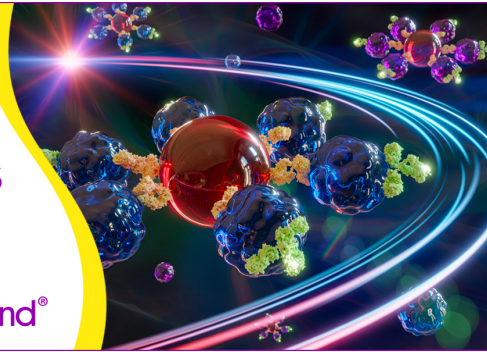


Advance allergy and  
asthma research by  
analyzing multiple cytokines  
with LEGENDplex™

Start multiplexing ▶

BioLegend®



## The Journal of Immunology

RESEARCH ARTICLE | JULY 01 2005

### Novel Innate Immune Functions for Galectin-1: Galectin-1 Inhibits Cell Fusion by Nipah Virus Envelope Glycoproteins and Augments Dendritic Cell Secretion of Proinflammatory Cytokines<sup>1</sup> **FREE**

Ernest L. Levrony; ... et. al

*J Immunol* (2005) 175 (1): 413–420.

<https://doi.org/10.4049/jimmunol.175.1.413>

#### Related Content

A novel mechanism for Galectin-1 inhibition of endothelial cell fusion in Nipah virus infection; a critical component of vascular mediated innate immunity and host defense (128.3)

*J Immunol* (April,2009)

The highly pathogenic Ebola and Nipah viruses hijack the host antiviral E3-ubiquitin ligase TRIM6 to enhance their own replication

*J Immunol* (May,2017)

The host ubiquitin system in innate immunity and virus replication: proviral and antiviral functions of the host E3-ubiquitin ligase TRIM family

*J Immunol* (May,2018)

# Novel Innate Immune Functions for Galectin-1: Galectin-1 Inhibits Cell Fusion by Nipah Virus Envelope Glycoproteins and Augments Dendritic Cell Secretion of Proinflammatory Cytokines<sup>1</sup>

Ernest L. Levroney,<sup>2\*</sup> Hector C. Aguilar,<sup>2\*</sup> Jennifer A. Fulcher,<sup>†</sup> Luciana Kohatsu,<sup>†</sup> Karen E. Pace,<sup>†</sup> Mabel Pang,<sup>†</sup> Kevin B. Gurney,<sup>\*</sup> Linda G. Baum,<sup>3†</sup> and Benhur Lee<sup>3\*†</sup>

**Galectin-1 (gal-1), an endogenous lectin secreted by a variety of cell types, has pleiotropic immunomodulatory functions, including regulation of lymphocyte survival and cytokine secretion in autoimmune, transplant disease, and parasitic infection models. However, the role of gal-1 in viral infections is unknown. Nipah virus (NiV) is an emerging pathogen that causes severe, often fatal, febrile encephalitis. The primary targets of NiV are endothelial cells. NiV infection of endothelial cells results in cell-cell fusion and syncytia formation triggered by the fusion (F) and attachment (G) envelope glycoproteins of NiV that bear glycan structures recognized by gal-1. In the present study, we report that NiV envelope-mediated cell-cell fusion is blocked by gal-1. This inhibition is specific to the Paramyxoviridae family because gal-1 did not inhibit fusion triggered by envelope glycoproteins of other viruses, including two retroviruses and a pox virus, but inhibited fusion triggered by envelope glycoproteins of the related Hendra virus and another paramyxovirus. The physiologic dimeric form of gal-1 is required for fusion inhibition because a monomeric gal-1 mutant had no inhibitory effect on cell fusion. gal-1 binds to specific *N*-glycans on NiV glycoproteins and aberrantly oligomerizes NiV-F and NiV-G, indicating a mechanism for fusion inhibition. gal-1 also increases dendritic cell production of proinflammatory cytokines such as IL-6, known to be protective in the setting of other viral diseases such as Ebola infections. Thus, gal-1 may have direct antiviral effects and may also augment the innate immune response against this emerging pathogen. *The Journal of Immunology*, 2005, 175: 413–420.**

**E**merging viral pathogens pose a threat to global health and the global economy. Nipah virus (NiV)<sup>4</sup> emerged in a 1999 outbreak among livestock workers in Malaysia and Singapore, resulting in 40% mortality among infected patients. In humans, NiV causes a severe encephalitic syndrome, with an average time of 9–10 days from fever onset to death (1). Livestock workers appeared to contract the virus from infected pigs; although NiV infection in animals causes respiratory illness and is not fatal, the transmission rate in animals approaches 100%. Thus, the threat to livestock workers (2) resulted in destruction of livestock worth more than \$100 million in the 1999 outbreaks (3). From 2001 to

2004, NiV outbreaks occurred in Bangladesh (4); in Bangladesh, pigs were not found to be infected, suggesting that the virus may have jumped directly from its natural host, the flying fox (genus *Pteropus*) (5), to humans. In more recent NiV outbreaks, mortality rates increased (up to 74% in Bangladesh) (4), similar to or exceeding those seen with other viruses such as Ebola (6). Thus, NiV has potential to be an agent of human and economic bioterrorism (3). In addition, recent outbreaks of viral diseases such as severe acute respiratory syndrome and avian influenza highlight the need for new antiviral strategies that exploit innate immunity (7, 8).

In humans, NiV infects endothelial cells, particularly in the CNS, lung, kidney, and heart. The pathognomonic feature of NiV infection is endothelial cell syncytia formation (1), resulting in endothelial cell destruction, inflammation, and hemorrhage. Coexpression of codon-optimized NiV-F and NiV-G alone results in syncytia formation of permissive cells; furthermore, we found that specific *N*-glycans in NiV-F and NiV-G are critical to the fusion process (H. C. Aguilar et al., manuscript in preparation).

Glycosylation of envelope glycoproteins of HIV, influenza, West Nile, and Ebola viruses regulates both fusogenicity of the envelope glycoproteins and viral pathogenicity (9, 10). Moreover, direct interaction of *N*-glycans on viral glycoproteins with cellular receptors has been implicated in Dengue virus entry into cells (11). The viral glycoproteins are recognized by endogenous lectins or carbohydrate-binding proteins, such as dendritic cell (DC)-specific ICAM-3 grabbing nonintegrin, that are expressed on the surface of the target cells and that mediate viral entry or viral transmission to other cell types (12).

Galectin-1 (gal-1) is a member of a family of lectins with a variety of immunomodulatory functions. gal-1 regulates pre-B cell

\*Department of Microbiology, Immunology, and Molecular Genetics, and <sup>†</sup>Department of Pathology and Laboratory Medicine, David Geffen School of Medicine at University of California, Los Angeles, CA 90095

Received for publication October 25, 2004. Accepted for publication April 12, 2005.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

<sup>1</sup> This work is supported by National Institutes of Health Grants AI059051 (to B.L.), GM63281 (to L.G.B.), AI06094 (to B.L. and L.G.B.), AI07323 (to L.K.), and AI61824 (to E.L.L.). B.L. is a Charles E. Culpepper Medical Scholar supported by the Rockefeller Brothers Fund and a recipient of the Burroughs Wellcome Fund Career Development Award.

<sup>2</sup> E.L.L. and H.C.A. contributed equally to this work.

<sup>3</sup> Address correspondence and reprint requests to Dr. Benhur Lee or Dr. Linda G. Baum, Department of Microbiology, Immunology, and Molecular Genetics, 3825 MSB, 609 Charles E. Young Drive East, University of California, Los Angeles, CA 90095. E-mail addresses: bleebl@ucla.edu or lbaum@mednet.ucla.edu

<sup>4</sup> Abbreviations used in this paper: NiV, Nipah virus; DC, dendritic cell; gal-1, galectin-1; wt, wild type; PNA, peanut lectin agglutinin; DMNJ, deoxymannojirimycin; MDDC, monocyte-derived DC; BS<sup>3</sup>, bis(sulfosuccinimidyl) suberate; sulfo-GMBS, *N*-( $\gamma$ -maleimidobutyryloxy)sulfosuccinimide ester; GNV, *Galanthus nivalis*; GnT V, *N*-acetylglucosaminyltransferase V; PHA-E, *Phaseolus vulgaris*.

development and thymocyte and T cell survival (13–17). Administration of gal-1 in animal models of autoimmune and transplant-related disease results in altered T cell cytokine production (18–21). However, the effects of gal-1 on innate immune function are not well understood, and no direct antimicrobial effects of gal-1 have been previously described. As both NiV-F and NiV-G bear complex *N*-glycans that can be recognized by the galectin family of mammalian lectins, we examined the effect of gal-1 on NiV-F/G-mediated cell fusion.

## Materials and Methods

### *NiV and HeV codon optimization and expression plasmids*

Codon optimization of *F* and *G* genes from NiV and HeV was performed by Geneart according to an in-house proprietary software that addresses codon usage, elimination of cryptic splice sites, as well as the stability of DNA/RNA secondary structures. The codon-optimized NiV and HeV *F* and *G* genes were synthesized chemically and subcloned into the pcDNA3.1 mammalian expression vector (Invitrogen Life Technologies). The sequences of the codon-optimized genes have been deposited into GenBank (*pcNiV-Fopt*, accession no. AY816748; *pcNiV-Gopt*, accession no. AY816746). The codon-optimized NiV-*F* and *G* were tagged at their C terminus with an AU1 sequence (DTYRYI).

### *Lectin-binding assay*

293T cells were transfected with codon-optimized, AU1-tagged NiV-*F* or NiV-*G*. Biotinylated lectins (Vector Laboratories) specific for the indicated glycans were added to NiV-F or NiV-G cell lysates. Lectin-bound NiV-F or NiV-G was precipitated with streptavidin beads and subsequently detected by immunoblotting with anti-AU1 mAb and <sup>125</sup>I-labeled protein A. The amount of lectin-precipitated protein was quantified by densitometry using a PhosphorImager (445SI; Molecular Dynamics) and ImageQuant (version 5.2).

### *Fusion assay*

Fusogenicity of wild-type (wt) or mutant NiV-F or NiV-G envelope glycoproteins was determined by transfecting 2 μg of a 1:15 ratio of NiV-*F:G* expression plasmids into Vero cells (6-well plates) and culturing for 16 h ( $n \geq 4$  for each mutant). After 4',6'-diamidino-2-phenylindole staining, nuclei inside syncytia per ×100 field were counted by fluorescence microscopy (at least 10 fields/condition). Syncytia were defined as four or more nuclei within a common cell membrane. The fusogenic index was defined by normalizing the number of nuclei in syncytia per field formed by the specified experimental condition or the various *N*-glycan mutants to that formed by the wt NiV-F and NiV-G proteins, which was set at 100%. The buffer control includes DTT; as gal-1 has unpaired cysteines in the binding pocket that, in the absence of saccharide ligand, can form intramolecular disulfide bonds and prevent saccharide ligand binding, we routinely store and prepare gal-1 in a buffer with DTT, as described in the original gal-1 purification (22). As gal-1 forms noncovalent dimers, reducing agents have no effect on gal-1 dimerization. Buffers with this concentration of DTT have no effect on fusion in the assays.

### *Recombinant dimeric and monomeric gal-1*

Recombinant human gal-1 was made exactly as described in Ref. 23. Monomeric gal-1 is the *N*-Gal-1 mutant (24) and was made as previously described, except that a 3 × 7-cm lactosyl-Sepharose affinity column was used to isolate the recombinant gal-1.

### *Production of N-glycan mutants*

There are five potential *N*-linked glycosylation sites in NiV-F. The *N*-glycan consensus sequence, NXS/T, where X is any amino acid except for proline, was altered specifically to produce F1 to F5 glycan mutants. In each case the arginine (N) was mutated conservatively to a glutamine (Q) by changing the AAT/AAC codons to CAG using the QuickChange Site-Directed Mutagenesis kit (Stratagene), according to the manufacturer's directions. Each mutant was sequenced in its entirety to confirm sequence fidelity.

### *Oligomerization of NiV envelope*

Increasing concentrations of gal-1 or *N*-Gal-1 were added to 293T cells expressing NiV-F or NiV-G for 30 min. Excess gal-1 was removed by washing with PBS, and the indicated amounts of the membrane imper-

meant cross-linkers (bis(sulfosuccinimidyl) suberate (BS<sup>3</sup>) for NiV-F and *N*-(γ-maleimidobutyryloxy)sulfosuccinimide ester (sulfo-GMBS) for NiV-G (Pierce)) were added to the cells. The reactions were quenched according to the manufacturer's directions, and cells were lysed in 1% Triton X-100. Lysates were separated by SDS-PAGE and NiV-F or -G detected by immunoblotting with monoclonal anti-AU1. Proteins were visualized and quantified by radiometric densitometry using <sup>125</sup>I-labeled protein G to detect the amount of bound anti-AU1.

### *Binding of NiV-F/G to gal-1*

Membrane proteins were isolated from 1 × 10<sup>7</sup> 293T cells expressing NiV-F or NiV-G (25). Isolated membrane proteins were solubilized in 0.1% Triton X-100 and applied to a gal-1 affinity column (22). After washing with wash buffer (PBS, 0.1% Triton X-100, and 0.02% Na<sub>3</sub>S), bound proteins were eluted with 0.1 M β-lactose or 0.1 M sucrose in wash buffer. Samples were resolved by 10% SDS-PAGE and blotted with anti-AU1 mAb. Proteins were visualized using ECL (Amersham Biosciences).

### *Coimmunoprecipitation of gal-1 and NiV-F*

To coprecipitate NiV-F and gal-1, 20 μM gal-1 was first added to 293T cells expressing equivalent amounts of F or F3 Env proteins for 1 h at 37°C. Cells were washed twice with PBS, lysed, and lysates immunoprecipitated with rabbit anti-gal-1 antiserum (1:100 in 10 mM NaPO<sub>4</sub>, 150 mM NaCl, 1% Triton X-100, 0.1% SDS, 0.5% sodium deoxycholate, and 1× Complete protease inhibitor mix (Roche)). Precipitated proteins were captured on protein G beads, washed three times with wash buffer 1 (100 mM Tris-HCl, 150 mM NaCl, 2 mM EDTA, and 0.2% Nonidet P-40), and three times with wash buffer 2 (100 mM Tris-HCl, 500 mM NaCl, 2 mM EDTA, and 0.2% Nonidet P-40). Bound material was separated by SDS-PAGE, and precipitated NiV-F was detected with an anti-AU1 mAb.

### *gal-1 stimulation of DCs*

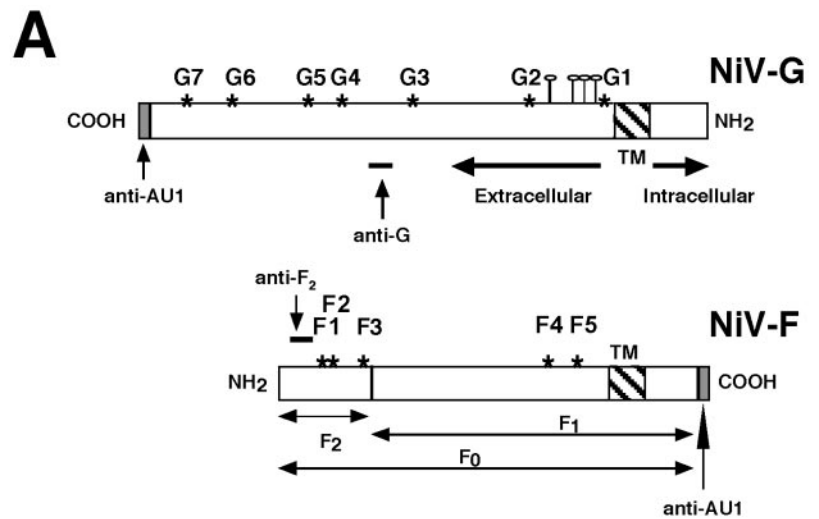
Monocytes were cultured for 5 days with IL-4 (100 ng/ml) and GM-CSF (50 ng/ml) in RPMI 1640 medium. At day 5, recombinant gal-1 (20 μM) or *N*-Gal-1 (20 μM) was added, and cells were cultured for an additional 48 h. In one well, 100 mM lactose were added 20 min before addition of recombinant gal-1. To control for potential endotoxin contamination in the gal-1 or *N*-Gal-1 stocks, all cells were preincubated with 10 μg/ml polymyxin B at 37°C for 30 min (26). Supernatant was collected after 48 h; IL-6 and TNF-α levels were measured with the cytometric bead array human inflammation kit (BD Biosciences). Values shown are averages from several independent experiments.

## Results

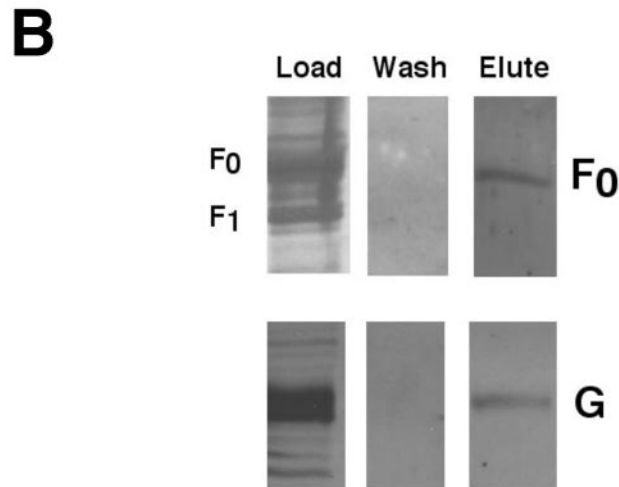
### *Glycosylation screen of NiV-F and NiV-G suggests the presence of gal-1 binding sites*

NiV-F and NiV-G are glycosylated heavily. NiV-F and NiV-G have five and seven *N*-linked glycosylation sites, respectively (Fig. 1), but the structures of the *N*-glycans at those sites are not known, nor is it known if NiV-F or NiV-G bear *O*-glycans, although potential sites of *O*-glycosylation have been identified in NiV-G (Fig. 1). We used a semiquantitative lectin-binding assay to characterize broad classes of glycans on NiV-F and NiV-G (Table I). To identify *O*-glycans, we used peanut lectin agglutinin (PNA), which recognizes the Galβ(1,3)GalNAc sequence on core 1 or core 2 *O*-glycans; the presence of PNA binding indicates that *O*-glycans are present, whereas the absence of PNA binding can indicate lack of *O*-glycans or capping of the Galβ(1,3)GalNAc sequence by sialic acid (27). As shown in Table I, NiV-G displayed greater PNA binding compared with NiV-F, indicating that NiV-G has more asialo *O*-glycans than NiV-F.

*N*-glycans can be high mannose, complex, or hybrid structures. Terminal mannose residues on high mannose or hybrid structures are recognized by the *Galanthus nivalis* (GNV) lectin, and both NiV-G and NiV-F bound GNV, although to different extents. Complex or hybrid structures can be modified by the *N*-acetylglucosaminyltransferase V (GnT V) enzyme to create the β(1,6)GlcNAc branch; this branch can be elongated with a poly-lactosamine sequence (28). The β(1,6)GlcNAc branch is recognized by the *Phaseolus vulgaris* (PHA-E) lectin (29). Both NiV-F and NiV-G



**FIGURE 1.** Schematic of NiV-G and NiV-F envelope glycoproteins. *A*, NiV-G is a type II transmembrane protein. NiV-F ( $F_0$ ) is a type I transmembrane protein cleaved into  $F_1$  and  $F_2$  by a host cell protease. \*, Potential *N*-linked glycosylation sites,  $F_1$  to  $F_5$  and  $G_1$  to  $G_7$ .  $\varphi$ , Potential NiV-G *O*-linked glycosylation sites. Epitopes of NiV-F and NiV-G antipeptide Abs are indicated. Codon-optimized NiV-G and NiV-F were AU1 epitope tagged at the indicated termini. *B*, NiV-F and NiV-G bind gal-1. Extracts of cell membranes from 293T cells expressing either NiV-F or NiV-G were applied to a gal-1 affinity column. Bound glycoproteins were eluted with  $\beta$ -lactose and detected with anti-AU1 mAb. No glycoproteins were eluted with sucrose wash.



bound PHA-E, demonstrating that both NiV-F and NiV-G bear processed *N*-glycans. Importantly, as the poly-lactosamine sequences preferentially added to the GnT V branch are recognized by gal-1 (30, 31), this screen suggested that both NiV-G and NiV-F could bind gal-1.

Thus, we sought to determine whether gal-1 could bind directly to NiV-F or NiV-G. The envelope glycoproteins were expressed in 293T cells, cell membrane extracts were applied to a gal-1 affinity matrix, and bound glycoproteins were eluted with lactose. Fig. 1*B* shows that both NiV-G and NiV-F bound to gal-1.

#### gal-1 inhibits envelope-mediated fusion

Endothelial cells express several galectins that recognize cell surface saccharides on bacteria, fungi, and parasites (19, 32, 33). As NiV-F and NiV-G bear complex *N*-glycans that could be recognized by gal-1 (Table I) and endothelial cells are a primary target for NiV infection, we asked whether gal-1 would affect syncytia formation mediated by NiV-F and NiV-G. Because endothelial cell syncytia is a pathognomonic feature of NiV infection (1), we reasoned that a syncytia formation assay would be relevant to test the antiviral activity of gal-1.

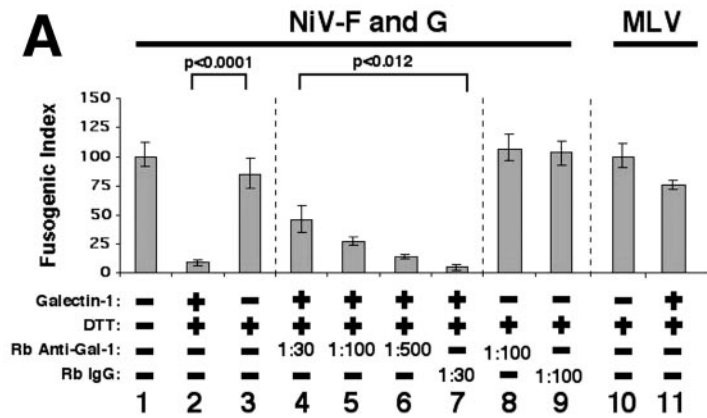
Addition of recombinant human gal-1 to Vero cells expressing NiV-F and NiV-G abrogated syncytia formation by ~90% (Fig. 2*A*). gal-1 also inhibited NiV-F/G-mediated fusion in other per-

missive cell lines such as human 293T and mouse NIH 3T3 cells (data not shown). The inhibitory effect of gal-1 was specifically antagonized by polyclonal anti-gal-1 antiserum. Importantly, anti-gal-1 antiserum alone in the absence of exogenous gal-1 did not inhibit syncytia formation, indicating that endogenous gal-1 (that can be secreted and bind back to cell surfaces) is not a viral receptor on the cell surface. Moreover, the inhibitory effects of gal-1 on NiV F/G-mediated cell fusion appeared specific for the Paramyxoviridae family (Fig. 2*B*). gal-1 inhibited fusion mediated by Hendra and human parainfluenza virus type III F and G glycoproteins (both are paramyxoviruses) but had no effect on fusion mediated by two retroviruses, human T lymphotropic virus II and

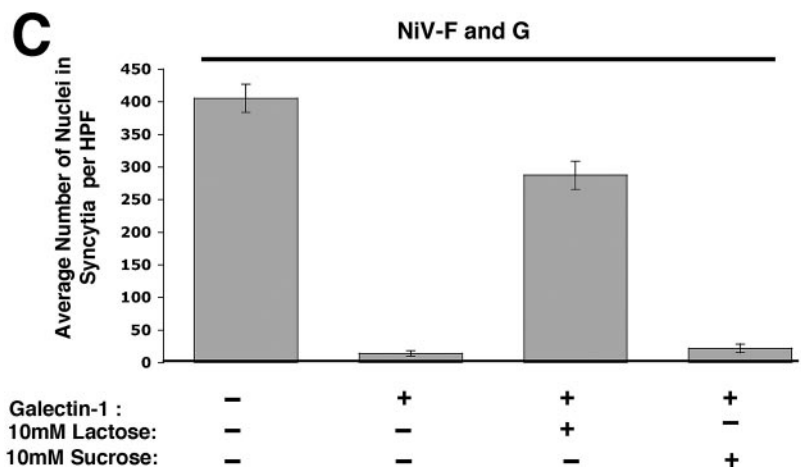
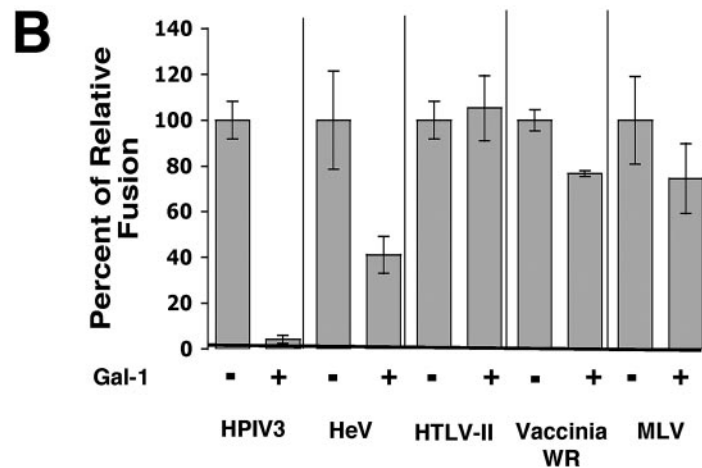
Table I. Glycosylation profiling by lectin-immunoprecipitation/immunoblot assay<sup>a</sup>

Lectin	Specificity	Binding to G	Binding to F
GNV	$\alpha(1,3)$ mannose	+	++
PHA-E	$\beta(1,6)$ GlcNAc	++	+++
PNA (peanut)	Gal $\beta(1,3)$ GalNAc	+++	+/-

<sup>a</sup> Relative amounts of NiV-F or -G precipitated by the indicated lectins were quantified by densitometry; arbitrary densitometric units: 1–40, +/-; 41–80, +; 81–120, ++; and 121–160, +++. Background is designated as –.



**FIGURE 2.** gal-1 blocks NiV envelope-mediated fusion. **A**, gal-1 inhibits NiV-F- and NiV-G-mediated fusion. Vero cells expressing NiV-F and NiV-G were cultured for 16 h in medium with gal-1 (20  $\mu$ M in 0.32 mM DTT) or buffer control. gal-1 has unpaired cysteines in the binding pocket that can form intra- or intermolecular disulfide binds in the absence of saccharide ligands, which can lead to inactivation of the binding site. Thus, the buffer control includes the same concentration of DTT, a reducing agent, which prevents gal-1 inactivation. To demonstrate the specificity of gal-1 inhibition, rabbit anti-gal-1 serum (in increasing amounts) or preimmune serum (IgG) were added simultaneously with gal-1. Fusion was quantified as described in *Materials and Methods*. Values of *p* were calculated using the Student *t* test (unpaired samples with unequal variance). Mean  $\pm$  SD of one representative experiment of three is presented. Note that anti-gal-1 sera alone did not inhibit NiV-G/F-mediated fusion. **B**, Specificity of gal-1 inhibition for Paramyxoviridae family: inhibition was observed only for fusion mediated by Hendra virus (HeV) glycoproteins and human parainfluenza virus type III (HPIV III), whereas no significant inhibition was seen for fusion mediated by glycoproteins from human T lymphotropic virus II (HTLV II), murine leukemia virus (MLV), or vaccinia (WR strain). Mean  $\pm$  SE of two determinations. **C**, Lactose reversal of gal-1 inhibition. A total of 10 mM lactose, a saccharide ligand for gal-1, or 10 mM sucrose, which does not bind gal-1, was added to fusion assays concurrently with gal-1. Fusion was quantified as above. Mean  $\pm$  SE of two determinations.



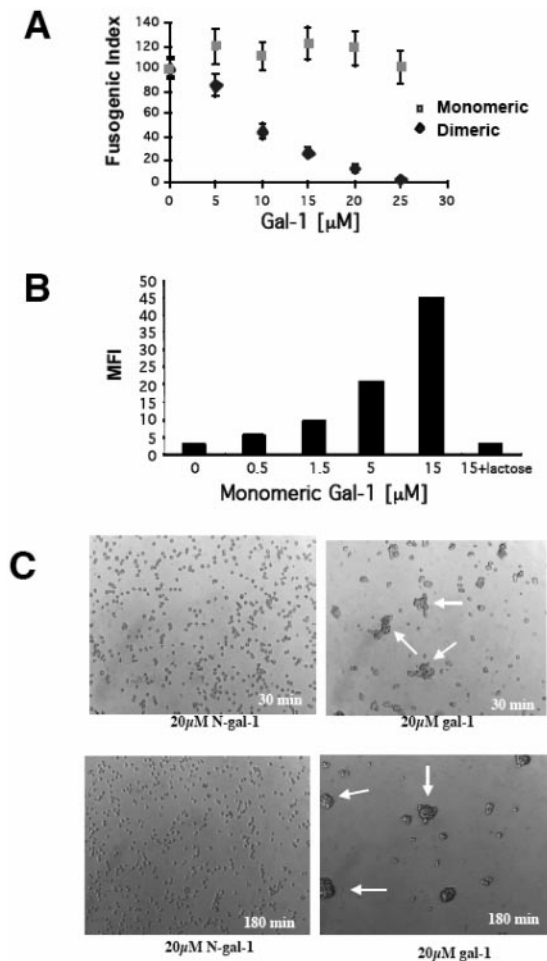
murine leukemia virus, or by a pox virus, vaccinia (WR strain) (Fig. 2B). In addition, gal-1 inhibition of NiV fusion is fully reversible with lactose, a cognate ligand for gal-1, but not sucrose (Fig. 2C), indicating that the fusion inhibitory effect of gal-1 is dependent on its interaction with carbohydrate moieties on the viral envelope glycoproteins.

The fusion inhibitory activity of gal-1 is dependent on its ability to dimerize. The  $IC_{50}$  of gal-1 in blocking cell fusion induced by NiV-G and NiV-F was in the 7–10  $\mu$ M range (Fig. 3A). gal-1 forms noncovalent homodimers at this concentration (34, 35), suggesting that dimeric gal-1 was essential for the inhibitory effect on fusion. We confirmed that dimeric gal-1 was required to inhibit NiV-F- and NiV-G-mediated fusion by using a mutant *N*-Gal-1

that remains monomeric < 250  $\mu$ M (24). *N*-Gal-1 had no effect on fusion of cells expressing NiV-F and NiV-G (Fig. 3A), although *N*-Gal-1 retained specific carbohydrate-binding activity (Fig. 3B). As also shown in Fig. 3C, the *N*-Gal-1 is monomeric because we observed no agglutination of T cells by the *N*-Gal-1 at concentrations up to 20  $\mu$ M, while we see clear T cell agglutination by native gal-1 at the same concentration. Thus, the inhibitory effect of gal-1 on syncytia formation is dependent on both the ability to dimerize and the ability to bind carbohydrate ligand (Fig. 2C).

#### gal-1 binds to specific N-glycans on NiV-F

To determine whether gal-1 preferentially binds to specific *N*-glycan sites on NiV-F, we performed a gal-1 dose-response curve for



**FIGURE 3.** Dimeric gal-1 is required to block NiV-F/G-mediated fusion. *A*, Monomeric (*N*-Gal-1) or dimeric gal-1 was added to Vero cells transfected with NiV-F and NiV-G. Fusion was quantified as in Fig. 2. Note that monomeric gal-1 had no effect on fusion even up to 25  $\mu$ M. Mean  $\pm$  SD of one representative experiment of three is presented. *B*, *N*-Gal-1 binds to T cells in a lactose inhibitable fashion. Increasing concentrations of biotinylated monomeric gal-1 were added to T cells. After binding for 30 min at 4°C, cells were washed once in 1 $\times$  PBS, and bound gal-1 was detected by addition of FITC-conjugated streptavidin and flow cytometry. The highest amount of *N*-Gal-1 binding observed was reversed by the addition of 0.1 mM lactose. MFI, mean fluorescence intensity. *C*, *N*-Gal-1 is monomeric. gal-1 or *N*-Gal-1 was added to CEM T cells for 30 or 180 min, and the cells examined by phase-contrast microscopy. Dimeric gal-1 agglutinated T cells (arrows) due to the ability to bind carbohydrate ligands on adjacent cells. *N*-Gal-1 was unable to agglutinate T cells, indicating that *N*-Gal-1 is monomeric at this concentration.

NiV-F and the NiV-F1, NiV-F2, NiV-F3, NiV-F4, and NiV-F5 mutants (see *Materials and Methods*). All the *N*-glycan mutants were similarly sensitive to gal-1 inhibition as wt NiV-F (Fig. 4A), with the exception of the NiV-F3 mutant (Fig. 4B). For example, note that 20  $\mu$ M gal-1 abrogated fusion for NiV-F and the NiV-F1, F2, F4, and F5 mutants (Fig. 4A), whereas the NiV-F3 mutant still exhibited 50% of control fusion activity at this gal-1 concentration (Fig. 4B) ( $p < 0.0125$ , Student's *t* test after Bonferroni inequality correction for paired-wise comparison of wt NiV-F against F1, F2, F3, F4, and F5 mutants). Thus, the NiV-F3 mutant was significantly more resistant to gal-1 inhibition, suggesting that the F3 glycan was important for gal-1 binding to NiV-F.

To directly examine gal-1 binding to the NiV-F3 mutant, gal-1 was added to intact 293T cells expressing either NiV-F or NiV-F3.

Cell lysates were immunoprecipitated with anti-gal-1 antiserum, and NiV-F or NiV-F3 was detected by immunoblotting. gal-1 precipitated significantly less NiV-F3 compared with wt NiV-F, indicating that gal-1 binds less efficiently to NiV-F3 (Fig. 4C). This result was not due to differential expression because NiV-F and NiV-F3 were expressed equivalently on the cell surface (data not shown). Our results indicate that the F3 *N*-glycan contributes significantly to gal-1 binding to NiV-F; however, the other NiV-F *N*-glycans can also contribute to gal-1 binding because a small amount of NiV-F3 is still coimmunoprecipitated with gal-1. To demonstrate that NiV-F binding requires complex *N*-glycans containing the preferred gal-1 saccharide ligands (see Table I), cells were treated with deoxymannojirimycin (DMNJ), which blocks the Golgi mannosidase II required for complex/hybrid *N*-glycan formation. As shown in Fig. 4D, DMNJ treatment significantly reduced the ability of gal-1 to coimmunoprecipitate with NiV-F. Next, we investigated the mechanism of gal-1 inhibition of NiV glycoprotein-mediated cell fusion.

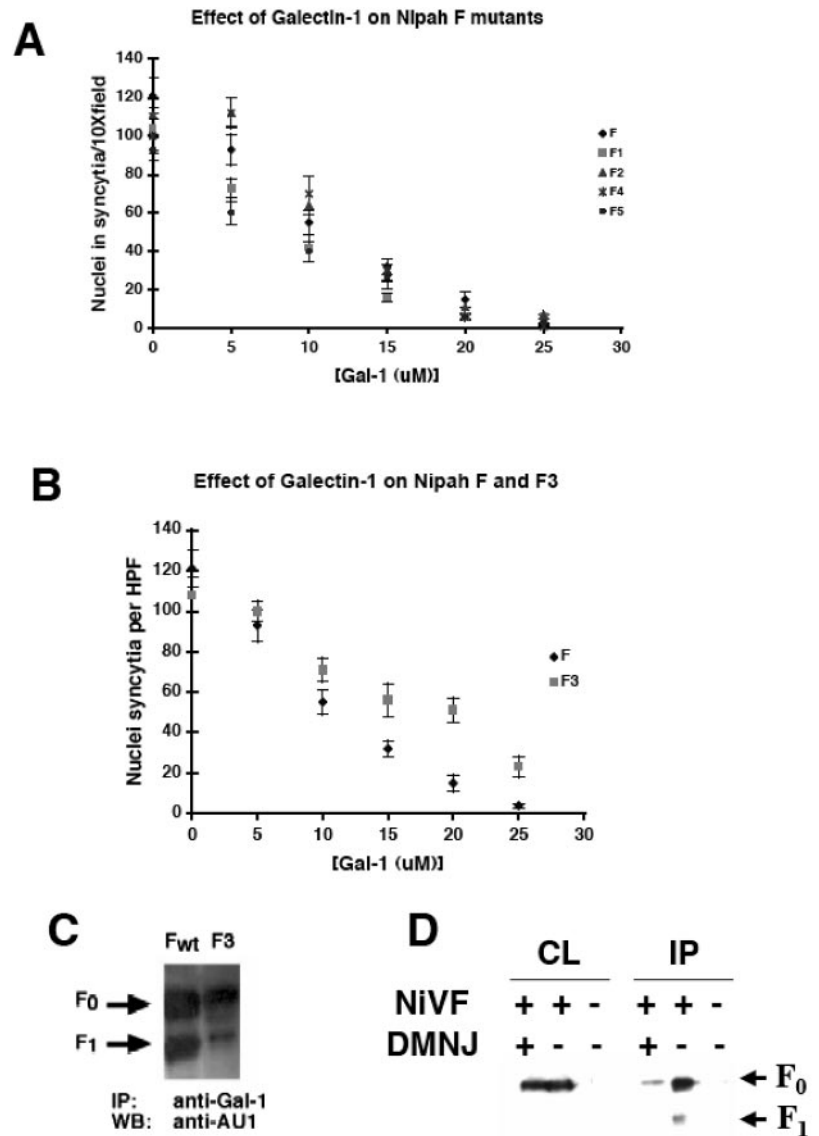
#### *Oligomerization of NiV-F/G is aberrantly modulated by gal-1*

NiV-F and NiV-G glycoproteins are closely associated on the plasma membrane because the two glycoproteins can be coimmunoprecipitated (Fig. 5A). Because the attachment (G) and fusion (F) proteins of paramyxoviral envelope glycoproteins are oligomeric and the oligomeric structure is required for cell fusion (36), we asked whether gal-1 inhibition of fusion resulted from altered oligomerization of NiV-F and/or NiV-G. As with other paramyxoviruses (37), the NiV-F glycoprotein forms trimers (Fig. 5B). However, in the presence of gal-1, an increased fraction of NiV-F was cross-linked into trimers; the ratio of NiV-F trimers to monomers increased 5-fold, from 0.35 to 1.75, in the presence of increasing amounts of gal-1 (Fig. 5B). gal-1 also markedly augmented the oligomerization of NiV-G. Although we detected tetramers of NiV-G in the absence of gal-1, the addition of gal-1 dramatically enhanced the amount of cell surface NiV-G tetramers; the ratio of NiV-G tetramers to monomers increased >400-fold, from 3.8 to 1665, with increasing amounts of gal-1 (Fig. 5B). Importantly, monomeric *N*-Gal-1 (10  $\mu$ M) had no effect on the cross-linking of NiV-F or NiV-G (Fig. 5B), demonstrating that dimeric gal-1 was required for enhanced oligomerization of NiV glycoproteins and indicating that altered oligomerization was likely responsible for the decreased cell fusion caused by dimeric gal-1.

#### *gal-1 induces DC secretion of proinflammatory cytokines*

CD45, an established cellular receptor for gal-1 (38, 39), is expressed on DCs (40). A link between CD45 cross-linking and IL-6 signaling has been established in certain cell types, and IL-6 is a proinflammatory cytokine in which increases correlate with increased probability of survival in Ebola-infected patients (41). gal-1 expression is increased at sites of inflammation (21). Thus, as DCs are likely to be exposed to high levels of gal-1 in inflamed tissues, we investigated the effects of gal-1 on the production of proinflammatory mediators by DCs. Monocyte-derived DCs (MDDCs) were matured as described in Fig. 6. gal-1 was added to differentiated MDDCs. As shown in Fig. 6, gal-1 increased MDDC production of IL-6 by at least two orders of magnitude and also stimulated TNF- $\alpha$  secretion. In contrast to gal-1, the monomeric *N*-Gal-1 mutant failed to induce significant IL-6 or TNF- $\alpha$  secretion, demonstrating that the dimeric form of gal-1 is required to stimulate cytokine secretion by MDDCs. Addition of 100 mM lactose blocked gal-1-induced IL-6 or TNF- $\alpha$  secretion, indicating that induction of proinflammatory cytokines required gal-1 binding to glycan ligands on the MDDCs.

**FIGURE 4.** gal-1 binds to a specific *N*-glycan site in NiV-F and NiV-G. *A*, F1, F2, F4, and F5 mutants are as sensitive to gal-1 inhibition as wt NiV-F. Vero cells transfected with NiV-G and wt NiV-F or the indicated NiV-F mutants (F1, F2, F4, or F5) were cultured with indicated concentrations of gal-1 and fusogenicity determined as in Fig. 1. Ten fields were counted for each mutant per condition. The number of nuclei in syncytia per field with no gal-1 treatment is normalized to 100% (fusogenic index). Mean  $\pm$  SD of one representative experiment of three is presented. *B*, NiV-F3 glycan mutant is resistant to gal-1 inhibition of fusion. For clarity, results are shown only for wt NiV-F and F3 only. The experiment was performed identically as described in *A*. *C*, The NiV-F3 mutant reduces gal-1 binding. gal-1 (20  $\mu$ M) was added to 293T cells expressing equivalent amounts of NiV-F or NiV-F3 protein, cell lysates were immunoprecipitated with rabbit anti-gal-1 antiserum, and NiV-F and NiV-F3 were detected with anti-AU1. *D*, gal-1 binding to NiV-F is dependent on lectin-glycan interaction. DMNJ (2 mM), which blocks the processing required for formation of complex/hybrid *N*-glycans, was added to 293T cells expressing NiV-F for 48 h. gal-1 (20  $\mu$ M) was added to the cells, the cells were lysed, and the lysates were immunoprecipitated with an anti-gal-1 Ab. Precipitated proteins were detected with anti-AU1. CL indicates the input cell lysate while IP indicated precipitated protein. Note that DMNJ treatment itself did not affect levels of NiV-F.

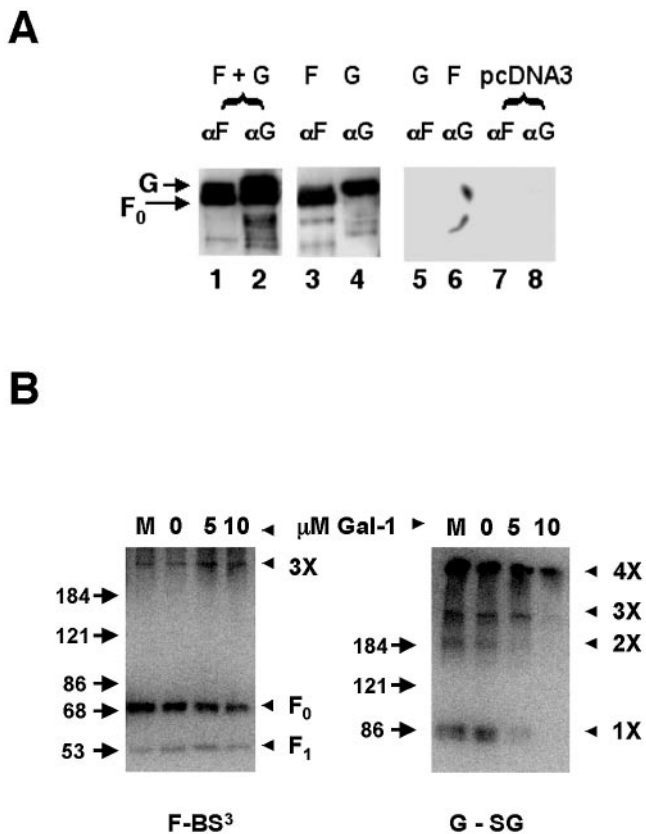


## Discussion

Glycan structures on the Nipah viral F and G envelope glycoproteins play critical roles in viral entry and cell fusion (H. C. Aguilar et al., manuscript in preparation). Some of these glycans bear oligosaccharide structures that can be bound by gal-1, an endogenous lectin expressed by a variety of cell types, including endothelial cells and DCs. The primary targets of NiV infection include endothelial cells and neurons, although the extensive lymphoid necrosis and positive immunostaining for viral Ags seen in lymphoid tissue suggest that this tissue could be similarly involved in primary replication (1). We found that gal-1 can bind directly to NiV-F and NiV-G and that this binding inhibited the cell-cell fusion mediated by NiV-F and NiV-G. It has been reported previously that endothelial cells increase gal-1 synthesis and export to the cell surface in the presence of inflammatory cytokines and that gal-1 is highly expressed by endothelial cells in inflamed lymph nodes (32). In addition, extracellular matrix can concentrate and present enough gal-1 to induce T cell apoptosis (42, 43), suggesting that the amounts of gal-1 that can be found in the extracellular matrix could potentially be sufficient to affect the efficiency of envelope-mediated fusion.

Given the histopathology associated with NiV infection and the elaboration of inflammatory cytokines that participate in the innate immune response to many viral pathogens, our results also suggest that up-regulation of gal-1 expression in the setting of NiV infection could be a mechanism to limit viral spread. The ability of gal-1 to directly block NiV-F/G-mediated cell-cell fusion may result from the ability of gal-1 to perturb NiV-F and NiV-G interactions on the cell surface necessary for membrane fusion. Increased NiV-F and NiV-G homo-oligomerization induced by gal-1 implies that gal-1 segregates NiV-F and NiV-G glycoproteins on the cell surface, perhaps by binding to specific glycans, e.g., the F3 *N*-glycan. The ability of gal-1 to aggregate cell surface glycoconjugates have been described for the interaction of gal-1 with CD45 on T cells (22). Our report is the first demonstration of a direct antiviral effect for gal-1 or any member of the galectin family. Moreover, the ability of gal-1 to inhibit viral-induced fusion is relatively specific and thus far restricted to Paramyxoviruses.

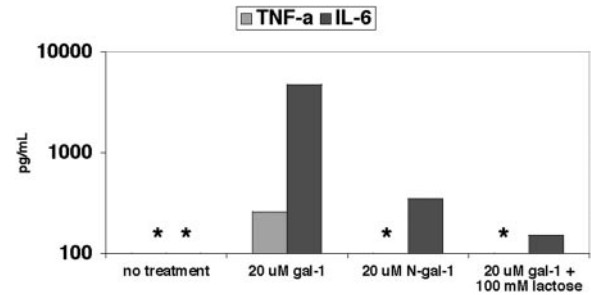
In addition to endothelial cells, we and others (Ref. 44 and data not shown) have detected gal-1 expression in DCs. Importantly, gal-1 may be an autocrine regulatory factor for endothelial cells



**FIGURE 5.** gal-1 modulates the oligomeric state of NiV-F and NiV-G. **A**, Association of NiV-F and NiV-G. Extracts of 293T cells expressing NiV-F and NiV-G (F + G), or NiV-F or NiV-G alone, were immunoprecipitated with anti-F peptide or anti-G peptide antisera. Precipitated proteins were detected with anti-AU1 to detect both NiV-F and NiV-G. Anti-F and anti-G coimmunoprecipitated NiV-G and NiV-F, respectively (lanes 1 and 2). When NiV-F and NiV-G were expressed alone, anti-F and anti-G precipitated only the relevant glycoproteins (lanes 3–6). No precipitated protein was seen in cells transfected with plasmid alone (lanes 7 and 8). **B**, gal-1 modulates oligomerization of NiV-F and NiV-G. gal-1 was added to 293T cells expressing NiV-F or NiV-G. Cell surface proteins were cross-linked using membrane impermeant cross-linkers (BS<sup>3</sup> for NiV-F and sulfo-GMBS (SG) for NiV-G), and NiV-F and NiV-G were detected with anti-AU1. Uncleaved NiV-F monomer (F<sub>0</sub>) and the estimated NiV-F trimer (3×) are indicated. Molecular mass markers are shown. Molecular mass estimates >180 kDa are not precise, but for NiV-G, the progressive ladder pattern suggests that monomers (1×), dimers (2×), trimers (3×), and tetramers (4×) are formed. M, 10 mM monomeric *N*-Gal-1, does not enhance oligomerization.

and DCs as it is for T cells. For example, Pircher and coworkers (45) found that CD8 cells secrete gal-1 after stimulation and suggested that gal-1 acts as an autocrine factor to control T cell survival after an immune response. Similarly, gal-1 alters T cell secretion of cytokines, including IFN- $\gamma$  and TNF- $\alpha$  (15, 21, 46). As DCs can express gal-1 and its cognate receptors (such as CD45 and CD43) and DCs adjacent to NiV-infected endothelial cells would be exposed to gal-1 secreted as part of the inflammatory response, we examined the effects of gal-1 on cytokine production by DCs.

In the present study, we report the novel observation that gal-1 dramatically increased IL-6 and TNF- $\alpha$  secretion by MDDCs. This indicates that, in addition to directly blocking NiV F- and G-mediated cell fusion, gal-1 may also promote an innate inflammatory response that would facilitate a successful host response to viral infection. Significantly, two groups have documented human cytokine responses that predict survival vs mortality in patients in-



**FIGURE 6.** gal-1 induces DC secretion of proinflammatory cytokines. Human PBMCs were cultured for 5 days with IL-4 (100 ng/ml) + GM-CSF (50 ng/ml). At day 5, 20  $\mu$ M gal-1 (with or without 100 mM lactose) or *N*-Gal-1 were added, and the culture was continued for an additional 48 h. Cytokine secretion was measured in tissue culture supernatant by cytometric bead array. One experiment of three is shown. \*, Lower limit of the linear range.

fectured with Ebola virus. Georges and coworkers (41) found that several proinflammatory cytokines, including IL-6 and TNF- $\alpha$ , are markedly elevated in survivors of Ebola infection, whereas both the Georges group and the Peters group (47) found that patients who succumbed to infection typically had increased serum IFN- $\gamma$  and IL-2 but little or no increase in IL-6 response (41, 47–49). Importantly, we and others (21, 50) have demonstrated that gal-1 decreases IL-2 and IFN- $\gamma$  production when administered in murine models of immune-mediated disease. The proinflammatory response triggered by gal-1 binding to MDDCs is a novel function for gal-1 and may be particularly important as DCs may be an early target of NiV during lymph node infection (1). Significantly, IL-6 is a prototypical proinflammatory mediator known to induce DC maturation and enhance Ag presentation function or allostimulatory capacity and has been used to generate clinical grade DCs for immunotherapy applications (51–54). Therefore, we hypothesize that gal-1 induction of IL-6 can be further protective during NiV infection by enhancing the Ag presentation function of cognate DCs and acting as an immune adjuvant. Thus, gal-1 may have multiple direct and indirect mechanisms, including inhibition of viral-mediated cell fusion and promotion of cytokine production, to enhance the innate immune system's response to emerging viral pathogens such as NiV.

## Acknowledgments

We thank Irvin Chen for comments, and Jessica Zellhoefer for technical assistance.

## Disclosures

The authors have no financial conflict of interest.

## References

- Wong, K. T., W. J. Shieh, S. Kumar, K. Norain, W. Abdullah, J. Guarner, C. S. Goldsmith, K. B. Chua, S. K. Lam, C. T. Tan, et al. 2002. Nipah virus infection: pathology and pathogenesis of an emerging paramyxoviral zoonosis. *Am. J. Pathol.* 161: 2153–2167.
- Chua, K. B., W. J. Bellini, P. A. Rota, B. H. Harcourt, A. Tamin, S. K. Lam, T. G. Ksiazek, P. E. Rollin, S. R. Zaki, W. Shieh, et al. 2000. Nipah virus: a recently emergent deadly paramyxovirus. *Science* 288: 1432–1435.
- Lam, S. K. 2003. Nipah virus: a potential agent of bioterrorism? *Antiviral Res.* 57: 113–119.
2004. Nipah virus outbreak(s) in Bangladesh, January–April 2004. *Wkly. Epidemiol. Rec.* 79: 168.
- Mackenzie, J. S., K. B. Chua, P. W. Daniels, B. T. Eaton, H. E. Field, R. A. Hall, K. Halpin, C. A. Johansen, P. D. Kirkland, S. K. Lam, et al. 2001. Emerging viral diseases of Southeast Asia and the Western Pacific. *Emerg. Infect. Dis.* 7(3 Suppl.): 497–504.
- Polesky, A., and G. Bhatia. 2003. Ebola hemorrhagic fever in the era of bioterrorism. *Semin. Respir. Infect.* 18: 206–215.



7. Hackett, C. J. 2003. Innate immune activation as a broad-spectrum biodefense strategy: prospects and research challenges. *J. Allergy Clin. Immunol.* 112: 686–694.
8. Hsu, V. P., M. J. Hossain, U. D. Parashar, M. M. Ali, T. G. Ksiazek, I. Kuzmin, M. Niezgodza, C. Rupprecht, J. Bresee, and R. F. Breiman. 2004. Nipah virus encephalitis reemergence, Bangladesh. *Emerg. Infect. Dis.* 10: 2082–2087.
9. Olofsson, S., and J. E. Hansen. 1998. Host cell glycosylation of viral glycoproteins: a battlefield for host defence and viral resistance. *Scand. J. Infect. Dis.* 30: 435–440.
10. Wei, X., J. M. Decker, S. Wang, H. Hui, J. C. Kappes, X. Wu, J. F. Salazar-Gonzalez, M. G. Salazar, J. M. Kilby, M. S. Saag, et al. 2003. Antibody neutralization and escape by HIV-1. *Nature* 422: 307–312.
11. Navarro-Sanchez, E., R. Altmeyer, A. Amara, O. Schwartz, F. Fieschi, J. L. Virelizier, F. Arenzana-Seisdedos, and P. Despres. 2003. Dendritic-cell-specific ICAM3-grabbing non-integrin is essential for the productive infection of human dendritic cells by mosquito-cell-derived dengue viruses. *EMBO Rep.* 4: 723–728.
12. Van Kooyk, Y., and T. B. Geijtenbeek. 2003. DC-SIGN: escape mechanism for pathogens. *Nat. Rev. Immunol.* 3: 697–709.
13. Gauthier, L., B. Rossi, F. Roux, E. Termine, and C. Schiff. 2002. Galectin-1 is a stromal cell ligand of the pre-B cell receptor (BCR) implicated in synapse formation between pre-B and stromal cells and in pre-BCR triggering. *Proc. Natl. Acad. Sci. USA* 99: 13014–13019.
14. Perillo, N. L., C. H. Uittenbogaart, J. T. Nguyen, and L. G. Baum. 1997. Galectin-1, an endogenous lectin produced by thymic epithelial cells, induces apoptosis of human thymocytes. *J. Exp. Med.* 185: 1851–1858.
15. Vespa, G. N., L. A. Lewis, K. R. Kozak, M. Moran, J. T. Nguyen, L. G. Baum, and M. C. Miceli. 1999. Galectin-1 specifically modulates TCR signals to enhance TCR apoptosis but inhibit IL-2 production and proliferation. *J. Immunol.* 162: 799–806.
16. Endharti, A. T., Y. W. Zhou, I. Nakashima, and H. Suzuki. 2005. Galectin-1 supports survival of naive T cells without promoting cell proliferation. *Eur. J. Immunol.* 35: 86–97.
17. Rubinstein, N., M. Alvarez, N. W. Zwirner, M. A. Toscano, J. M. Ibarregui, A. Bravo, J. Mordoh, L. Fainboim, O. L. Podhajcer, and G. A. Rabinovich. 2004. Targeted inhibition of galectin-1 gene expression in tumor cells results in heightened T cell-mediated rejection: a potential mechanism of tumor-immune privilege. *Cancer Cell* 5: 241–251.
18. Hernandez, J. D., and L. G. Baum. 2002. Ah, sweet mystery of death! Galectins and control of cell fate. *Glycobiology* 12: 127R–136R.
19. Almkvist, J., and A. Karlsson. 2004. Galectins as inflammatory mediators. *Glycoconj. J.* 19: 575–581.
20. Hsu, D. K., and F. T. Liu. 2004. Regulation of cellular homeostasis by galectins. *Glycoconj. J.* 19: 507–515.
21. Rabinovich, G. A., L. G. Baum, N. Tinari, R. Paganelli, C. Natoli, F. T. Liu, and S. Iacobelli. 2002. Galectins and their ligands: amplifiers, silencers or tuners of the inflammatory response? *Trends Immunol.* 23: 313–320.
22. Pace, K. E., C. Lee, P. L. Stewart, and L. G. Baum. 1999. Restricted receptor segregation into membrane microdomains occurs on human T cells during apoptosis induced by galectin-1. *J. Immunol.* 163: 3801–3811.
23. Pace, K. E., H. P. Hahn, and L. G. Baum. 2003. Preparation of recombinant human galectin-1 and use in T cell death assays. *Methods Enzymol.* 363: 499–518.
24. Cho, M., and R. D. Cummings. 1996. Characterization of monomeric forms of galectin-1 generated by site-directed mutagenesis. *Biochemistry* 35: 13081–13088.
25. De Maio, A., H. Lis, J. M. Gershoni, and N. Sharon. 1986. Identification of glycoproteins that are receptors for peanut agglutinin on immature (cortical) mouse thymocytes. *FEBS Lett.* 194: 28–32.
26. Jacobs, D. M., and D. C. Morrison. 1977. Inhibition of the mitogenic response to lipopolysaccharide (LPS) in mouse spleen cells by polymyxin B. *J. Immunol.* 118: 21–27.
27. Gillespie, W., J. C. Paulson, S. Kelm, M. Pang, and L. G. Baum. 1993. Regulation of  $\alpha 2,3$ -sialyltransferase expression correlates with conversion of peanut agglutinin (PNA)<sup>+</sup> to PNA<sup>-</sup> phenotype in developing thymocytes. *J. Biol. Chem.* 268: 3801–3804.
28. Partridge, E. A., C. Le Roy, G. M. Di Guglielmo, J. Pawling, P. Cheung, M. Granovsky, I. R. Nabi, J. L. Wrana, and J. W. Dennis. 2004. Regulation of cytokine receptors by Golgi N-glycan processing and endocytosis. *Science* 306: 120–124.
29. Cummings, R. D., and S. Kornfeld. 1982. Characterization of the structural determinants required for the high affinity interaction of asparagine-linked oligosaccharides with immobilized *Phaseolus vulgaris* leucoagglutinating and erythroagglutinating lectins. *J. Biol. Chem.* 257: 11230–11234.
30. Di Virgilio, S., J. Glushka, K. Moremen, and M. Pierce. 1999. Enzymatic synthesis of natural and 13C enriched linear poly-N-acetylglucosamines as ligands for galectin-1. *Glycobiology* 9: 353–364.
31. Amano, M., M. Galvan, J. He, and L. G. Baum. 2003. The ST6Gal I sialyltransferase selectively modifies N-glycans on CD45 to negatively regulate galectin-1-induced CD45 clustering, phosphatase modulation, and T cell death. *J. Biol. Chem.* 278: 7469–7475.
32. Baum, L. G., J. J. Seilhamer, M. Pang, W. B. Levine, D. Beynon, and J. A. Berliner. 1995. Synthesis of an endogenous lectin, galectin-1, by human endothelial cells is up-regulated by endothelial cell activation. *Glycoconj. J.* 12: 63–68.
33. Zuniga, E., A. Gruppi, J. Hirabayashi, K. I. Kasai, and G. A. Rabinovich. 2001. Regulated expression and effect of galectin-1 on *Trypanosoma cruzi*-infected macrophages: modulation of microbicidal activity and survival. *Infect. Immun.* 69: 6804–6812.
34. Cho, M., and R. D. Cummings. 1995. Galectin-1, a  $\beta$ -galactoside-binding lectin in Chinese hamster ovary cells. I. Physical and chemical characterization. *J. Biol. Chem.* 270: 5198–5206.
35. Perillo, N. L., K. E. Pace, J. J. Seilhamer, and L. G. Baum. 1995. Apoptosis of T cells mediated by galectin-1. *Nature* 378: 736–739.
36. Lamb, R. A., and D. Kolakofsky. 2001. Paramyxoviridae: The Viruses and Their Replication. In *Fundamental Virology*. D. M. Knipe and P. M. Howley, eds. Lippincott, Williams & Wilkins, Philadelphia, pp. 689–724.
37. Russell, R., R. G. Paterson, and R. A. Lamb. 1994. Studies with cross-linking reagents on the oligomeric form of the paramyxovirus fusion protein. *Virology* 199: 160–168.
38. Walzel, H., U. Schulz, P. Neels, and J. Brock. 1999. Galectin-1, a natural ligand for the receptor-type protein tyrosine phosphatase CD45. *Immunol. Lett.* 67: 193–202.
39. Nguyen, J. T., D. P. Evans, M. Galvan, K. E. Pace, D. Leitenberg, T. N. Bui, and L. G. Baum. 2001. CD45 modulates galectin-1-induced T cell death: regulation by expression of core 2 O-glycans. *J. Immunol.* 167: 5697–5707.
40. Hart, D. N. J., K. MacDonald, S. Vuckovic, and G. J. Ckark. 2001. Phenotypic characterization of dendritic cells. In *Dendritic Cells*. M. T. Lotze and A. W. Thomson, eds. Academic Press, San Diego, pp. 97–117.
41. Baize, S., E. M. Leroy, A. J. Georges, M. C. Georges-Courbot, M. Capron, I. Bedjabaga, J. Lansoud-Soukate, and E. Mavoungou. 2002. Inflammatory responses in Ebola virus-infected patients. *Clin. Exp. Immunol.* 128: 163–168.
42. Collins, B. E., and J. C. Paulson. 2004. Cell surface biology mediated by low affinity multivalent protein-glycan interactions. *Curr. Opin. Chem. Biol.* 8: 617–625.
43. He, J., and L. G. Baum. 2004. Presentation of galectin-1 by extracellular matrix triggers T cell death. *J. Biol. Chem.* 279: 4705–4712.
44. Dietz, A. B., P. A. Bulur, G. J. Knutson, R. Maticic, and S. Vuk-Pavlovic. 2000. Maturation of human monocyte-derived dendritic cells studied by microarray hybridization. *Biochem. Biophys. Res. Commun.* 275: 731–738.
45. Blaser, C., M. Kaufmann, C. Muller, C. Zimmermann, V. Wells, L. Mallucci, and H. Pircher. 1998.  $\beta$ -Galactoside-binding protein secreted by activated T cells inhibits antigen-induced proliferation of T cells. *Eur. J. Immunol.* 28: 2311–2319.
46. Rabinovich, G. A., A. Ariel, R. Hershkovitz, J. Hirabayashi, K. I. Kasai, and O. Lider. 1999. Specific inhibition of T cell adhesion to extracellular matrix and proinflammatory cytokine secretion by human recombinant galectin-1. *Immunology* 97: 100–106.
47. Villinger, F., P. E. Rollin, S. S. Brar, N. F. Chikkala, J. Winter, J. B. Sundstrom, S. R. Zaki, R. Swanepoel, A. A. Ansari, and C. J. Peters. 1999. Markedly elevated levels of interferon (IFN)- $\gamma$ , IFN- $\alpha$ , interleukin (IL)-2, IL-10, and tumor necrosis factor  $\alpha$  associated with fatal Ebola virus infection. *J. Infect. Dis.* 179(Suppl. 1): S188–S191.
48. Baize, S., E. M. Leroy, M. C. Georges-Courbot, M. Capron, J. Lansoud-Soukate, P. Debre, S. P. Fisher-Hoch, J. B. McCormick, and A. J. Georges. 1999. Defective humoral responses and extensive intravascular apoptosis are associated with fatal outcome in Ebola virus-infected patients. *Nat. Med.* 5: 423–426.
49. Leroy, E. M., S. Baize, V. E. Volchkov, S. P. Fisher-Hoch, M. C. Georges-Courbot, J. Lansoud-Soukate, M. Capron, P. Debre, J. B. McCormick, and A. J. Georges. 2000. Human asymptomatic Ebola infection and strong inflammatory response. *Lancet* 355: 2210–2215.
50. Baum, L. G., D. P. Blackall, S. Arias-Magallano, D. Nanigian, S. Y. Uh, J. M. Browne, D. Hoffmann, C. E. Emmanouilides, M. C. Territo, and G. C. Baldwin. 2003. Amelioration of graft versus host disease by galectin-1. *Clin. Immunol.* 109: 295–307.
51. Nieda, M., M. Tomiyama, and K. Egawa. 2003. Ex vivo enhancement of antigen-presenting function of dendritic cells and its application for DC-based immunotherapy. *Hum. Cell* 16: 199–204.
52. Sorg, R. V., Z. Ozcan, T. Brefort, J. Fischer, R. Ackermann, M. Muller, and P. Wernet. 2003. Clinical-scale generation of dendritic cells in a closed system. *J. Immunother.* 26: 374–383.
53. Luft, T., M. Jefford, P. Luetjens, H. Hochrein, K. A. Masterman, C. Maliszewski, K. Shortman, J. Cebon, and E. Maraskovsky. 2002. IL-1 $\beta$  enhances CD40 ligand-mediated cytokine secretion by human dendritic cells (DC): a mechanism for T cell-independent DC activation. *J. Immunol.* 168: 713–722.
54. Jonuleit, H., U. Kuhn, G. Muller, K. Steinbrink, L. Paragnik, E. Schmitt, J. Knop, and A. H. Enk. 1997. Pro-inflammatory cytokines and prostaglandins induce maturation of potent immunostimulatory dendritic cells under fetal calf serum-free conditions. *Eur. J. Immunol.* 27: 3135–3142.