Sialic Acid Associated with αᵥβ₃ Integrin Mediates HIV-1 Tat Protein Interaction and Endothelial Cell Proangiogenic Activation*

Received for publication, December 23, 2011, and in revised form, April 3, 2012 Published, JBC Papers in Press, April 23, 2012, DOI 10.1074/jbc.M111.337139

Paola Chiodelli‡, Chiara Urbinati‡, Stefania Mitola‡, Elena Tanghetti‡, and Marco Rusnati‡

From the Units of General Pathology and Immunology and Histology, Department of Biomedical Sciences and Biotechnology, School of Medicine, University of Brescia, 25123 Brescia, Italy

Background: HIV-1 Tat engages αᵥβ₃, leading to endothelial cell (EC) proangiogenic activation. 

Results: Sialic acid (NeuAc)-binding lectins and neuraminidase partially inhibit Tat/αᵥβ₃ interaction and consequent EC proangiogenic activation.

Conclusion: Endothelial αᵥβ₃-associated NeuAc is involved in Tat interaction and consequent EC proangiogenic activation.

Significance: Integrin-associated NeuAc can be considered a target for the development of new treatments for angiogenesis/AIDS-associated pathologies.

Sialic acid (NeuAc) is a major anion on endothelial cells (ECs) that regulates different biological processes including angiogenesis. NeuAc is present in the oligosaccharidic portion of integrins, receptors that interact with extracellular matrix components and growth factors regulating cell adhesion, migration, and proliferation. Tat is a cationic polypeptide that, once released by HIV-1+ cells, accumulates in the extracellular matrix, promoting EC adhesion and proangiogenic activation by engaging αᵥβ₃. By using two complementary approaches (NeuAc removal by neuraminidase or its masking by NeuAc-binding lectin from Maackia amurensis, MAA), we investigated the presence of NeuAc on endothelial αᵥβ₃ and its role in Tat interaction, EC adhesion, and proangiogenic activation. αᵥβ₃ immunoprecipitation with biotinylated MAA or Western blot analysis of neuraminidase-treated ECs demonstrated that NeuAc is associated with both the αᵥ and the β₃ subunits. Surface plasmon resonance analysis demonstrated that the masking of αᵥβ₃-associated NeuAc by MAA prevents Tat/αᵥβ₃ interaction. MAA and neuraminidase prevent αᵥβ₃-dependent EC adhesion to Tat, the consequent FAK and ERK1/2 phosphorylation, and EC proliferation, migration, and regeneration in a wound-healing assay. Finally, MAA inhibits Tat-induced neovascularization in the ex vivo human artery ring sprouting assay. The inhibitions are specific because the NeuAc-unrelated lectin from Ulex europaeus is ineffective on Tat. Also, MAA and neuraminidase affect only weakly integrin-dependent EC adhesion and proangiogenic activation by fibronectin. In conclusion, NeuAc is associated with endothelial αᵥβ₃ and mediates Tat-dependent EC adhesion and proangiogenic activation. These data point to the possibility to target integrin glycosylation for the treatment of angiogenesis/AIDS-associated pathologies.

Polyanionic macromolecules are extremely abundant in the extracellular environment, readily accessible to many proteins for interactions implicated in various biological functions. Among polyanions, sialic acid (NeuAc)-bearing gangliosides and glycoproteins are widely distributed in biological fluids, extracellular matrix, and cell membrane, where they act as receptors for various physiological ligands and for many human viruses, bacteria, and protozoa (1–3).

The term NeuAc encompasses a large family of sugars characterized by a nine-carbon sugar acid common in higher animals and some microorganisms (4). NeuAc is found mainly as a terminal component of ganglioside and glycoproteins, where it regulates various molecular and cellular interactions (5).

NeuAc is the major surface anion on the endothelial cell (EC)² surface. Accordingly, the lectin from Maackia amurensis (MAA), which specifically binds NeuAc residues attached to galactose through an α(2→3) linkage, binds to ECs of retina, brain, and myocardium (6). NeuAc expression on ECs isregulated during ontogenesis, inflammation (7–9), and possibly neovascularization, as suggested by the observation that the binding of the NeuAc-binding lectin from Limax flavus to ECs increases during angiogenesis in the chick embryo chorioallantoic membrane (8).

NeuAc is involved in different physiological and pathological functions of the endothelium; in its ganglioside- or glycoprotein-associated form, it mediates EC infection by different microorganisms (10) and the transport of HIV-1 or of its proteins across the blood–brain barrier (11, 12). In its ganglioside-associated form, NeuAc takes part in the regulation of neovascularization (13–15). When associated with integrin subunits (including α₂ (16), α₂v (17), α₃ (18), α₄ (19), α₅ (20), β₁ (21)

*This work was supported by grants from Ministero dell’Istruzione dell’Università e della Ricerca, Istituto Superiore di Sanità (AIDS Project), and Cassa di Risparmio delle Provincie Lombarde (to M. R.).
1 To whom correspondence should be addressed: General Pathology and Immunology, Dept. of Biomedical Sciences and Biotechnology, viale Europa 11, 25123 Brescia, Italy. Tel: 39-30-3717315; Fax: 39-30-3717747; E-mail: rusnati@med.unibs.it.
(17, 18, 20), \( \beta_2 \) (21), and \( \beta_4 \) (16, 20)), NeuAc contributes to leukocyte and tumor cell extravasation during inflammation and metastasization, respectively.

Integrins are widely distributed receptors that interact with extracellular matrix components, growth factors, and microbial proteins regulating adhesion, migration, and proliferation of various normal and transformed cell types (22). Among the various integrins, \( \alpha_\beta_3 \) expressed on the surface of ECs plays a central role in neovascularization (23). Interestingly, NeuAc has been found associated with \( \alpha_\beta_3 \) integrin from melanoma metastatic cell surface (18), but no data are available for \( \alpha_\beta_3 \) from ECs.

HIV-1 Tat is a cationic protein that, once released by HIV-1-infected cells (24), targets ECs, causing a variety of pathological effects that, in turn, lead to different angiogenesis-related AIDS-associated diseases such as Kaposi sarcoma and ocular microangiopathies. Extracellular Tat accumulates in the extracellular matrix where, by binding to endothelial \( \alpha_\beta_3 \) integrin, it promotes EC adhesion and proangiogenic activation (25–27). Tat/\( \alpha_\beta_3 \) interaction occurs both via the RGD motif and the basic domain (RRKQRQRRR) of Tat (25). On the basis of what is described above, in this study, we decided to evaluate the presence of NeuAc on integrin \( \alpha_\beta_3 \) expressed at the EC surface and to investigate its role in Tat engagement and consequent biological activities.

**EXPERIMENTAL PROCEDURES**

**Chemicals**—Synthetic 86-amino acid Tat was from Xeptagen (Venezia, Italy). The recombinant wild type 86-amino acid form of HIV-1 Tat and its mutants Tat 1e (characterized by the deletion of the amino acid sequence that contains the RGD sequence) and Tat R—A (in which the arginine residues 49, 52, 53, 55, 56, and 57 within the basic domain were mutated to alanine residues) were purified from Escherichia coli as glutathione S-transferase (GST) fusion proteins (28). GST moiety does not interfere with Tat molecular interactions and biological activities (25). Anti-vascular endothelial growth factor receptor-2 (VEGFR2) antibody was gifted by Prof. H. A. Weich, National Research Centre for Biotechnology, Braunschweig, Germany. The heptapeptides GRGDSPK and GRADSPK were from Neosystems Laboratoires, Strasbourg, France, K5NOSH was from Glycores 2000, Milan, Italy, specific \( \alpha_\beta_3 \) antagonist SCH221153 and its inactive analog SCH21668 (27) were from Schering-Plough (Kenilworth, NJ), anti-phospho-FAK antibody was from Santa Cruz Biotechnology (Santa Cruz, CA), anti-phospho-ERK1/2 antibody and anti-phospho-VEGFR2 antibody were from Cell Signaling Technology (Danvers, MA), biotinylated MAA was from Vector Laboratories (Burlingame, CA), streptavidin-Sepharose, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride, and N-hydroxy-succinimide were from EC Healthare, anti-paxillin antibody was from Upstate Biotech Millipore (Lake Placid, NY), purified human \( \alpha_\beta_3 \) integrin, anti-\( \alpha_\beta_3 \) LM 609, anti-fascin, anti-\( \alpha_5 \), and anti-\( \beta_3 \) antibodies were from Chemicon, Millipore (Billerica, MA), glucosyl ceramide synthase inhibitor \( \Delta \)-threo-1-phenyl-2-decanoyl-amino-3-morpholino-1-propanol (PDMP) and \( \Delta \)-1-threo-1-phenyl-2-hexadecanoylamino-3-pyrrolidino-1-propanol-HCl (PPPP) were from Matreya, LLC (Pleasant Gap, PA), anti-\( \alpha \)-tubulin antibody, TRITC-phalloidin, FITC-conjugated anti-mouse IgG, neuraminidase from Clostridium perfringens, MAA, lectin from Ulex europeaus (UEA), poly-L-lysine, fibrinogen, fibronectin (FN), phosphor myristate acetate, 4-6-diamidino-2-phenylindole (DAPI), phenylmethylsulfonyl fluoride (PMSF), amino-n-caproic acid, leupeptin, Na\(_3\)VO\(_4\), and NaF were from Sigma.

**Surface Plasmon Resonance (SPR) Analysis**—A BIAcore X instrument (GE Healthcare) was used. Two different immobilizations were used to study the Tat/\( \alpha_\beta_3 \) interaction. (i) As described previously (25), synthetic Tat (40 \( \mu \)g/ml) was allowed to react with a CM5 sensor chip activated with 50 \( \mu \)l of a mixture of 0.2 M 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride and 0.5 M N-hydroxy-succinimide, leading to the immobilization of 6,470 resonance units (0.35 pmol/mm\(^2\)) of protein. Similar results were obtained for the immobilization of bovine serum albumin (BSA), used as a negative control and for blank subtraction. Increasing concentrations of integrin \( \alpha_\beta_3 \) in 10 mM Tris, pH 7.8, containing 10 mM Mn\(^{2+}\) were injected over the Tat or BSA surfaces in the absence or in the presence of MAA (250 nM) for 4 min and then washed until dissociation. In parallel experiments, increasing concentrations of integrin \( \alpha_\beta_3 \) in 10 mM Tris, pH 7.8, containing 10 mM Mn\(^{2+}\) were injected for 1 h with neuraminidase (500 milliunits/ml) before injection. Samples containing \( \alpha_\beta_3 \) to which neuraminidase was added only before injection were used as controls, demonstrating that the presence of the enzyme does not interfere significantly with Tat/\( \alpha_\beta_3 \) interaction. After every run, the sensor chip was regenerated by injection of 2.0 M NaCl in 10 mM HEPES, 3 mM EDTA, 150 mM NaCl, 0.005% surfactant P20. The dissociation constant (\( K_d \)) of the Tat/\( \alpha_\beta_3 \) interaction was calculated by the Scatchard plot analysis of the steady-state SPR data. (ii) Anti-GST antibody was immobilized on a CM5 surface using standard amine-coupling chemistry allowing the immobilization of 25,000 resonance units, equal to 0.98 pmol. Then, wild type GST-Tat, GST-Tat 1e, and GST-Tat R—A (120 \( \mu \)g/ml in Tris 10 mM pH 7.8 containing 10 mM Mn\(^{2+}\)) were injected over the anti-GST surface at a flow rate of 10 \( \mu \)l/min, allowing the immobilization of about 800 resonance units (equal to about 0.023 pmol) for wild type GST-Tat and GST-Tat R—A, and 1,300 resonance units (equal to 0.037 pmol) for GST-Tat 1e and for the GST moiety alone, used for blank subtraction.

**Cell Culture**—Transformed fetal bovine aortic endothelial GM7373 cells (obtained from the Human Genetic Mutant Cell Repository, Institute for Medical Research, Camden, NJ) (29), were grown in Dulbecco’s modified minimum essential medium (DMEM), 10% fetal calf serum (FCS), and antibiotics (Invitrogen, Paisley, UK). Removal of NeuAc from the cell surface was obtained by a 1-h incubation at 37 °C of cells with phosphate-buffered saline (PBS) containing neuraminidase from C. perfringens (from 125 to 500 milliunits/ml) and used for the various assays described below.

**Detection of NeuAc on Integrin \( \alpha_\beta_3 \)—GM7373 ECs (1 \times 10^6 cells/sample) were treated with neuraminidase (from 125 to 500 milliunits/ml), washed, scraped in 50 \( \mu \)l of 50 mM Tris-HCl, pH 7.4, containing 150 mM NaCl, 1% Nonidet P-40, 0.25% sodium deoxycholate, 1 mM PMSF, 4 mM amino-n-caproic acid,
**Endothelial αvβ3 Sialic Acid and HIV-1 Tat**

10 μg/ml leupeptin, 1 mM Na3VO4, 50 mM NaF (radioimmuno-precipitation modified lysis buffer) and centrifuged (10 min at 12,000 rpm). Cell extracts (30-μg aliquots) were analyzed on nonreducing SDS-6% PAGE followed by Western blot (WB) with anti-αv or anti-β3 antibodies. Human purified αvβ3 (100 ng) was incubated for 1 h at 37 °C with neuraminidase (125 milliunits/ml in PBS) and used as control.

For immunoprecipitation analysis, GM7373 EC cultures were lysed in radioimmunoprecipitation modified lysis buffer and centrifuged (10 min at 12,000 rpm). Cell extracts (400 μg) were incubated for 1 h at 25 °C with biotinylated MAA (1 μg/sample) and for an additional 16 h at 4 °C with streptavidin-Sepharose (30 μl/sample), centrifuged (1 min at 3,000 rpm), and analyzed on nonreducing SDS-6% PAGE followed by WB with anti-αv or anti-β3 antibodies. Human purified αvβ3 integrin (250 ng) incubated with biotinylated MAA was used as control.

**Cell Adhesion Assay**—Adhesion assay was performed with GM7373 ECs on polystyrene nontissue culture microtiter plates coated with Tat, with the αvβ3-ligand FN, or with the αvβ3 ligand fibrinogen, as described (30).

**Immunocytochemistry**—GM7373 ECs (40,000/cm²) were allowed to adhere to glass coverslips coated with Tat or FN for 4 h in DMEM containing 1% FCS. Cells were then treated with neuraminidase (125 milliunits/ml), washed, fixed, permeabilized, saturated (27), stained for actin by a 30-min incubation at room temperature with 0.9 μg/ml TRITC-phalloidin in PBS containing 3% BSA (PBS/BSA), co-stained for paxillin by a 1-h incubation at room temperature with anti-paxillin antibody (1:800 in PBS/BSA), further incubated for 45 min at room temperature with FITC-conjugated anti-mouse IgG (1:200 in PBS/BSA), and photographed under an Axiosplan 2 microscope equipped for epifluorescence (Carl Zeiss, Gottingen, Germany).

**FAK, ERK1/2, and VEGFR2 Phosphorylation Analysis**—Confluent GM7373 EC cultures were maintained in serum-free DMEM for 16 h, detached, and resuspended in DMEM 1% FCS. Aliquots of 1,000,000 cells were treated with neuraminidase (125 milliunits/ml) and washed, fixed, permeabilized, saturated, and stained for actin by a 30-min incubation at room temperature with 0.9 μg/ml TRITC-phalloidin in PBS containing 3% BSA (PBS/BSA), co-stained for paxillin by a 1-h incubation at room temperature with anti-paxillin antibody (1:800 in PBS/BSA), further incubated for 45 min at room temperature with FITC-conjugated anti-mouse IgG (1:200 in PBS/BSA), and photographed under an Axiosplan 2 microscope equipped for epifluorescence (Carl Zeiss, Gottingen, Germany).

**EC Proliferation Assay**—GM7373 ECs (25,000/well) were seeded onto Tat- or FN-coated polystyrene nontissue culture microtiter plates and incubated for 4 h in DMEM 1% FCS. Then, cells were treated with neuraminidase (125 milliunits/ml) and further incubated for 24 or 48 h in DMEM, 0.4% FCS containing 5-bromo-2-deoxyuridine in the presence of MAA or UE (62 nm). Incorporation of 5-bromo-2-deoxyuridine was detected with the colorimetric cell proliferation ELISA kit (Roche Applied Science, Mannheim, Germany).

**EC Membrane Ruffling and Wound-healing Assays**—Confluent cultures of GM7373 ECs were allowed to adhere to Tat- or FN-coated 35-mm polystyrene nontissue culture plates, treated with neuraminidase (125 milliunits/ml), wounded with a rubber policeman, and incubated with DMEM, 0.4% FCS for 3 or 48 h for membrane ruffling and wound-healing assay, respectively. Alternatively, neuraminidase-untreated ECs were incubated as described above in the presence of MAA or UE (62 nm). For membrane ruffling assay, at the end of the 3-h incubation, ECs were stained for nuclei (DAPI), immunostained for fascin (by a 1-h incubation with anti-fascin antibody followed by a further 45-min incubation with TRITC-conjugated anti-mouse IgG antibody), and photographed under an Axioscan 2 microscope equipped for epifluorescence (Carl Zeiss). Then, the number of ruffling-positive cells was counted. For the wound-healing assay, at the end of the 48-h incubation, wounded monolayers were photographed under the inverted microscope. The extent of wound repair (due to both EC migration and proliferation (31)) was evaluated by measuring the area of the wound by computerized image analysis using the Image Pro-Plus analysis system (Media Cybernetics).

**MTS Assay**—Tat-adherent GM7373 ECs were treated with neuraminidase or lectins as described above and then incubated for 24 or 48 h in DMEM, 0.4% FCS. Then, cell viability was assessed by using the CellTiter 96 AQueous kit from Promega (Madison, WI).

**Human Artery Ring Sprouting Assay**—This assay was performed as already described (32). Briefly, 1-mm-thick human umbilical artery rings were embedded in fibrin gel and cultured in human EC serum-free medium (Invitrogen) in the absence or in the presence of Tat (5 nM) and the lectins MAA or UE (62 nM). After 6 days, rings were photographed at 100× magnification using an AxioVert 200M microscope equipped with a 20× objective (LD A PLAN 20/0.30PH1, Zeiss), and EC sprouts were counted.

**RESULTS**

**NeuAc Is Associated with Endothelial αvβ3 Integrin**—To evaluate the presence of NeuAc on glycan(s) linked to αvβ3 of the endothelial surface, ECs were lysed, immunoprecipitated with the NeuAc-binding lectin MAA, and blotted with anti-αv or anti-β3 antibodies. As shown in Fig. 1A, MAA binds to both αv and β3 subunits, similarly to the α subunit from human placenta integrin. In a second set of experiments, ECs were treated with neuraminidase (500 milliunits/ml), lysed, and analyzed by WB with anti-αv or anti-β3 antibodies. Neuraminidase treatment causes a decrease of the molecular mass of both αv (from 134 to 126 kDa) and β3 (from 70 to 67 kDa). Similar results were obtained with αvβ3 purified from human placenta (Fig. 1B). The
NeurAc Is Involved in \( \alpha_v\beta_3 \)/Tat Interaction—Tat binds to \( \alpha_v\beta_3 \) (25). The contribution of the RGD motif and of the basic domain to \( \alpha_v\beta_3 \) binding and cell-adhesive capacity was here characterized by two experimental approaches. First, the mutants GST-Tat 1e (in which the RGD sequence has been deleted) and GST-Tat R–A (in which the arginine residues of the basic domain have been substituted with alanine residues) were evaluated for their \( \alpha_v\beta_3 \) binding capacity in SPR. As shown in Fig. 2A and Table 1, the two mutants retain the capacity to bind to \( \alpha_v\beta_3 \) although decreased in respect to wild type GST-Tat. Second, a cell adhesion assay was performed in the presence of the peptide GRGDSPK (which competes with the RGD motif of Tat for the binding to \( \alpha_v\beta_3 \)) or in the presence of the K5 derivative KSNOSH (which inhibits EC adhesion to Tat (33) by binding to the basic domain of the transactivating factor). As shown in Fig. 2B, when assayed at the doses of 12 \( \mu M \) (GRGDSPK) and 75 nM (KSNOSH), the two compounds slightly inhibit EC adhesion to Tat, but when assayed together, slightly inhibit EC adhesion to Tat, but when assayed together,

![FIGURE 1. Detection of NeuAc on integrin \( \alpha_v\beta_3 \). A, GM7373 ECs or purified human integrin \( \alpha_v\beta_3 \) were immunoprecipitated with biotinylated MAA and analyzed in WB with anti-\( \alpha_v \) or anti-\( \beta_3 \) antibodies. B and C, alternatively, they were incubated with 500 milliunits/ml (mU/ml) (8) or with the indicated concentrations (C) of neuraminidase, lysed, and analyzed in WB with anti-\( \alpha_v \) or anti-\( \beta_3 \) antibodies. D, GM7373 ECs were treated with neuraminidase (neu., 500 milliunits/ml) washed, further incubated for the indicated periods of time in the absence of the enzyme (rescue), lysed, and analyzed in WB with anti-\( \alpha_v \) or anti-\( \beta_3 \) antibodies. In panels A, B, and D, the data shown are representative of 2–3 other experiments that gave similar results. In C, data are expressed as the percentage of the molecular mass of the \( \alpha_v \) subunit from neuraminidase-treated ECs with respect to the intact protein from untreated ECs.](image1)

![FIGURE 2. Role of \( \alpha_v\beta_3 \)-associated NeuAc in Tat interaction. A, overlay of blank-subtracted sensograms showing the binding of \( \alpha_v\beta_3 \) (10 nm) to sensor chip-immobilized wild type GST-Tat or its mutants GST-Tat 1e or GST-Tat R→A. B, GM7373 ECs were allowed to adhere to Tat in the absence (ctrl) or in the presence of the indicated inhibitors. C, overlay of blank-subtracted sensograms showing the binding of native or neuraminidase-treated (neu.) \( \alpha_v\beta_3 \) (12.5 nm) to sensor chip-immobilized synthetic Tat in the absence or in the presence of MAA (250 nm). The sensograms generated by injecting MAA (250 nm) or neuraminidase (500 milliunits/ml) on the Tat surface are also shown. D, saturation curves obtained using the values of resonance units (RU) bound at equilibrium from injection of increasing concentrations of native or neuraminidase-treated (neu.) \( \alpha_v\beta_3 \) (12.5 nm) to sensor chip-immobilized synthetic Tat in the absence or in the presence of MAA (250 nm). The sensograms generated by injecting MAA (250 nm) or neuraminidase (500 milliunits/ml) on the Tat surface are also shown. E, overlay of blank-subtracted sensograms showing the binding of native or neuraminidase-treated (neu.) \( \alpha_v\beta_3 \) (12.5 nm) to sensor chip-immobilized synthetic Tat in the absence or in the presence of MAA (250 nm). The sensograms generated by injecting MAA (250 nm) or neuraminidase (500 milliunits/ml) on the Tat surface are also shown.](image2)
Endothelial $\alpha_\beta_3$ Sialic Acid and HIV-1 Tat

TABLE 1

| Ligand                  | Analyte       | $K_d$ (nM) |
|-------------------------|---------------|------------|
| Synthetic Tat           | Native $\alpha_\beta_3$ | 19.9       |
| $\alpha_\beta_3$        | after neuraminidase treatment | 157.1     |
| GST-Tat wild type       | Native $\alpha_\beta_3$ | 40.3       |
| GST-Tat R→A             |               | 102.6      |
| GST-Tat 1e              |               | 136.2      |
| GST-Tat wild type       | Heparin       | 16.0       |

The values of dissociation constant ($K_d$) reported have been calculated by the Scatchard plot analysis of the steady-state SPR data in the different experimental conditions adopted. For a comparison, the $K_d$ of the Tat/heparin interaction previously calculated by Scatchard plot analysis of the steady-state SPR (33) is also reported.

FIGURE 3. Role of NeuAc in $\alpha_\beta_3$-dependent EC adhesion to Tat and cytoskeleton organization. A, GM7373 ECs were treated with the indicated concentrations of neuraminidase and subjected to cell adhesion assay on Tat or FN. Alternatively, cells were subjected to adhesion assay in the presence of anti-VEGFR2 antibody (400 μg/ml, white arrow) or with anti-α,β3 antibody (100 μg/ml, black arrow). B, GM7373 ECs were allowed to adhere to Tat or FN, incubated for 1 h with increasing concentrations of neuraminidase, and then further incubated in the absence of the enzyme for 24 h. C, GM7373 ECs were treated with PDMP (10 μM for 72 h), PPPP (1 μM for 48 h), or vehicle (ctrl) and subjected to cell adhesion assay on wells without coating (−) or coated with Tat or fibrinogen (FG). D, At the end of the incubations, adherent cells were counted. D, GM7373 ECs were treated with neuraminidase, allowed to adhere to Tat or FN, co-stained for nuclei (blue), paxillin (green), and actin (red), and photographed (630×). E, GM7373 ECs were incubated for 2 h at 37°C with increasing concentrations of MAA (circles) or UEA (triangles) and then allowed to adhere to Tat (black symbols) or FN (white symbols). F, GM7373 ECs were incubated for 2 h at 37°C with increasing concentrations of MAA and allowed to adhere onto the indicated GST-Tat proteins. In panels A–C, E, and F, each point is the mean ± S.E. of 3–4 independent experiments in duplicate (* = $p < 0.05$, ** = $p < 0.01$, with respect to untreated controls, Student’s t test).

they completely inhibit the same process. Taken together, these data indicate that both the RGD and the basic domain of Tat contribute to its cell-adhesive capacity, the presence of one of the two domains being enough to ensure a partial $\alpha_\beta_3$ binding and cell-adhesive capacity to Tat.

Besides $\alpha_\beta_3$, the basic domain also mediates the binding of Tat to the polyanionic heparin/heparan sulfate (28), the two interactions occurring with similar affinities (Table 1). These observations suggest that the basic domain of Tat, besides interacting with the negatively charged sulfated groups of heparin, may as well make contact with the negatively charged NeuAc residues of $\alpha_\beta_3$. To evaluate this possibility, two different experimental approaches were exploited. (i) We first evaluated the effect of neuraminidase and MAA on $\alpha_\beta_3$/Tat interaction. Preliminary SPR analyses demonstrated that neuraminidase treatment of the integrin, as well as the presence of MAA, significantly inhibits its interaction with Tat. It is important to note that MAA and neuraminidase do not bind directly to Tat (Fig. 2C). In a second set of experiments, increasing concentrations of native or neuraminidase-treated $\alpha_\beta_3$ were injected onto the Tat surface in the absence or in the presence of MAA. Then, the values of steady-state SPR data were used to generate the saturation curves shown in Fig. 2D. Scatchard plot analysis demonstrated that neuraminidase treatment, as well as MAA, decreases the affinity of the $\alpha_\beta_3$/Tat interaction (Table 1). Interestingly, the reduction of the affinity observed for $\alpha_\beta_3$/Tat interaction in the absence or in the presence of MAA (2.48 times) is in the same order of magnitude as the difference of the affinity measured for the interaction of $\alpha_\beta_3$ with wild type GST-Tat or with GST-Tat R→A (2.53 times) (Table 1), suggesting that MAA hampers the interaction of NeuAc residues of $\alpha_\beta_3$ to the basic domain of Tat. (ii) The two Tat mutants GST-Tat 1e and GST-Tat R→A where evaluated for their capacity to induce EC adhesion in the presence of MAA. Similarly to what was observed with synthetic Tat, at 250 nM, MAA partially inhibits the binding of $\alpha_\beta_3$ to wild type GST-Tat (Fig. 2E). At the same concentration, MAA exerts a weak inhibition on $\alpha_\beta_3$/GST-Tat R→A interaction (possibly because this occurs only via the RGD sequence), whereas it efficiently inhibits $\alpha_\beta_3$/GST-Tat 1e interaction (possibly because this can occur only via the basic domain) (Fig. 2E). Taken together, these data indicate that at low concentrations, MAA binds to NeuAc residues of $\alpha_\beta_3$, inhibiting the interaction of the integrin with the basic domain of Tat but leaving unaffected that with the RGD motif.

NeuAc Mediates $\alpha_\beta_3$-dependent EC Adhesion to Substrate-immobilized Tat—Substrate-immobilized Tat induces EC adhesion in an $\alpha_\beta_3$-dependent manner (25). Accordingly, by using specific anti-$\alpha_\beta_3$ or anti-VEGFR2 antibodies, here we confirmed that EC adhesion to Tat specifically depends on the integrin but not on VEGFR2 (Fig. 3A). Also, our unpublished experiments with silencing RNAs directed against enzymes of the biosynthetic pathway of heparan sulfate demonstrated that these receptors are not involved in EC adhesion to Tat (data not shown).
The removal of NeuAc from the EC surface by neuraminidase prevents EC adhesion to Tat, only slightly affecting that to the α5β1-ligand FN, here used as a control (Fig. 3A). Neuraminidase also causes the detachment of ECs already adhered to Tat over a 24-h period of incubation (Fig. 3B), without causing significant cell death (as assessed by MTS assay, data not shown).

Besides integrins, NeuAc is also associated with gangliosides. To evaluate the possible involvement of these structures in EC adhesion to Tat, the cells were treated with PDMP or PPPP (which prevent ganglioside biosynthesis without affecting intracellular levels of ceramide (34)) and then evaluated for their capacity to adhere to Tat. When used at doses that effectively hamper the expression of NeuAc-bearing gangliosides (34), PDMP and PPPP do not affect ECs adhesion to Tat or to fibrinogen (another α5β3 ligand here used as a control, Fig. 3C). These results rule out the possibility that ganglioside-associated NeuAc is responsible for the observed EC adhesion to Tat.

EC adhesion to Tat induces cytoskeleton organization with the assembly of actin stress fibers and focal adhesion plaques containing integrins and paxillin (35). We then evaluated the involvement of NeuAc in cytoskeleton organization of Tat-adherent ECs. When tested at concentrations that do not hamper EC adhesion (125 milliunits/ml), neuraminidase prevents the proper organization of actin stress fibers and paxillin-containing focal adhesion plaques. The effect is specific because neuraminidase did not alter focal adhesion plaque formation in FN-adherent ECs (Fig. 3D).

In a second set of experiments, we also evaluated whether MAA affects α5β3-dependent EC adhesion to Tat. As shown in Fig. 3E, MAA inhibits EC adhesion to Tat in a dose-dependent way. The inhibition is specific because MAA does not affect EC adhesion to FN and UEA (a lectin that specifically binds to α-linked fucose) does not affect EC adhesion to Tat. In agreement with the results of the SPR analyses, MAA inhibits EC adhesion to GST-Tat 1e with a potency (ID50 = 62 nM) that is higher than those with which it inhibits EC adhesion to GST-Tat R→A and to wild type GST-Tat (ID50 = 198 and 182, respectively) (Fig. 3F).

NeuAc Is Required for Signal Transduction Triggered by Tat/α5β3 Interaction in ECs—We evaluated the role of NeuAc in Tat/α5β3-dependent phosphorylation of FAK, a second messenger involved in Tat/α5β3-dependent EC cytoskeleton organization and proangiogenic activation (25, 36). Preliminary experiments confirmed that FAK undergoes phosphorylation in ECs adherent to Tat but not in ECs maintained in suspension or adherent to poly-1-lysine (an integrin-independent adhesive molecule). When used at 125 milliunits/ml (a dose that prevents cytoskeleton organization without hampering EC adhesion to Tat; Fig. 3), neuraminidase inhibits FAK phosphorylation in Tat-adherent ECs (Fig. 4A). Accordingly, at 62 nm, MAA, but not UEA, prevents FAK phosphorylation (Fig. 4B). It is relevant to note that at 62 nm, MAA does not hamper EC adhesion to Tat (Fig. 3E). Besides FAK, EC adhesion to Tat induces the activation of ERK1/2, another second messenger involved in Tat-dependent EC proangiogenic activation (37). Neuraminidase pretreatment (Fig. 4C) and MAA, but not UEA (Fig. 4D), prevent ERK1/2 phosphorylation in Tat-adherent EC.

In the same experimental conditions, ERK1/2 phosphorylation is inhibited by the specific α5β3 antagonist SCH221153 but not by its inactive analog SCH216687, indicating that ERK1/2...
activation is dependent, at least in part, on \( \alpha_3 \). Also, MAA does not affect ERK1/2 phosphorylation induced by phorbol myristate acetate in EC adherent to tissue culture plastic (Fig. 4E), suggesting that the specificity of the inhibitory effect exerted by MAA is likely due to its interaction with \( \alpha_3 \beta_3 \), which prevents the binding of the integrin to Tat (Fig. 2).

Besides integrin \( \alpha_3 \), the proangiogenic activity of Tat is also mediated by VEGFR2 activation (38). Interestingly, this receptor bears NeuAc residues (39). On these bases, we evaluated the effect of the removal of NeuAc residues of VEGFR2 on its phosphorylation driven by Tat. As shown in Fig. 4F, neuraminidase effectively removes NeuAc from VEGFR2, as assessed by the decrease of the molecular mass of the band corresponding to the receptor. However, neuraminidase treatment does not hamper VEGFR2 phosphorylation in response to Tat, which is instead even increased (Fig. 4F).

EC NeuAc Is Involved in \( \alpha_3 \beta_3 \)-dependent Proangiogenic Activity of Tat—EC adhesion, proliferation, and migration represent essential steps of neovascularization (40). Accordingly, ECs adherent to substrate-immobilized Tat are induced to migrate and proliferate in an \( \alpha_3 \beta_3 \)-dependent way (27). Preliminary experiments confirmed that Tat-adherent ECs proliferate more efficiently than those adherent to FN both at 24 h and at 48 h (Fig. 5A). Pretreatment with neuraminidase (125 milliunits/ml) inhibits the proliferation of Tat-adherent cells in a specific way because it leaves unaffected the basal proliferation of FN-adherent ECs or of ECs adherent on tissue culture plates (data not shown). Besides neuraminidase, MAA (62 nM), but not UEA (assayed at both 62 nM and 100 nM), inhibits the proliferation of Tat-adherent ECs (Fig. 5A).

Cell membrane ruffling, which precedes the migration of EC body, is considered a morphological phenotype of motile cells (41) and has been already exploited to characterize the migration of Tat-adherent ECs (27). As shown in Fig. 5D, when adherent to Tat or to FN, ECs rapidly form membrane ruffles at the edge of a wounded monolayer. Pretreatment with neuraminidase (125 milliunits/ml) inhibits membrane ruffling in Tat-adherent ECs and, to a lesser extent, in FN-adherent ECs (Fig. 5B). Accordingly, MAA (62 nM), but not UEA, inhibits membrane ruffling only in Tat- but not FN-adherent ECs (Fig. 5C).

The ability of a substrate-immobilized protein to stimulate proliferation and motility in adherent ECs leads to an increased capacity of a mechanically wounded EC monolayer to cover the denuded area, a biological activity referred to as “motogenic activity” that has been used as a surrogate marker of angiogenesis (31). Substrate-immobilized Tat induces motogenesis of adherent ECs in an \( \alpha_3 \beta_3 \)-dependent way (25).

When allowed to adhere to Tat, treated with neuraminidase (250 milliunits/ml), and wounded, ECs show a decreased motogenic activity. The effect is specific because, in the same experimental conditions, neuraminidase does not affect the motogenic activity of FN-adherent ECs (Fig. 6A). In this regard, it is important to recall that when added after EC adhesion has occurred, neuraminidase at 250 nM does not cause the detachment of Tat-adherent ECs (Fig. 3B) nor affect their viability over a 48-h period of time (data not shown). MAA (62 nM) inhibits the motogenic activity of Tat. The effect is specific because MAA does not affect viability of Tat-adherent ECs (data not shown) nor the motogenic activity of FN-adherent ECs (Fig. 6, B and C). Also, UEA is ineffective on Tat-adherent ECs (Fig. 6, B and C).

Tat induces neovascularization via \( \alpha_3 \beta_3 \) (27). We then decided to evaluate the involvement of NeuAc in Tat proangiogenic activity by using neuraminidase to remove NeuAc from VEGFR2.

![Figure 5. Role of NeuAc in Tat/\( \alpha_3 \beta_3 \)-dependent proliferation and migration of ECs. GM7373 ECs adherent to the indicated proteins were treated as follows. A, cells were treated with neuraminidase (neu., 125 milliunits/ml) and incubated in the absence of the enzyme for an additional 24 h. Alternatively, cells were directly incubated for 24 or 48 h with the indicated concentrations of MAA or UEA. B, cells were incubated in the absence (ctrl) or in the presence of neuraminidase (125 milliunits/ml), wounded, and further incubated for 30 min. C, cells were wounded and incubated for 30 min in the absence (ctrl) or in the presence of MAA or UEA (62 nM). At the end of the incubations, EC proliferation (A) or the number of ruffling-positive ECs (B and C) were evaluated. Each point is the mean ± S.E. of 3–4 independent experiments in duplicate (\(* p < 0.01, ** p < 0.001, *p < 0.01, *p < 0.001, \text{Student’s t test}\)). In panel A, white arrowheads point to the proliferation measured in cells adherent to FN. D, representative epifluorescence microphotographs (630×) of ECs at the edge of a wounded monolayer incubated in the absence (ctrl) or in the presence of MAA or UEA (62 nM) and stained with DAPI (blue) and with anti-fascin antibody (red). Arrows point to the most prominent ruffles.](image-url)

---

**Endothelial \( \alpha_3 \beta_3 \) Sialic Acid and HIV-1 Tat**
DISCUSSION

$\alpha_3\beta_3$ mediates EC adhesion and proangiogenic activation by binding cationic angiogenic growth factors including FGF2 and HIV-1 Tat (27, 30). On the other hand, NeuAc has so far been identified on $\alpha_3\beta_3$ of melanoma metastatic cell surface, where it regulates cell adhesion (18, 20). We thus decided to investigate whether NeuAc is also associated with $\alpha_3\beta_3$ integrin of ECs and whether it is involved in $\alpha_3\beta_3$/ Tat interaction and in the consequent proangiogenic activation of ECs. These possibilities were investigated by two complementary approaches, consisting of the use of neuraminidase from *C. perfringens* (an enzyme that removes NeuAc from the cell surface) and of MAA (a lectin that specifically binds NeuAc residues).

In ECs, the removal of $\alpha_3\beta_3$-associated NeuAc by neuraminidase is rapid and efficient, occurring in less than 2 h. The biochemical features of bacterial neuraminidase surely contribute to this (43), but so does the rapid recycling of integrins that are classically internalized to the early endosomes to be immediately returned to the plasma membrane (44, 45). In this way, endogenous $\alpha_3\beta_3$ remains continuously exposed at the cell surface, accessible to exogenous neuraminidase.

Fully sialylated $\alpha_3\beta_3$ integrins are re-exposed on ECs only 48 h after neuraminidase treatment. The long-lasting nature of $\alpha_3\beta_3$ desialylation is likely due to the fact that after de novo synthesis, integrins must undergo sialylation by sialyltransferases in the Golgi apparatus (46). Whatever its cause, the long-lasting desialylation of $\alpha_3\beta_3$ by neuraminidase is in agreement with the capacity of the enzyme to inhibit processes, such as EC proliferation and motogenesis, that occur over 24–48 h periods.

MAA immunoprecipitates both $\alpha_3$ and $\beta_3$ subunits from ECs, indicating the presence of $\alpha(2\rightarrow3)$-linked NeuAc on the two chains. We also obtained similar results with the lectin from *Sambucus nigra* that binds instead to $\alpha(2\rightarrow6)$-linked NeuAc (data not shown). Accordingly, neuraminidase from *C. perfringens*, which hydrolyzes both $\alpha(2\rightarrow3)$-linked and $\alpha(2\rightarrow6)$-linked NeuAc, causes a decrease of the molecular mass of both $\alpha_3$ subunit (~7 kDa, corresponding to about 25 NeuAc residues) and $\beta_3$ subunit (~3 kDa, corresponding to about 10 NeuAc residues). These data are in agreement with the observations that $\alpha_3$ and $\beta_3$ subunits possess 13 and 6 putative N-glycosylation sites, respectively, with biantennary structures (UniProt accession number P05106) (18).

Neuraminidase cleaves $\alpha(2\rightarrow3)$-linked NeuAc residues faster than $\alpha(2\rightarrow6)$ linkages (47). Accordingly, neuraminidase operates a complete removal of $\alpha_3\beta_3$-associated NeuAc only when used at high concentration (>250 milliunits/ml), whereas when used at suboptimal concentrations (125 milliunits/ml), desialylation of $\alpha_3\beta_3$ remains incomplete. Interestingly, when used at higher concentrations, neuraminidase directly prevents (and even disrupts) $\alpha_3\beta_3$-dependent EC adhesion to Tat (Fig. 3, A and B), whereas at lower concentrations, it leaves EC adhesion unaffected, although inhibiting Tat/ $\alpha_3\beta_3$-dependent proangiogenic activation of Tat-adherent ECs. Taken together, these data suggest that the complete removal of $\alpha_3\beta_3$-associated NeuAc prevents the binding of the integrin to Tat and the consequent EC adhesion. Instead, a partial removal of NeuAc...
residues allows an “unproductive” Tat/αβ3 interaction enough for cell adhesion, but that does not mediate the signal transduction cascade required for EC proangiogenic activation.

By removing NeuAc residues from αβ3, neuraminidase causes a decrease of the affinity of αβ3/Tat interaction and its inhibition (Fig. 2). Accordingly, by binding NeuAc residues of αβ3, MAA causes the same effects, also if at a lesser extent. The inhibition exerted by neuraminidase and MAA on αβ3/Tat interaction is mirrored by their capacity to inhibit several αβ3-dependent biological activities of Tat, connecting the two processes. In effect, MAA shares with neuraminidase the capacity to differently affect Tat/αβ3 interaction and consequent biological activities in a concentration-dependent way. At higher concentrations (125–250 nM), it directly inhibits αβ3-mediated EC adhesion to Tat, whereas at a lower concentration (less than 62 nM), it leaves cell adhesion unaffected, although inhibiting the αβ3-dependent signal transduction and proangiogenic activation of Tat-adherent ECs. Interestingly, MAA scarcely affects αβ3 interaction with (and EC adhesion to) GST-Tat R → A (in which the intact RGD motif mediates integrin interaction and EC adhesion), whereas it exerts a stronger inhibition on GST-Tat1e that, lacking the RGD motif, binds to integrin and mediates EC adhesion via its basic domain. These findings, together with the notion that αβ3 interaction occurs via both the RGD motif and the basic domain of Tat (25) (Fig. 2B), suggest that at appropriate concentrations, MAA succeeds in inhibiting the interaction of the basic domain of Tat with NeuAc residues of αβ3, leaving unaffected the RGD-dependent αβ3/Tat interaction. This generates an unproductive Tat/αβ3 interaction enough to promote cell adhesion but not adequate to trigger signal transduction and hence EC proangiogenic activation.

Besides integrin αβ3, a wide variety of cell surface sialoglycoproteins can be affected by neuraminidase and MAA, possibly impacting the phenomena reported here. Although we cannot completely rule out this possibility, it is, however, relevant to point out that neuraminidase does not significantly affect (or even increase) Tat-driven phosphorylation of VEGFR2, a tyrosine kinase receptor that bears NeuAc residues (39) and whose activation is required for Tat proangiogenic activity (38). Curiously, the proangiogenic fibroblast growth factor (FGF) receptor-1 also possesses NeuAc-bearing glycans whose removal leads to an increased capacity to bind its ligand FGF2 (48). Also, hypo- or desialylation of αβ3 integrin increases its affinity for the natural ligand FN (49). Relevant to this point, FN acts as entry receptors for several viruses (59), suggesting the possibility of blocking viral infection by means of NeuAc-binding lectin-like compounds or by NeuAc analogues. Relevant to this point, the NeuAc derivative NMSO3 has been demonstrated to exert a potent inhibition against HIV-1 (42).

In conclusion, the results presented in this study open up the possibility that modulation of integrin glycosylation could be a promising strategy for regulating angiogenesis and viral infection.

Acknowledgments—We thank Mauro Giacca (International Centre for Genetic Engineering and Biotechnology, Trieste, Italy) for the E. coli strain expressing the various GST-Tat mutants, Pasqua Oreste for the KSNOSH, Alessandra Armato for technical assistance, and Eugenio Monti and Marco Presta (University of Brescia) for helpful discussion.

REFERENCES

1. Urbinati, C., Chiodelli, P., and Rusnati, M. (2008) Polyanionic drugs and viral oncogenesis: a novel approach to control infection, tumor-associated inflammation, and angiogenesis. Molecules 13, 2758–2785
2. dos Santos, W. L., Rahman, J., Klein, N., and Male, D. K. (1995) Distribution and analysis of surface charge on brain endothelium in vitro and in situ. Acta Neuropathol. 90, 305–311
3. Vorbrodt, A. W. (1989) Ultracytochemical characterization of anionic sites in the wall of brain capillaries. J. Neurocytol. 18, 359–368
4. Travaglini, A., and Schauer, R. (1988) Structure, function, and metabolism of sialic acids. Cell. Mol. Life Sci. 54, 1330–1349
5. Schauer, R. (2009) Sialic acids as regulators of molecular and cellular interactions. Curr. Opin. Struct. Biol. 19, 507–514
6. Murean, V., and Simionescu, N. (1987) High and low molecular weight tracers for the electron microscopical detection of sialoglycoconjugates. Histochem. J. 19, 170–178
7. Welim, H. B., Thies, M., and Herken, R. (1989) Appearance of lectin-binding sites during vascularization of the primordium of the central nervous system in 10–12-day-old mouse embryos. Cell Tissue Res. 255, 627–630
8. Henry, C. B., and DeFouw, D. O. (1996) Distribution of anionic sites on microvascular endothelium of the chick chorioallantoic membrane. Tissue Cell. 28, 449–454
9. Doiron, A. L., Kirkpatrick, A. P., and Rinker, K. D. (2004) TGF-β and TNF-α affect cell surface proteoglycan and sialic acid expression on vas-
cells transformed in vitro by benzo(a)pyrene. J. Cell Physiol. 114, 328–338
30. Rusnati, M., Tanghetti, E., Dell’Era, P., Guandalini, A., and Presta, M. (1997) $\alpha_\beta_3$ integrin mediates the cell-adhesive capacity and biological activity of basic fibroblast growth factor (FGF-2) in cultured endothelial cells. Mol. Biol. Cell 8, 2449–2461
31. Lauder, H., Frost, E. E., Hiley, C. R., and Fan, T. P. (1998) Quantification of the repair process involved in the repair of a cell monolayer using an in vitro model of mechanical injury. Angiogenesis 2, 67–80
32. Mitola, S., Moroni, E., Ravelli, C., Andres, G., Belleri, M., and Presta, M. (2008) Angiopoietin-1 mediates the proangiogenic activity of the bone morphogenetic protein antagonist Dmr. Blood 112, 1154–1157
33. Urbani, C., Bugatti, A., Oreste, P., Zoppetti, G., Waltenberger, J., Mitola, S., Ribatti, D., Presta, M., and Rusnati, M. (2004) Chemically sulfated Escherichia coli KS polysaccharide derivatives as extravascular HIV-1 Tat protein antagonists. FEBS Lett. 586, 171–177
34. Kopitz, J., Bergmann, M., and Gabius, H. J. (2010) How adhesion/growth-regulatory galectins-1 and -3 attain cell specificity: case study defining their target on neoplasma cells (SK-N-MC) and marked affinity regulation by affecting microdomain organization of the membrane. JIBMB Life 62, 624–628
35. Urbani, C., Ravelli, C., Tanghetti, E., Belleri, M., Giacopuzzi, E., Monti, E., Presta, M., and Rusnati, M. (2012) Substrate-immobilized HIV-1 Tat drives VEGFR2/$\alpha_\beta_3$ integrin complex formation and polarization in endothelial cells. Arterioscler. Thromb. Vasc. Biol. 32, e25–e34
36. Angelucci, A., and Bologna, M. (2007) Targeting vascular cell migration as a strategy for blocking angiogenesis: the central role of focal adhesion protein tyrosine kinase family. Curr. Pharm. Des. 13, 2129–2145
37. Rusnati, M., Urbani, C., Musulin, B., Ribatti, D., Albini, A., Noonan, D., Marchisone, C., Waltenberger, J., and Presta, M. (2001) Activation of endothelial cell mitogen-activated protein kinase ERK1/2 by extravascular HIV-1 Tat protein. Endotheilum 8, 65–74
38. Albini, A., Soldi, R., Giunciglio, D., Giraudo, E., Benelli, R., Primo, L., Noonan, D., Salio, M., Camussi, G., Rockl, W., and Bussolino, F. (1996) The angiogenesis induced by HIV-1 Tat protein is mediated by the Fk/1 KDR receptor on vascular endothelial cells. Nat. Med. 2, 1371–1375
39. Nacev, B. A., Grassi, P., Dell’Era, P., Sarti, L., Pilla, M., and Liu, J. O. (2011) The antifungal drug itraconazole inhibits vascular endothelial growth factor receptor 2 (VEGFR2) glycosylation, trafficking, and signaling in endothelial cells. J. Biol. Chem. 286, 44045–44056
40. Folkman, J., and Klagsbrun, M. (1987) Angiogenic factors. Science 235, 442–447
41. Ridley, A. J., Paterson, H. F., Johnston, C. L., Diekmann, D., and Hall, A. (1992) The small GTP-binding protein rac regulates growth factor-induced membrane ruffling. Cell 70, 401–410
42. Terada, M., Fujita, S., Suda, I., and Mastico, R. (2005) Polysulfated sialic acid derivatives as anti-human immunodeficiency virus. Biomed. Pharma. 59, 423–429
43. Bouwstra, J. B., Deyl, C. M., and Vliegenthart, J. F. (1987) Purification and kinetic properties of sialidase from Clostridium perfringens. Biol. Chem. Hoppe Seyler 368, 269–275
44. Caswell, P. T., Vadrevu, S., and Norman, J. C. (2009) Integrins: masters and slaves of endocytic transport. Nat. Rev. Mol. Cell Biol. 10, 843–853
45. Roberts, M., Barry, S., Woods, A., van der Sluijs, P., and Norman, J. (2001) PDGF-regulated rab4-dependent recycling of $\alpha_\beta_3$ integrin from early endosomes is necessary for cell adhesion and spreading. Curr. Biol. 11, 1392–1402
46. Christie, D. R., Shaikh, F. M., Lucas, J. A., 4th, Lucas, J. A., 3rd, and Bellis, M. (1987) Glycoproteins and glycoconjugates in metastatic melanoma WM9 and WM239 cell lines. Biochim. Biophys. Acta 941, 9969–9978
47. Corfield, A. P., Higa, H., Paulson, J. C., and Schauer, R. (1983) The specificity of viral and bacterial sialidases for $\alpha_2$-$\omega_3$- and $\alpha_2$-$\omega_6$-linked sialic acids in glycoproteins. Biochim. Biophys. Acta 744, 121–126
48. Duchesne, L., Tissot, B., Rudd, T. R., Dell, A., and Fernig, D. G. (2006) N-Glycosylation of fibroblast growth factor receptor 1 regulates ligand and heparan sulfate co-receptor binding. J. Biol. Chem. 281, 27178–27189
49. Semel, A. C., Seales, E. C., Singhal, A., Eklund, E. A., Colley, K. J., and Bells, M.
Endothelial $\alpha_\beta_3$ Sialic Acid and HIV-1 Tat

S. L. (2002) Hyposialylation of integrins stimulates the activity of myeloid fibronectin receptors. J. Biol. Chem. 277, 32830–32836

Scatena, M., Almeida, M., Chaisson, M. L., Fausto, N., Nicosia, R. F., and Giachelli, C. M. (1998) NF-κB mediates $\alpha_\beta_3$ integrin-induced endothelial cell survival. J. Cell Biol. 141, 1083–1093

Monti, E., Bonten, E., D’Azzo, A., Bresciani, R., Venerando, B., Borsani, G., Schauer, R., and Tettamanti, G. (2010) Sialidases in vertebrates: a family of enzymes tailored for several cell functions. Adv. Carbohydr. Chem. Biochem. 64, 403–479

Renkonen, R., Mattila, P., Majuri, M. L., Räbinä, J., Toppila, S., Renkonen, J., Hirvas, L., Niittymäki, J., Turunen, J. P., Renkonen, O., and Paavonen, T. (1997) In vitro experimental studies of sialyl Lewis x and sialyl Lewis a on endothelial and carcinoma cells: crucial glycans on selectin ligands. Glycoconj. J. 14, 593–600

Sakarya, S., Rifat, S., Zhou, J., Bannerman, D. D., Stamatos, N. M., Cross, A. S., and Goldblum, S. E. (2004) Mobilization of neutrophil sialidase activity desialylates the pulmonary vascular endothelial surface and increases resting neutrophil adhesion to and migration across the endothelium. Glycobiology 14, 481–494

Lang, Z., Guerrera, M., Li, R., and Ladisch, S. (2001) Ganglioside GD1a enhances VEGF-induced endothelial cell proliferation and migration. Biochem. Biophys. Res. Commun. 282, 1031–1037

Rusnati, M., and Presta, M. (2006) Extracellular angiogenic growth factor interactions: an angiogenesis interactome survey. Endothelium 13, 93–111

Harduin-Lepers, A., Mollicone, R., Delannoy, P., and Oriol, R. (2005) The animal sialyltransferases and sialyltransferase-related genes: a phylogenetic approach. Glycobiology 15, 805–817

Brockhausen, I., Lehotay, M., Yang, J. M., Qin, W., Young, D., Lucien, J., Coles, J., and Paulsen, H. (2002) Glycoprotein biosynthesis in porcine aortic endothelial cells and changes in the apoptotic cell population. Glycobiology 12, 33–45

Auzzas, L., Zanardi, F., Battistini, L., Burreddu, P., Carta, P., Rassu, G., Curti, C., and Casiraghi, G. (2010) Targeting $\alpha_\beta_3$ integrin: design and applications of mono- and multifunctional RGD-based peptides and semi-peptides. Curr. Med. Chem. 17, 1255–1299

Stewart, P. L., and Nemerow, G. R. (2007) Cell integrins: commonly used receptors for diverse viral pathogens. Trends Microbiol. 15, 500–507