Cytokine-induced β-Galactoside α-2,6-Sialyltransferase in Human Endothelial Cells Mediates α2,6-Sialylation of Adhesion Molecules and CD22 Ligands

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Sialic acids decorating blood and cell surface proteins can play important roles in various biological processes. The inflammatory cytokines tumor necrosis factor-α (TNF-α) and interleukin-1, as well as bacterial lipopolysaccharide, can activate vascular endothelium, increasing expression of several surface glycoproteins. Here we show that treatment of cultured human endothelial cells (HEC) with TNF-α, interleukin-1, or lipopolysaccharide causes increased expression of the enzyme β-galactoside α-2,6-sialyltransferase (α2-6STN). TNF-α was most effective, inducing a 3.5-fold enhancement of cell-associated sialyltransferase activity by 72 h. In addition, activated HEC secreted a large portion of the induced sialyltransferase activity into the medium. Analysis of labeled HEC showed both a relative and an absolute increase of α2,6-linked sialic acid on N-linked oligosaccharides after TNF-α stimulation. This coincided with increased expression of endothelial glycoproteins bearing N-linked glycans with α2,6-linked sialic acid detected by the lectin Sambucus nigra agglutinin. The cytokine-induced endothelial cell adhesion molecules E-selectin, ICAM-1, and VCAM-1 are among these glycoprotein substrates for α2-6STN. These changes also correlated with a substantial increase in binding sites for CD22β, a mammalian lectin known to recognize oligosaccharides carrying multiple copies of α2,6-linked sialic acid. Northern analysis revealed increased levels of mRNA encoding α2-6STN. Thus, activation of endothelial cells during inflammatory and immunological processes may induce α2-6STN, which can participate in sialylation of other activation-dependent molecules.

Recent studies have demonstrated that specific terminal oligosaccharide sequences of glycoproteins and glycolipids participate in various adhesion events (1–4). The sialic acids are a family of closely related nine-carbon carboxylated sugars found at terminal positions of mammalian cell surface sugar chains (5). N-Acetylneuraminic acid (Neu5Ac) is the most common sialic acid and appears to serve as a biosynthetic precursor of the other family members. Due to their negative charge, sialic acids can inhibit cell-cell interactions in a nonspecific (5, 6) or specific (7) fashion. However, sialic acids can also act as ligands for cell-cell recognition. Several adhesion molecules bind to sugar chains with sialic acids as critical components (1–4, 8). The slectins, found on endothelium (E- and P-selectin), platelets (P-selectin), and leukocytes (L-selectin), can bind to ligands that include oligosaccharide structures such as sialyl Lewis-x (Neu5Acα2-3Galβ1,4GlcNAcβ1-2-4Galβ1,4GlcNAcβ1- (2–4)). Also, oligosaccharides containing α2,6-linked sialic acid are critical components of ligands for CD22, an immunoglobulin (Ig) superfamily member found on a subset of B cells (8–12), and the sequence Neu5Acα2-3Galβ1,3GalNAcβ-serves as a primary recognition unit for the macrophage-specific adhesion molecule, sialoadhesin (13, 14).

Sialyltransferases add sialic acid to terminal positions on the oligosaccharide chains of glycoproteins and glycolipids (15). β-Galactoside α-2,6-sialyltransferase (α2-6STN) specifically catalyzes the reaction, CMP - Neu5Ac + Galβ1,4GlcNAcβ1- → CMP + Neu5Acα2-6Galβ1,4GlcNAcβ1- (16). Within the Golgi complex, α2-6STN is found as a 47-kDa type II transmembrane protein (17). Hepatocytes also release a 41-kDa soluble form of α2-6STN resulting from proteolytic cleavage of the catalytic domain from its membrane anchor (17, 18). In addition, soluble α2-6STN is one of a subset of serum glycoproteins known as acute phase reactants that are elevated during a variety of pathological processes (19–21). Acute phase reactants are generally assumed to be derived from hepatocytes (20).

Activation of vascular endothelium by cytokines and bacterial products results in an orchestrated display of new cell surface glycoproteins (22–24). In the present study, we address the effects of endothelial activation on cell surface carbohydrates and demonstrate that inflammatory cytokines act on endothelial cells to induce increased expression of cellular α2-6STN. This enhances the amount of α2,6-linked sialic acids as well as its display on newly activated adhesion molecules and on ligands of CD22β.

EXPERIMENTAL PROCEDURES

Endothelial Cell Culture and Stimulation—Primary cultures of HEC from Clonetics Corp. (San Diego, CA) were grown in dishes coated with 0.1% gelatin (Fisher, Pittsburgh, PA) using Medium 199 (Life Technologies, Invitrogen) with 20% fetal calf serum (FCS; Hyclone Laboratories, Logan, UT), 50 μg/ml endothelial cell growth supplement, and 100 μg/ml porcine heparin (Sigma), and subcultured using trypsin/versene (Life Technologies, Inc.) – II-1, interleukin-1; LPS, lipopolysaccharide; mAb, monoclonal antibody; PBS, phosphate-buffered saline; PAGE, polyacrylamide gel electrophoresis; TBS, Tris-buffered saline, TNF-α, tumor necrosis factor-α; VCAM-1, vascular cell adhesion molecule-1; BSA, bovine serum albumin; HPLC, high performance liquid chromatography.

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Cytokine-induced α2,6-Sialyltransferase in Endothelial Cells

R. M. Miquel, J. S. Badger, D. J. P. Paulson, Cytel Corp., San Diego, CA. After sialidase treatment, the radiactivity of remaining [3H]sialylated AGP was measured by using a filtration procedure as described above. Under the conditions used, the Newcastle disease virus sialidase released 100% of the α2,3-linked and less than 2% of the α2,6-linked sialic acid, whereas the A. ureafaciens sialidase released 100% of both. These specificities were unchanged after binding to sialyltransferase activity from HEC, but the amount of cell lysate protein or pure α2-6STN (rat liver-derived; TosoHaas), as described previously (30, 31). Although this system can detect most major forms of substituted sialic acids (30, 31), the major sialic acid in isolated N-linked oligosaccharides from resting and TNF-stimulated HEC was Neu5Ac. Therefore, quantification of the released sialic acid was done with known standards of derivatized Neu5Ac. The ratio of α2,3 and α2,6 linkage of sialic acids on the same preparations of HEC were isolated as described above (10). Sialidase-released [3H]sialic acids were purified away from desialylated HEC-linked oligosaccharides on a Bio-Gel P-4 (Bio-Rad) column in 0.1 M ammonium formate, pH 6.5. The radioactivity of eluted [3H]sialic acids was monitored. Radioactivity released by Newcastle disease virus sialidase (α2,3-linked sialic acids) or A. ureafaciens sialidase (α2,3- and α2,6-linked sialic acids) was subtracted from the background activity in reactions including exogenous acceptors. Results are expressed as nmol of CMP-Neu5Ac transferred/mg of HEC protein/1 h. The specific activity of conditioned medium samples was calculated as the total enzyme activity in conditioned medium minus the activity of Medium 199, 20% FCS alone divided by the total HEC protein content in the particulate dish.

Assessment of Sialyltransferase Activity by Lectin Precipitation and Sialidase Digestion of [3H]Sialylated AGP—HEC were incubated with or without 200 units/ml TNF-α for 72 h, and the cell lysate and conditioned medium samples were prepared as described above. Sialyltransferase activity (4 h at 37 °C) were performed under a constant current (1.7 mA/cm²) in the blotting buffer containing 40 mM EDTA for 13 h at 37 °C. Following glycosidase treatment of immobilized proteins was performed as follows. First 2 ml of effluent was discarded, and the next 1.5 ml containing [3H]sialylated AGP was collected. The isolated [3H]sialylated AGP (0.5 ml) was incubated with 15 μl of agarose conjugated with Sambucus nigra agglutinin (E. Y. Laboratories, Inc., San Mateo, CA) for 12 h at 4 °C. After five cycles of PBS washing and centrifugation, lectin-bound glycoproteins were eluted with 0.2 M lactose in PBS and the radioactivity was counted. S. nigra agglutinin-bound radioactivity was not eluted with PBS alone. In some cases, [3H]sialylated AGP (100 μl) was prepared and isolated as described above, with the same volume of 0.1 M ammonium acetate (to give a final pH of 5.4) and then digested with 20 millunits of Arthrobacter ureafaciens sialidase (Boehringer Mannheim) for 12 h at 37 °C. A separate aliquot was mixed with the same volume of 0.2 M sodium cacodylate (to give a final pH of 6.9) and digested with 6 millunits of Newcastle disease virus sialidase as described above. The mixture was centrifuged at 12 h at 37 °C. The A. ureafaciens sialidase cleaves α2,3- and α2,6-, and α2,8-linked sialyl residues, whereas the Newcastle disease virus sialidase hydrolyzes predominantly α2,3- and α2,8-linked sialyl residues. The specificities were validated by digesting AGP, which had been purified from sialidase-AGP (asialo-AGP; prepared by mild acid hydrolysis of human AGP (Sigma)) as a control. Under these conditions, incorporation was linear with respect to incubation time as well as to the amount of cell lysate protein or pure α2,6STN (rat liver-derived; Boehringer Mannheim). The reactions were conducted by addition of 1 ml of ice-cold 5% phosphotungstic acid in 2 N HCl. Reaction mixture were applied to 2.4-cm Whatman filter discs (GF/C), which were then washed twice with 5% trichloroacetic acid. Filters were rinsed with water and ethanol, dried, and processed for scintillation counting. Endogenous activity (determined in the absence of exogenous acceptors, usually below 5%) was subtracted from the exogenous acceptor activity in reactions including exogenous acceptors. Results are expressed as pmol of CMP-Neu5Ac transferred/mg of HEC protein/1 h. The specific activity of conditioned medium samples was calculated as the total enzyme activity in conditioned medium minus the activity of Medium 199, 20% FCS alone divided by the total HEC protein content in the particulate dish.
Immuno precipitation of Endothelial Cell Adhesion Molecules—HEC
lysates (0.7 mg of protein; ~1 ml) were precloured using protein A-Sepharose (Pierce Chemical Co.) and incubated for 1 h at 4 °C with one of the following monoclonal antibodies (mAbs): anti-E-selectin mAb H118/7 (IgG2a; ammonium sulfate-precipitated Ig, 4 µg (33)), anti-ICAM-1 mAb E17 (IgG2a; purified Ig, 2 µg (49, 35), and anti-ICAM-1 mAb 3G1 (ammonium sulfate-precipitated Ig, 4 µg (34, 35)). Protein A-Sepharose was added and the mixture was incubated for 1 h at 4 °C. After centrifugation at 10,000  x  g for 3 min, the supernatant was collected. The Sepharose was washed with lysine buffer and the immunoprecipitated proteins were eluted by boiling in Laemmli sample buffer. Samples were analyzed using SDS-PAGE and stained with S. nigra agglutinin as described above.

Assay for CD22-Immunoglobulin Chimera (CD22-Ig) Binding to HEC—Development and characterization of a soluble CD22-Ig has been described previously (8, 10). Confluent HEC in 96-well plates were stimulated for 48 h. After washing 3 times with Hanks’ balanced salt solution, cells were incubated with 5 µg/ml of CD22-Ig in Hanks’ balanced salt solution, 1% BSA for 2 h at 4 °C. The cells were washed with Hanks’ balanced salt solution and incubated with peroxidase-conjugated goat anti-human IgG Fe fragment-specific antibody (Jackson Immunoresearch Laboratories, Inc., West Grove, PA) at 1/6,000 dilution. After 45 min at 4 °C, the plates were washed 3 times with a balanced salt solution followed by the addition of 0.8 mg/ml of o-phenylenediamine (Sigma) in 50 mM sodium citrate, 50 mM sodium phosphate buffer, pH 5.0, containing 0.015% (v/v) H2O2 (200 µl/well). The reaction was stopped by addition of 4 x H2SO4 (50 µl/well), and the plates were read at 490 nm using a Vmax microplate reader (Molecular Devices, Menlo Park, CA). Data were expressed as Δabs value after subtracting from the background value (obtained in the presence of goat antibody alone).

Northern Analysis—Total cellular RNA was prepared from HEC grown in 150-mm diameter dishes according to the method of Chomczynski and Sacchi (36). Total RNA (10 µg) was fractionated on a 1.5% agarose gel containing formaldehyde and transferred onto nylon membranes (GeneScreen Plus; DuPont NEN). After immobilization of the RNA by UV cross-linking, hybridization was performed overnight at 42 °C in 50% formamide, 4 x SSC (standard saline citrate; 15 mM sodium citrate and 150 mM NaCl), 5 x Denhardt’s solution, 50 µg/ml HEPES pH 7.3, 0.5% SDS, and 200 µg/ml denatured herring sperm DNA. Human α2-6STDN and β-actin cDNA (a gift from Dr. J. Lawler, Boston, MA) were labeled with α-[32P]dCTP (DuPont NEN, 300 Ci/nmol) using a random primer labeling system. After hybridization, the membrane was washed twice with 1 x SSC containing 0.5% SDS at room temperature, rinsed twice with 0.1 x SSC containing 0.1% SDS at 50 °C, and studied by autoradiography.

RESULTS

Activation of HEC Increases Sialyltransferase Activity—Endothelial sialyltransferase activity was assessed by incorporation of label from CMP-[14C]Neu5Ac into acceptor glycoproteins. TNF-α treatment resulted in a time-dependent increase in cell-associated activity directed against the exogenous acceptor asialofetuin (Fig. 1A). Induction of enzymatic activity was detectable by 6–12 h and continued up to 72 h (since some detachment of cells occurred beyond 72 h, we did not follow activity at later time points). The response was dose-dependent, with a half-maximal effect at 5 units/ml of TNF-α (data not shown). HEC incubated in control medium demonstrated little or no increase in cell-associated activity during this period. Abundant sialyltransferase activity was found in the conditioned medium of the HEC by 48–72 h and was much increased in media from TNF-stimulated monolayers (Fig. 1B). As shown in Table I, treatment of HEC with TNF-α for 72 h resulted in a 3- to 5-fold increase in cell-associated sialyltransferase activity when assessed either with asialofetuin, which contains three triantennary complex-type N-linked glycans and three O-linked glycans (37), or with asialo-AGP, which contains only N-linked glycans (38). LL-1 as well as bacterial endotoxin (LPS) also stimulated increases in activity in cells (Table I) and in conditioned medium (data not shown) using both acceptors.

Induced HEC Sialyltransferase Adds α2,6-Linked Sialic Acid to Acceptor Glycoproteins—The majority of cell surface glycoproteins contain sialic acids linked α2,3 or α2,6 to Gal or GalNAc. Certain cells also contain extended chains of α2,8-linked sialic acid (polysialic acid) (7). These different linkages are generated by distinct sialyltransferases. The linkage specificity of the induced endothelial sialyltransferase activity was examined using specific lectins and sialidases. The lectin S. nigra agglutinin recognizes α2,6-linked sialic acid but not α2,3-linked sialic acid (39). Incorporation of [14C]sialic acid into asialo-AGP by TNF-induced (72 h) HEC sialyltransferase increased S. nigra agglutinin-precipitable counts (cell lysates, 4-fold; conditioned medium, 2.5-fold) compared with the sialyltransferase activity of resting HEC. The [14C]sialylated AGP was treated with sialidases from A. ureafaciens, which hydrolyzes α2,3-, α2,6-, and α2,8-linked sialic acid, or with Newcastle disease virus sialidase, which cleaves α2,3- and α2,8-linkages (28). As shown in Fig. 2, A. ureafaciens sialidase completely removed the sialic acids from the [14C]sialylated AGP products, whereas Newcastle disease virus sialidase had little or no effect. Thus, the measured endothelium-derived sialyltransferase activity adds predominantly α2,6-linked sialic acid to the acceptor.

Effect of TNF-α on the Content of α2,3- and α2,6-Linked Sialic Acid in N-Linked Oligosaccharides of Glycoproteins—N-Linked oligosaccharides were released by peptide-N-glycosidase F from total glycoproteins of resting or TNF-stimulated HEC labeled with [3H]glucosamine for 72 h. The total amount and ratio of α2,3- to α2,6-linked sialic acids in the released oligosaccharides were examined as described under “Experimental Procedures.” As summarized in Table II, the N-linked oligosaccharides carried a surprisingly large fraction of their
were treated with buffer alone and isolated on PD-10 columns. Aliquots (100 pl) of the [14C]sialylated columns, the mean activity remaining with [14C]sialylated AGP was then measured. Data are cell lysate virus sialidase HEC were incubated with or without 200 units/ml TNF-a for 72 h, and [14C]Sialylated AGP was prepared from asialo-AGP using these samples (hatched columns) or A. ureafaciens sialidase (open columns), as described under "Experimental Procedures." The radioactivity remaining with [14C]sialylated AGP was then measured. Data are the mean ± S.D. from a representative measurement performed in triplicate. Similar results were obtained in two additional experiments.

**TABLE II**

Effect of TNF-a stimulation on the content of a2,3- and a2,6-linked sialic acid in N-linked oligosaccharides of total HEC glycoproteins

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>a2,3-Linked</th>
<th>a2,6-Linked</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0.71 ± 0.04</td>
<td>0.76 ± 0.08</td>
</tr>
<tr>
<td>TNF-a</td>
<td>0.75 ± 0.06</td>
<td>1.07 ± 0.09*</td>
</tr>
</tbody>
</table>

*In N-linked oligosaccharides.

sialic acids in a2,3 linkage (endosialidase treatments ruled out the presence of extended a2,8-linked polysialic acids, data not shown). However, TNF-a activation induced a significant increase only in the relative and absolute amount of a2,6-linked sialic acid (Table II).

**Activation of HEC Increases Expression of Specific Glycoproteins with a2,6-Linked Sialic Acid—**HEC lysates, prepared from unstimulated and TNF-stimulated (24 h) monolayers, were resolved by SDS-PAGE, blotted onto membranes, and stained with two lectins: S. nigra agglutinin, which recognizes a2,6-linked sialic acid (39), and M. amurensis agglutinin, which recognizes sialic acid in a2,3 linkage to Galβ1,4GlcNAc (40). As shown in Fig. 3A, S. nigra agglutinin staining of endothelial glycoproteins was considerably enhanced by TNF-mediated activation. Notably, several glycoproteins of estimated molecular weight between 100 and 120 kDa stained prominently on blots prepared from activated endothelial cells but were barely detected on those prepared from control endothelial cells. At least two other glycoproteins (approximately 140 and 170 kDa) showed substantial staining in control preparations, which were enhanced in activated endothelial cells. A prominent band(s) at approximately 60 kDa showed little or no increase in S. nigra agglutinin staining after endothelial activation. Similar patterns of S. nigra agglutinin staining were observed after activation by IL-1 or LPS (data not shown). By contrast, TNF-a activation resulted in little or no effect on M. amurensis agglutinin staining, which predominantly recognized lower molecular weight glycoproteins (Fig. 3B).

**Fig. 2. Effect of sialidase treatment on the [14C]sialylated AGP.**

PEC were incubated with or without 200 units/ml TNF-a for 72 h, and cell lysate (A) and conditioned medium samples (B) were prepared. [14C]Sialylated AGP was prepared from asialo-AGP using these samples and isolated on PD-10 columns. Aliquots (100 pl) of the [14C]sialylated AGP were treated with buffer alone (black columns), Newcastle disease virus sialidase (hatched columns) or A. ureafaciens sialidase (open columns), as described under "Experimental Procedures." The radioactivity remaining with [14C]sialylated AGP was then measured. Data are the mean ± S.D. from a representative measurement performed in triplicate. Similar results were obtained in two additional experiments.

**Fig. 3. S. nigra agglutinin and M. amurensis agglutinin lectin blots of endothelial glycoproteins.** HEC were incubated with or without 200 units/ml TNF-a for 24 h. Cell lysate proteins were resolved by SDS-PAGE (4–20% polyacrylamide gradient) and blotted on membranes. After treatment with (+) or without (−) sialidase, the membranes were stained with S. nigra agglutinin (A) or M. amurensis agglutinin (B). Molecular weight markers were: myosin (200 kDa), phosphorylase b (97 kDa), BSA (69 kDa), ovalbumin (46 kDa), and carbonic anhydrase (30 kDa).

**Induced Endothelial Cell Adhesion Molecules Show Incorporation of a2,6-Linked Sialic Acid**—The cytokine-induced expression and apparent molecular weights (100–120 kDa) of certain S. nigra agglutinin-reactive glycoproteins suggested their identities. The endothelial adhesion molecules E-selectin (41), ICAM-1 (42), and VCAM-1 (34, 35, 43) were immunoprecipitated from HEC with specific mAbs and stained with S. nigra agglutinin after SDS-PAGE and blotting. As shown in Fig. 5A, each of these adhesion molecules could be stained with S. nigra agglutinin. In agreement with its known kinetics of expression (33), E-selectin and its associated S. nigra agglutinin reactivity were found in greater abundance in endothelial preparations that had been stimulated with TNF-a for 4 h.
Effect of TNF-α on the α2-6STN mRNA Levels in HEC—Northern blot analysis of total RNA from HEC (Fig. 7A) showed that TNF-α treatment caused a time-dependent increase in expression of α2-6STN mRNA. Increased mRNA was detected by 6 h, slightly preceding the increase in cellular sialyltransferase activity (Fig. 1A). The induction of mRNA continued up to 72 h. IL-1 and LPS also enhanced the expression of α2-6STN mRNA (Fig. 7B). The level of α2-6STN mRNA in unstimulated HEC was not altered during a 72-h incubation (data not shown). Thus, the increase of α2-6STN activity is associated with increase of the corresponding mRNA.

**DISCUSSION**

Endothelial cells undergo profound changes in response to cytokines like IL-1 and TNF (22–24). These changes allow the endothelium to help orchestrate the movement of fluid and leukocytes that characterizes inflammatory and immunological responses. Here we have demonstrated that cytokines also regulate endothelial glycosylation. Specifically, TNF-α and IL-1, as well as LPS, were shown to increase α2-6STN mRNA and sialyltransferase activity. The TNF-α-induced sialyltransferase activity was detected using as acceptor proteins asialofetuin, which contains both N- and O-linked glycans (37), and asialo-AGP, which contains only N-linked glycans (38). Treatments with linkage-specific sialidases and binding to the lectin S. nigra agglutinin demonstrated that most, if not all, of the sialic acid incorporated into asialo-AGP by sialyltransferase was in α2,6-linkage. Induction of endothelial α2,6-sialyltransferase activity was found to coincide with the following observations. First, TNF-α stimulation resulted in an enhancement of α2,6-linked sialic acid content in N-linked oligosaccharides isolated from glycoproteins (Table II). Second, TNF-α induced an increase in the S. nigra agglutinin lectin staining of certain endothelial glycoproteins (Fig. 3A). In most of these proteins, the binding of S. nigra agglutinin was susceptible to peptide-N-glycosidase F, indicating that the α2,6-linked sialic acids were on N-linked oligosaccharides. In contrast, the binding activity of M. amurensis agglutinin lectin (α2,3-linked sialic acid-specific) was not altered by TNF-α stimulation (Fig. 3B).
TNF-α activation resulted in increased binding of CD22-Ig, which recognizes specific N-linked oligosaccharide structures containing α2,6-linked sialic acid (10, 12). Taken together, these data indicate that cytokine activation of vascular endothelial cells increases the expression of α2-6STN, which participates in glycosylation of several glycoproteins. The expression patterns of other glycosyltransferases in activated vascular endothelium deserves attention in the future.

Although α2-6STN is synthesized as a transmembrane molecule and probably functions in this form within the Golgi apparatus (44), a 41-kDa active form is released by the action of a cathepsin D-like proteinase (21). Recent studies have identified this soluble α2-6STN as one of the acute-phase reactants (19). The elevation of α2-6STN in the blood of patients with various acute inflammatory conditions appears to depend on its inductive expression and active release from cells (19, 45, 46). Previous studies have shown that hepatocytes respond to glucocorticoids and hepatocyte-stimulating factor with a marked increase in production of a group of glycoproteins that includes α2-6STN (47, 48). In the present study, treatment of HEC with inflammatory mediators caused a substantial increase in α2-6STN enzyme activity in the conditioned medium. In fact, the amounts found in the conditioned medium at 72 h of stimulation were about 20 times that remaining in the cells (Fig. 1). Thus, it is likely that activated vascular endothelial cells contribute to the increased serum α2-6STN levels in acute phase reactions. In addition, normal serum levels of this enzyme may be explained, in part, by the constitutive synthesis and release of α2-6STN from endothelial cells.

Lectin blotting experiments revealed the presence of α2,6-linked sialic acid both on constitutively expressed proteins and on the cytokine-inducible adhesion molecules E-selectin, ICAM-1, and VCAM-1. Unactivated endothelial cells express little or no E-selectin. With activation, E-selectin expression is abundant by 4–6 h and declines toward basal levels by 24–48 h (33). Modest amounts of ICAM-1 and VCAM-1 are typically found on unstimulated cultured endothelial cells, and exposure to activating cytokines causes dramatic increases, which are sustained for several days (24). The kinetics of expression of endothelial sialyltransferase activity, as well as of the mRNA encoding α2-6STN, are similar to those of ICAM-1 and VCAM-1. Unstimulated cells were found to contain low levels of mRNA for α2-6STN and low levels of sialyltransferase activity. TNF-α treatment resulted in large increases that were first observed after 6–12 h and continued through 72 h. Thus, cytokine-activated endothelial cells coordinately express adhesion molecules and α2-6STN that contributes to their post-translational modification. The constitutively expressed α2-6STN is likely to be responsible for sialylation of induced molecules during the early hours of activation and, therefore, may be particularly important for E-selectin. It is also likely that the induced α2-6STN contributes to the sialylation of other induced or noninduced glycoproteins (140- and 170-kDa proteins in Fig. 3A) or of glycolipids in the activated endothelial cells. Considerable changes have been reported in the expression of glycoproteins in migrating endothelial cells (49) and of glycosphingolipids in interferon-γ-treated HEC (50).

In human B lymphocytes, sialyltransferase activity is up-regulated, and soluble enzyme is released into the medium during activation (51). Expression of the α2-6STN in B cells has been reported to regulate the generation of multiple cell surface differentiation antigens, such as CDw75 and CD76 (52, 53). The induced expression of α2-6STN on activated endothelium may also have functional consequences. First, the present study demonstrated an increase of CD22-Ig binding to cytokine-activated endothelial cells in an α2,6-linked sialic acid-dependent manner (Fig. 6), suggesting that cytokine-induced α2-6STN contributes to generation of ligand(s) for CD22. Since CD22 is expressed on resting mature B cells and is thought to play a role in cell-cell interactions and B cell activation (54), it is tempting to speculate that CD22 on B cells may bind to cytokine-activated vascular endothelium. In this regard, our recent work indicates that the number and distribution of α2,6-linked sialic acids can dramatically alter the recognition of oligosaccharides...
by CD22 (12). This may also explain why a relatively small increase (1.4-fold) in the amount of α2,6-linked sialic acids after TNF stimulation (Table 1) causes such a marked increase (>4-fold) in CD22-Ig binding. Also, it has been suggested that L-selectin (expressed on leukocytes (3)) can recognize a cytokine-inducible and sialidase-sensitive ligand on endothelial cells (55). The possibility that α2-6STN contributes to the generation of ligand(s) for L-selectin deserves attention. Finally, increasing evidence suggests that oligosaccharides modify the expression and function of a variety of cell surface and soluble proteins (56). Ultimately, studies of activation-induced changes in endothelial glycosylation must be interpreted in the context of the multifaceted changes that occur at vessel wall during inflammatory and immunological processes.

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