renilla* luciferase as described previously (27). NiV strain Malaysia (NiV_{MAL}) was kindly provided by the Special Pathogens Branch, Centers for Disease Control and Prevention, Atlanta, GA, and propagated in Vero E6 cells. All work with infectious NiV was carried out under biosafety level 4 (BSL-4) conditions in the Robert E. Shope BSL-4 and in the Galveston National Laboratory BSL-4 laboratories at the University of Texas Medical Branch.

Quantitation of viral entry. Cells plated in 48-well plates were infected with pseudotyped virions in PBS plus 1% FBS for 2 h at 25°C at 2,000 rpm (spinoculation). After 2 h, the cells were washed, appropriate growth medium was added, and cells were incubated at 37°C. At 24 h postinfection (hpi), cells were lysed, and luciferase activity was measured as relative light units (RLU) using a *Renilla* luciferase detection system (Promega, Madison, WI) and a Veritas microplate luminometer (Turner Biosystems, Sunnyvale, CA). Galectin-1, buffer control, 100 mM lactose, and 100 mM sucrose were added just prior to spinoculation.

Reduction in complex N-glycans. Cells (Vero cells, 293T cells, and HUVECs) were treated with kifunensine (2 μ g/ml) for 24 h. To confirm loss of complex N-glycans on the cell surface, cells were suspended in PBS with 5 mM EDTA, washed, and resuspended in biotinylated l-phytohemagglutinin (L-PHA) in PBS (1:1,000) plus 1% bovine serum albumin (BSA; Gemini Scientific) for 1 h at 4°C. Cells were washed in PBS, and bound PHA was detected with a fluorescein isothiocyanate-conjugated streptavidin (1:60) in PBS plus 1% BSA for 1 h at 4°C. Cells were washed in PBS with 1% BSA, and flow cytometric analysis was performed on a FACScan using CellQuest software (Becton Dickinson). Pseudotyped VSV expressing NiV-G and NiV-F was produced by infecting kifunensine-treated or control cells with VSV-rLuc virions for 24 h. Supernatants were collected, and virions were purified by centrifugation through 20% sucrose in NaCl-Tris-EDTA (NTE) buffer. To examine loss of N-glycan processing by immunoblot, NiV-G and NiV-F were separated and detected as previously described (24).

Rescue of recombinant Nipah virus (rNiV) and *in vitro* luciferase assay. Recombinant Nipah virus expressing secreted *Gaussia* luciferase (rNiV-GLuc) was rescued at BSL-4 (28). Briefly, HEK293T cells were transfected with the following plasmids: T7-driven positive-sense rNiV antigenome (3.5 μ g), codon-optimized pCAGGS/T7 (1 μ g), and the T7-driven support plasmids encoding NiV nucleoprotein (1 μ g), phosphoprotein (0.2 μ g), and polymerase (0.4 μ g). Transfection was performed using TransIT-LT1 transfection reagent (Mirus) according to the manufacturer's instructions. After 4 days posttransfection, *Gaussia* luciferase expression was confirmed using cell supernatant and a BioLux *Gaussia* luciferase assay kit (New England BioLabs). Collected supernatant was used for subsequent virus preparation in Vero CCL-81 cells. HUVECs were infected with rNiV-GLuc at different multiplicity of infections (MOIs), and cell supernatants were collected at the indicated intervals. Luciferase assays were performed using a BioLux *Gaussia* luciferase assay kit (New England BioLabs) according to the manufacturer's protocol, and expression was measured on Modulus luminometer (Turner Biosystems, Inc.).

NiV plaque assay. For titrations, confluent monolayers of Vero CCL-81 cells were infected with 100 μ l of serial 10-fold dilutions of virus-

FIG 1 Galectin-1 enhances infection of NiVpp in a carbohydrate binding-dependent manner. (A) Quantification of galectin-1 enhancement of NiVpp infection. NiVpp was titrated such that viral inoculum that gave luciferase activity in the linear range at 24 hpi was used. NiVpp was added to monolayers of Vero cells, and virus entry in the absence (white bars) or presence (black bars) of increasing concentrations of galectin-1 was quantified by measuring luciferase activity in infected cell lysate 24 hpi as described in Materials and Methods. The data from one of three replicate experiments are presented as mean fold increase ($\pm$ the standard deviations [SD] of triplicate samples) over the virus-only (i.e., no galectin-1) condition. Significant *P* value determined by two-way ANOVA. (B) Galectin-1 enhancement of NiVpp infection is carbohydrate binding dependent. NiVpp (white bars) or 10 μ M galectin-1 (black bars) was added to Vero cells in the presence of 100 mM lactose or 100 mM sucrose. The data are presented as in panel A as means $\pm$ the SD of triplicate samples from one of three replicate experiments is shown. (C) Galectin-1 added during spinoculation (at 4°C) shows an increase in NiVpp infection on

containing cell supernatant. After 1 h of incubation at 37°C and 5% CO₂, the inocula were removed, and the wells were overlaid with a mixture of one part 1.0% methylcellulose (Fisher Scientific) and one part 2× MEM (Gibco/Invitrogen) supplemented with 2% FBS and 2% penicillin-streptomycin. The plates were incubated at 37°C and 5% CO₂ for 3 days and stained with 0.25% crystal violet in 10% buffered formalin. The plates were then washed and the plaques enumerated.

Wild-type NiV galectin-1 experiments. HUVECs were infected with NiV_{MAL} at an MOI of 0.1 for 1 h at 37°C. Infections were performed either in the presence of various galectin-1 concentrations (1, 7, and 20 μ M) or cells were treated with galectin-1 postinfection. In the case of infections performed in the presence of galectin-1, the inoculum was removed at 1 hpi, and the cells were washed three times with 0.1 M β -lactose, followed by incubation in complete EGM-2 medium. The cells infected only with NiV were washed three times with medium and incubated in complete EGM-2 medium containing different concentrations of galectin-1 (1, 7, or 20 μ M). Supernatant aliquots were harvested at 1, 12, 24, and 36 hpi and stored at -80°C until titration. DTT was used as a negative control, and experiments were performed in triplicates.

For DAPI (4',6'-diamidino-2-phenylindole) staining, HUVECs were grown on 12-well plates and incubated with NiV for 1 h at 37°C. Galectin-1 treatment was performed as described above. At 24 hpi, the cells were fixed in 10% buffered formalin, the nuclei were stained with DAPI (Sigma) at 20 $\mu\text{g}/\text{ml}$, and the samples were analyzed by fluorescence microscopy.

RESULTS

Galectin-1 increases viral infection of endothelial cells. Galectin-1 inhibits endothelial cell syncytium formation mediated by NiV envelope glycoproteins (11). Although galectin-1 can bind to both NiV-F and -G, we showed that inhibition of cell-cell fusion was mediated largely by galectin-1 binding to abundant and accessible lactosamine moieties on complex N-glycans at the "F3" N-glycan site (⁹⁹NNT¹⁰¹) on NiV-F (11, 24). The F3 N-glycan site is close to the cathepsin L cleavage site ¹⁰³DLVGDVR¹⁰⁹. Binding of dimeric galectin-1 to F3 N-glycans on different NiV-F protomers likely accounts for the suite of inhibitory activities that galectin-1 exerts on NiV-F-mediated fusion (11). We therefore expected galectin-1 to inhibit NiV infection just as potently. Surprisingly, when we examined the function of galectin-1 in NiV entry using NiV-F/G-pseudotyped particles (NiVpp) (29), we found that the addition of recombinant galectin-1 significantly increased infection of target cells in a concentration-dependent manner (Fig. 1A).

We confirmed that the galectin-1-mediated increase in infection was carbohydrate dependent. Lactose, but not sucrose, specifically abrogated the galectin-1-mediated increase in infection, whereas lactose alone in the absence of galectin-1 had no effect (Fig. 1B). Thus, exogenous galectin-1 increased viral infection in a dose-dependent and carbohydrate binding-dependent manner.

Galectin-1 increases viral attachment to host cells. Entry of NiV into host cells requires two steps, viral attachment to the target cell surface mediated by NiV-G followed by membrane fusion mediated by NiV-F. Viral attachment is largely energy independent, whereas virus-cell fusion is an energy-dependent process (30). To examine the point at which galectin-1 enhancement occurs, NiVpp was centrifuged onto target cells in the presence or

Vero cells. NiVpp infection in the absence (white bars) or presence (black bars) of 10 μ M galectin-1 added during or after spinoculation, i.e., during or after attachment of virus to cells. The data are means $\pm$ the SD of triplicate samples from one of three replicate experiments. Significant *P* values were determined by using the Student *t* test.

FIG 2 Galectin-1 enhances infection of NiVpp by bridging the virus to the cell through binding of viral surface and cell surface complex N-glycans. Flow cytometric analysis of L-PHA binding to 293T cells (A) or Vero cells (C) treated with kifunensine (dashed black line) shows loss of cell surface complex N-glycans compared to L-PHA binding to untreated parental cells (bold black outlined histograms). Gray-filled histogram represents negative control with secondary only. (B) Galectin-1 mediated enhancement of NiVpp infection is dependent upon complex N-glycans found on the surface of the virus. Virus infection of Vero cells in the absence (white) or presence of 20 μ M galectin-1 (black) is shown for NiVpp bearing three types of envelope glycoproteins: wild-type NiV-F+-G, NiV-F3 mutant (+ wild-type G), or NiV-F+-G devoid of complex N-glycans. The data from one of three replicate experiments are presented as mean fold increases in infection ($\pm$ the SD of triplicate samples) over the virus-only (no galectin-1) condition. (D) Galectin-1-mediated enhancement of NiVpp infection is dependent upon complex N-glycans on the surface of Vero cells. Virus infection in the absence (white bars) or presence (black bars) of 20 μ M galectin-1 is shown for wild-type Vero cells and Vero cells devoid of complex N-glycans. The data (means $\pm$ the SD of triplicate samples) from one of three replicate experiments are presented exactly as described for panel B.

absence of galectin-1 for 2 h at 4°C to allow viral attachment but not virus-target cell fusion. Alternatively, virus was centrifuged onto target cells in the absence of galectin-1 and then incubated with or without galectin-1 for 1 h at 37°C to permit fusion. As shown in Fig. 1C, galectin-1 present during the 4°C spinoculation step clearly enhanced viral entry. In contrast, galectin-1 added after spinoculation at 4°C and present only during the 37°C fusion step, did not increase viral entry. These experiments suggest that galectin-1 increased infection by enhancing viral attachment to host cells. Moreover, that no inhibition was seen suggests that there are functional NiV-F envelope spikes on the virion particles that are not bound by galectin-1 and are still capable of being triggered by receptor binding to NiV-G.

Galectin-1 bridges N-glycans on viral envelope glycoproteins to glycoproteins on target cells. Galectin-1 stabilizes HIV-1 attachment and adsorption to host cells by binding specifically to clustered complex N-glycans on gp120 and CD4, bridging the virus to the target cell (20–22). We sought to determine whether complex N-glycans that bear glycan ligands recognized by galectin-1 were involved in the galectin-1-mediated increase in viral

entry of NiVpp. To eliminate complex N-glycans from viral envelope glycoproteins, virus was produced in 293T cells treated with kifunensine to block processing of high-mannose N-glycan precursors into complex N-glycans (31). We detected equivalent expression levels of NiV-F by immunoblotting from cells treated with or without kifunensine (data not shown). We confirmed that kifunensine blocked N-glycan processing in 293T cells by flow cytometry with the lectin L-PHA (Fig. 2A); loss of L-PHA binding indicates the loss of complex N-glycans.

Vero cells were infected with NiVpp virus, with NiVpp virus lacking complex N-glycans from kifunensine-treated 293T cells, or with NiVpp virus specifically lacking the NiV-F3 N-glycan in the presence or absence of exogenous galectin-1 (Fig. 2B). As expected, addition of galectin-1 resulted in a 12.6-fold increase in target cell infection of virus with a full complement of complex N-glycans (Fig. 2B, wild-type NiVpp). Remarkably, the loss of the F3 N-glycan site on NiV-F resulted in a >80% reduction in galectin-1-mediated enhancement (3.2-fold versus 12.6-fold enhancement for NiV-F3pp versus wt NiVpp, respectively), underscoring the contribution of the complex N-glycan at the F3 site in the

FIG 3 Galectin-1 enhances NiVpp and live Nipah virus infection of HUVECs. (A) NiVpp was added to monolayers of HUVECs in the absence (white bar) or presence (black bars) of 20 μ M galectin-1 for 1 h, and infection was quantified by measuring *Renilla* luciferase activity at 24 hpi. The data are presented as the mean fold increases ($\pm$ the SD of triplicate samples) in infection over the virus-only (no galectin-1) condition. The results from one of three replicate experiments are shown. (B) Quantification of galectin-1 enhancement of recombinant GLuc reporter NiV (rNiV-GLuc) infection of HUVECs. (B) rNiV-GLuc was added to monolayers of HUVECs in the absence (white bar) or the presence (black bars) of the indicated amounts of galectin-1 for 1 h, and infection was quantified by measuring the *Gaussia* luciferase activity. The data are presented as mean fold increases ($\pm$ the SD of triplicate samples) in infection over the virus-only (no galectin-1) condition. The results of one of three replicate experiments are shown. (C) Galectin-1-mediated enhancement of rNiV-GLuc infection is dependent upon complex N-glycans on the surface of HUVECs and on the surface of the virus. Virus or HUVECs deficient in complex N-glycans were made in the presence of 20 μ M kifunensine (Virus Kif+ and Cells Kif+, respectively). rNiV-GLuc infection in the absence or presence of increasing concentrations of galectin-1 is shown for wild-type rNiV-GLuc and HUVECs (Virus wt/Cells wt, black bars), wild-type rNiV-GLuc and complex N-glycan-deficient HUVECs (Virus wt/Cells Kif+, dark gray bars), complex N-glycan-deficient rNiV-GLuc and wild-type HUVECs (Virus Kif+/Cells wt, light gray bars), and complex N-glycan-deficient rNiV-GLuc and HUVECs (Virus Kif+/Cells Kif+, white bars). The data represent the average fold increases in infection determined as in panel B and are presented as means $\pm$ the SD of triplicate samples from one of three replicate experiments.

enhancement of virus entry, as well as fusogenicity (11, 27). However, the galectin-1-mediated increase was further reduced ($\sim$ 95% reduction) when cells were infected with virus lacking complex N-glycans (Fig. 2B, complex N-glycan-less NiVpp), indicating that other complex N-glycans on NiV-F or NiV-G also contributed to the galectin-1 enhancement effect.

To examine the role of host cell complex N-glycans in galectin-1-mediated enhancement of viral infection, we treated uninfected Vero cells with kifunensine to reduce complex N-glycans on the target cells. A reduction of complex N-glycans was confirmed by flow cytometry with L-PHA, as described above (Fig. 2C). A >10 -fold reduction in complex N-glycans from target Vero cells reduced galectin-1-mediated enhancement of infection by ca. 30% (Fig. 2D). Thus, complex N-glycans on host cell glycoproteins participate in the galectin-1 enhancement of viral infection; however, since 10-fold reduction of complex N-glycans on kifunensine-treated host cells partially reduced the galectin-1 enhancement effect, other host cell glycans, likely O-glycans, may also play a role on the host cell side (32). Collectively, the data in

Fig. 2 demonstrate that glycans on both the viral envelope and on the target cell surface are important for galectin-1 enhancement. Our results support a model in which the enhancement effect results from dimeric galectin-1 acting as a bridge or bivalent attachment factor between the virus and the target cell, with complex N-glycans contributing to attachment on both the virus and the host cell.

Galectin-1 enhances Nipah virus infection of human endothelial cells. Endothelial cells are primary targets of NiV infection *in vivo*, due to expression of the viral attachment receptor ephrinB2 (4). To determine whether galectin-1 would enhance Nipah virus infection of physiologically relevant target cells, we infected primary HUVECs with NiVpp (Fig. 3A) in the presence or absence of exogenous galectin-1. The addition of galectin-1 resulted in a 6-fold increase in infection of HUVECs, similar to the effect we had observed with Vero cells as targets of infection.

Thus far, we used NiVpp, rather than replication-competent, wild-type infectious NiV. NiVpp is a VSV-based pseudotyped virus capable only of single cycle of infection, and the bullet-shaped

VSV-based particles may not fully recapitulate the morphology or envelope glycoprotein densities present on native pleomorphic paramyxovirus particles (33). To determine whether galectin-1 modulates infection by replication competent NiV, we first generated and rescued, under BSL4 conditions, a recombinant NiV expressing secreted *Gaussia* luciferase (rNiV-GLuc; see Materials and Methods). To facilitate the generation of additional recombinant NiVs such as the one bearing the F3 N-glycan, we also developed an efficient and robust reverse genetics system for henipaviruses (28).

This rNiV-GLuc replicated to the same endpoint titers as the parental NiV Malaysia strain (data not shown) and was pathogenic in a lethal hamster challenge model (34); using rNiV-GLuc allowed us to accurately quantify enhancement or inhibition of live virus entry by sampling the infected cell culture supernatant for GLuc activity. We first sought to determine whether rNiV-GLuc would also exhibit enhanced infection of endothelial cells in the presence of galectin-1. HUVECs infected at an MOI of 0.05 with rNiV-GLuc showed a dose-dependent enhancement of infection in the presence of galectin-1 at 24 hpi. The enhancement effect plateaued at 7 to 10 μ M galectin-1, with a 6-fold increase in infection compared to controls lacking exogenous galectin-1 (Fig. 3B). This level of enhancement was similar to that observed for NiVpp in HUVECs, albeit in the presence of 20 μ M galectin-1.

Next, we reasoned that, if galectin-1 was bridging virions to the cell surface, as suggested by the data in Fig. 2, then the relative level of enhancement should be increased when the amount of viral inoculum was decreased. That is, when the number of infectious viral particles is more limiting, the effect of galectin-1 on bridging viruses to the cell surface should become more apparent. Thus, we reduced the viral inoculum by 5-fold and quantified the corresponding effect of galectin-1 on virus infection. Indeed, at an MOI of 0.01, 10 μ M galectin-1 enhanced rNiV-GLuc infection by up to 40-fold (Fig. 3C, black bars) compared to the 6-fold enhancement seen with an equivalent dose of galectin-1 when a higher viral inoculum (MOI of 0.05) was used (Fig. 3B).

Galectin-1 enhances rNiV-GLuc virus infection by bridging complex N-Glycans. To confirm that galectin-1 enhances rNiV-GLuc infection by bridging complex N-glycans present on virions and the endothelial cell surface, as we had seen in infection of Vero cells with NiVpp (Fig. 2), we generated either HUVECs or viruses deficient in complex N-glycans by treating HUVECs with kifunensine (denoted by “Cells Kif+” in Fig. 3) or growing rNiV-GLuc in the presence of kifunensine (Virus Kif+). Complex N-glycanless Nipah virus (Virus Kif+) was equally infectious when titers were determined on Vero cells (data not shown). As shown in Fig. 3C, depletion of complex N-glycans on target cells (Cells Kif+) moderately reduced galectin-1-enhanced rNiV-GLuc infection of HUVECs (black bars versus dark gray bars), as we had seen with Vero cells. Depleting virus of complex N-glycans (Virus Kif+) also reduced galectin-1 enhancement of infection (light gray bars) more significantly, as we had seen with NiVpp. When both virus and HUVECs lack complex N-glycans (Virus Kif+/Cells Kif+), galectin-1 had the least enhancement effect (white bars). *In toto*, our data reveal that galectin-1 enhanced infection of both rNiV-GLuc and NiVpp by the same mechanism, i.e., the interaction between galectin-1 and Nipah virus results primarily from galectin-1 binding to complex N-glycans on NiV envelope glycoproteins, and that enhancement of infection involves galectin-1-mediated bridging of viral glycans to glycans on the host cell

surface. The remaining degree of galectin-1-mediated enhancement seen with kifunensine treated cells and virus suggest that lactosamine residues on O-linked glycans might also serve as bridging receptors for galectin-1's infection enhancement effect. However, the role of these putative O-glycans may only be unmasked when cognate complex N-glycans are limiting.

Timing of galectin-1 exposure has opposing effects in NiV replication. Previous work in our labs has shown that galectin-1 inhibited syncytium formation mediated by NiV-F+G expressed by endothelial cells (11, 24). Galectin-1 reduced NiV-F mediated fusion of endothelial cells by retarding NiV-F maturation, reducing the lateral mobility of NiV-F in the plasma membrane, and inhibiting triggering of NiV-F that results in fusion of the virus with the host cell plasma membrane. Moreover, endogenous galectin-1 produced by endothelial cells was sufficient to inhibit syncytium formation (11). Importantly, all of the galectin-1-mediated effects that we had previously analyzed would occur following NiV infection and was studied only in a cell-cell fusion context. Thus, while the data presented demonstrate that galectin-1 enhances viral entry when present at initial infection, we sought to determine whether downstream events, i.e., syncytium formation and viral replication in human endothelial cells infected with NiV_{MAL}, were affected by the presence of galectin-1.

To examine the effect on viral replication, HUVECs infected with the NiV strain Malaysia (NiV_{MAL}) were incubated postinfection with galectin-1 or buffer control. The presence of exogenous galectin-1 postinfection at early time points (12 and 24 hpi) inhibited virus replication titers below the limit of detection, effectively reducing virus titers by >3 logs at 24 hpi. This inhibitory effect of galectin-1 persisted at 36 hpi as a 15-fold reduction in virus titers (Fig. 4A).

To examine the effect of galectin-1 on syncytium formation, wild-type NiV_{MAL}-infected HUVECs were analyzed microscopically in three conditions: no exogenous galectin-1 added, galectin-1 added before infection, or galectin-1 added postinfection. The addition of exogenous galectin-1 before infection enhanced syncytium formation (Fig. 4B, panel 2), a finding consistent with the effect of galectin-1 on increasing NiV infection of endothelial cells (Fig. 3). In contrast, the addition of exogenous galectin-1 after NiV infection essentially abrogated syncytium formation (Fig. 4B, panel 3). This inhibitory effect of galectin-1 on endothelial cell fusion (quantified in Fig. 4C) is consistent with our previous observation using pseudotyped virus (11).

To determine whether the NiV-F3 N-glycan contributes to the differential effect of galectin-1 on syncytium formation in the context of live virus infection, we generated and rescued a rNiV-GLuc bearing the NiV-F3 N-glycan mutant. Infection with the rNiV-GLuc F3 N-glycan mutant virus resulted in an overall increase in syncytium formation, compared to wild-type NiV_{MAL}, a finding consistent with the hyperfusogenic phenotype previously observed for the NiV-F3 mutant (27). However, the addition of galectin-1 before infection no longer enhanced syncytium formation, in contrast to the enhancement seen with the wild-type virus in the presence of galectin-1 (Fig. 4C). Galectin-1 was still able to inhibit syncytium formation by the mutant virus when added after infection, although the lack of N-glycans at the F3 site made the mutant virus more resistant to the inhibitory effect of galectin-1 (Fig. 4C).

Collectively, our data indicate that the timing of galectin-1 exposure differentially modulates NiV entry, syncytium formation,

FIG 4 Galectin-1 can have opposing effects on Nipah virus production and syncytium formation. (A) Effect of galectin-1 added postinfection on the replicative spread of NiV. HUVECs were infected with NiV_{MAL} for 1 h at 37°C and washed to remove excess virus, and 20 μM galectin-1 (black bars) or buffer (virus only, white bars) was added to the medium only postinfection. Virus titers (log scale) were quantified by plaque assay after 0, 12, 24, and 36 hpi. (B) Syncytium formation induced by NiV infection. HUVECs infected with live NiV_{MAL} or NiV_{MAL} F3 mutant virus in the absence (no galectin-1), presence of galectin-1 added before infection, or presence of galectin-1 added after infection. Cells were fixed at 24 hpi and stained with DAPI to reveal nuclei. (C) Quantitation of Fig. 4C. Nuclei per syncytium per field were enumerated for the three separate conditions.

and replicative spread in endothelial cells. Galectin-1 present at the attachment/entry step of viral infection enhanced NiV entry into endothelial cells and also enhanced syncytium formation, likely due to increased virus envelope production in initially infected cells. In contrast, galectin-1 added after NiV infection of endothelial cells inhibited both syncytium formation and the replicative spread of progeny virus such that virus titers at 24 and 36 hpi lagged by at least 4 logs and 1 log, respectively.

DISCUSSION

Innate immune mediators such as galectins have many functions, including triggering endothelial activation, inducing cytokine production and release, promoting leukocyte infiltration of infected tissues, and mediating pathogen clearance (10, 14, 16, 35, 36). Since galectin-1 can recognize both self (mammalian) and nonself (pathogen) glycans, depending on factors such as glycan ligand density and presentation (37), galectin-1 has been proposed to be both a damage-associated molecular pattern that is released from injured cells, and a pattern recognition receptor, recognizing pathogen associated molecular patterns, such as specific viral glycans (11). Thus, galectin-1 can have dual functions in innate immunity (38). Our current data also demonstrate that galectin-1 has complex and dichotomous roles in NiV infection. Galectin-1 enhanced initial NiV attachment to and entry into hu-

man endothelial target cells (Fig. 3), while subsequently inhibiting cell syncytium formation (Fig. 4) (11, 24), a key sequel of viral replicative spread. The ability of galectin-1 to produce these divergent effects depends on when it is present during the viral replicative life cycle.

There are several examples of galectin-1 having complex or opposing roles in different viral infections, via binding to glycan ligands on both the virus and the target cell. Galectin-1 bound to influenza virus reduced virus entry into host cells; moreover, intranasal administration of galectin-1 enhanced survival of mice infected with a lethal dose of influenza virus, and galectin-1-null mice were more susceptible to influenza virus infection than were wild-type mice (39). In contrast, galectin-1 increased HIV-1 infection of monocyte-derived macrophages through stabilization of virus attachment and adsorption (20, 21). That galectin-1 stabilized HIV adsorption to macrophages suggests that dimeric galectin-1 can act as a bridge between virus glycans and host cell surface glycans, although this was not directly demonstrated in those studies.

Here we examined whether galectin-1 formed a bridge between viral particles and host cells to enhance infection. The enhancement effect was clearly glycan dependent, since the specific inhibitor lactose, but not the noncognate disaccharide sucrose,

FIG 5 Modeling of the glycosylated prefusion trimeric NiV-F spike and galectin-1. (A) A model of the glycosylated NiV-F ectodomain in the prefusion state was created with the Swiss-Model server (52) using the structure of the parainfluenza virus 5 F protein in the metastable, prefusion conformation (PDB accession number 2B9B) (53) as a template. The model of the NiV-F ectodomain is shown in the surface representation, with each protomer colored a different shade of gray. For each protomer, structures of complex-type glycans (54) were placed at N-linked glycosylation sites F2-F4 and oligomannose-type glycans (55) were placed at F5. Glycans at sites F2 and F4 are pink, F3 are red, and at F5 are green. Residues corresponding to the putative fusion peptide (residues 103 to 128) are colored blue. The distance between equivalent F3 glycans is ca. 100 Å. (B) Homodimeric crystal structure of C2S human galectin-1 in complex with lactose (PDB accession number 1W60) (56). Galectin-1 is shown as a surface representation with the two protomers colored different shades of gray. β -Lactose is bound to both protomers and is shown as pink sticks. The distance between equivalent binding sites is ca. 50 Å. Structures and models were rendered with PyMOL (www.pymol.org).

blocked the enhancement of viral infection (Fig. 1B). To specifically address the step at which galectin-1 binding promoted infection, we bound virus to host cells at 4°C and initiated viral entry at 37°C (Fig. 1C). Galectin-1 added only during the attachment step at 4°C enhanced infection, while galectin-1 added after viral attachment, but during fusion at 37°C, did not enhance infection. These data support a model whereby galectin-1 enhancement occurs as a result of bridging glycoproteins on the virus to glycoproteins on the host cell. To determine whether the essential glycans recognized by galectin-1 were on the virus, the host cell, or both, we eliminated complex N-glycans on viral particles and on the surface of the target cells by treatment with the α -mannosidase I inhibitor kifunensine. Complex N-glycans on viral glycoproteins were clearly required for galectin-1 enhancement of infection (Fig. 2B and 3C). We have previously demonstrated that galectin-1 specifically interacts with the F3 N-glycan on the NiV-F glycoprotein and that the F3 N-glycan site is occupied by complex N-glycans that bears cognate ligands for galectin-1 (11, 27). In the present report, we show that this F3 glycan was the most significant viral determinant of galectin-1 mediated enhancement of infection (Fig. 2B and 4C). On the target cells, both complex N-glycans and O-glycans appear to contribute to galectin-1 enhancement of infection (Fig. 2D and 3C) (40). Although several endothelial cell surface glycoprotein receptors for galectin-1 have been identified (41), the entire repertoire of cell surface glycoproteins that could bind to galectin-1 to facilitate viral “bridging” and increase virus attachment is not known. In addition, little is known about the N- or O-glycan composition of the Nipah cellular receptor ephrinB2 (42) and whether galectin-1 can bind to ephrinB2 on the surface of endothelial cells.

Lectin-glycan interactions play complex roles in virus-host pathogenesis, and other types of innate immune lectins have apparently dichotomous roles in viral pathogenesis. For example, mannan-binding lectin, a soluble innate immune lectin, binds to influenza A virus and leads directly to virus inactivation (43). In contrast, another mannose-binding human lectin, macrophage mannose receptor (MMR) potentiates HIV binding to target cells (44–46), and MMR can also potentiate infection of macrophages by influenza virus (47), so that different mannose-binding lectins

can bind to viruses to either reduce or potentiate infection. Considering the long-standing evolutionary relationship between virus and host, it is not entirely surprising that viruses have learned to co-opt host innate immune defenses for increased target cell entry and replication.

In the present study, we demonstrate that galectin-1 enhanced the infection of endothelial cells by live NiV (Fig. 3C); since inflammation is known to increase endothelial cell production of galectin-1 (11), we speculate that, *in vivo*, galectin-1 produced by endothelial cells may increase target cell infection by increasing viral attachment to host target cells. However, galectin-1 can also inhibit syncytium formation among endothelial cells infected with NiV *in vitro* (Fig. 4). This effect may limit the extent or severity of NiV pathogenicity *in vivo*, potentially reducing endothelial cell damage and the resulting hemorrhagic diathesis. Moreover, the opposing effects of increased viral entry by facilitating viral attachment without affecting viral fusion with the endothelial cell membrane versus inhibition of membrane fusion during syncytium formation of infected cells suggests that these two types of fusion events are not identical.

It is remarkable that complex N-glycans on a specific site in NiV-F (F3) can account for the majority of galectin-1's effect. To gain further insight into how galectin-1 binding to F3 N-glycan mediates its dichotomous role in NiV infection and spread, we modeled a trimeric spike with the cognate N-glycans at the relevant sites. Figure 5A shows that the F3 N-glycan (red) is positioned right “above” the fusion peptide (blue), and proximal to the putative target cell membrane. The distance between glycan binding sites on the dimeric galectin-1 is ~ 50 Å (Fig. 5B), which is too short to span the F3 N-glycans between any two F protomers on the same trimeric NiV-F spike. Thus, galectin-1 likely binds to the F3 N-glycans on NiV-F protomers from distinct F spikes, forming a glycoprotein lattice that blocks the triggering of the fusion peptide (Fig. 5A, legend). We posit that glycoprotein lattice formation predominates when galectin-1 binds to NiV-F expressed on infected cells, thereby inhibiting syncytium formation (11). However, on the virion surface that is tightly packed with envelope glycoproteins, glycoprotein lattice formation may be less efficient, and the ability of galectin-1 to bridge virus predominates.

The latter can lead to an enhancement of infection when receptor binding triggers fusion by the other virion-associated F spikes that are not bound by galectin-1.

As mentioned above, although endothelial cells express abundant galectins, inflammation in general and viral infection specifically are known to significantly increase endothelial cell expression of several galectins, including galectin-1. Moreover, since secreted galectins bind back to glycoproteins on the plasma membrane, as well as to glycans on extracellular matrix glycoproteins, galectins can be highly concentrated at the cell surface. Thus, the galectin-1 concentration used in these studies may reflect the physiologic concentration of galectin-1 encountered by a virus at the endothelial cell membrane (48–51).

The present study further characterizes the complex interaction between galectin-1 and NiV. Although other lectins have been shown to have effects both beneficial and detrimental to the host in microbial infections (20, 21, 36, 39), we report these opposing effects for a single virus, NiV, and a single lectin, galectin-1, in the same host cell. These findings may help to explain the range of clinical outcomes of NiV infection, since some patients recover fully after infection, while other patients succumb. Perhaps differential expression of galectin-1 in different individuals at different time points during infection either promotes virus-endothelial cell interactions or prevents endothelial cell damage. Our findings suggest a complex spatiotemporal interplay between an immune regulator and a virus that may ultimately determine the outcome of infection.

ACKNOWLEDGMENTS

This study was supported by National Institutes of Health (NIH) grant R01AI060694 (to L.G.B. and B.L.), Ruth L. Kirschstein National Research Service award T32HL69766 (to O.B.G.), NIH grant R01AI109022 (to H.C.A.), and Medical Research Council grant MR/L009528/1 (to T.A.B.). A.N.F. acknowledges partial support by a grant from the NIAID/NIH through the Western Regional Center of Excellence for Biodefense and Emerging Infectious Disease Research (U54 AI057156).

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