Tumor Necrosis Factor α Increases the Expression of Glycosyltransferases and Sulfotransferases Responsible for the Biosynthesis of Sialylated and/or Sulfated Lewis x Epitopes in the Human Bronchial Mucosa*

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There is increasing evidence that inflammation may affect glycosylation and sulfation of various glycoproteins. The present study reports the effect of tumor necrosis factor α (TNF-α), a proinflammatory cytokine, on the glycosyl- and sulfotransferases of the human bronchial mucosa responsible for the biosynthesis of Lewis x epitope and of its sialylated and/or sulfated derivatives, which are expressed in human bronchial mucins. Fragments of macroscopically normal human bronchial mucosa were exposed to TNF-α at a concentration of 20 ng/ml. TNF-α was shown to increase α1,3-fucosyltransferase activity as well as expression of the two α1,3-fucosyltransferase genes expressed in the human airway, FUT3 and FUT4. It had no influence on α1,2-fucosyltransferase activity or expression. It also increased α2,3-sialyltransferase activity and the expression of ST3Gal-IV and, more importantly, ST3Gal-III and both N-acetylglucosamine 6-O-sulfotransferase and galactose 3-O-sulfotransferase. These results are consistent with the observation of oversialylation and increased expression sialyl-Lewis x epitopes on human airway mucins secreted by patients with severe lung infection such as those with cystic fibrosis, whose airways are colonized by Pseudomonas aeruginosa. However, other cytokines may also be involved in this process.

Human bronchial mucins represent a very broad family of polydisperse high molecular weight glycoproteins that are part of the innate airway immunity. Apomucins, which correspond to their peptide part, are encoded by at least six mucin genes (MUC1, MUC2, MUC4, MUC5B, MUC5AC, and MUC7). Bronchial mucins are highly glycosylated and contain from one single to several hundreds of carbohydrate chains. These carbohydrate chains that cover the apomucins are extremely diverse, adding to the complexity of these molecules. Currently, more than 150 different O-linked carbohydrate chains have been described, and the mucins from a single individual probably contain a few hundred different carbohydrate chains (1). Due to their wide structural diversity forming a combination of carbohydrate determinants, as well as their location at the surface of the airways, mucins may be involved in multiple interactions with microorganisms and are very important in the protection of the underlying airway mucosa.

The biosynthesis of these carbohydrate chains is a stepwise process involving many glycosyl- and sulfotransferases. The only structural element shared by all mucin glycan chains is an N-acetylgalactosamine residue linked to a serine or threonine residue of the apomucin. The nonreducing end of the chains, which corresponds to the termination of the biosynthetic process, may bear different carbohydrate structures, such as blood groups A, B, or O determinants; H and sulfated H determinants; and Lewis a, Lewis b, Lewis y, and various derivatives of the Lewis x epitope. The synthesis of these different terminal determinants involves different pathways utilizing a variety of transferases (1, 2).

Bronchial mucins may express the Lewis x epitope as well as its sialylated and/or sulfated derivatives. Some of these determinants are ligands for L-selectin (3). The biosynthesis of these different Lewis x derivatives involves four types of transferases: (i) α2,3-sialyltransferase, (ii) α1,3-fucosyltransferase, (iii) galactose-3-O-sulfotransferase, and (iv) N-acetylgalactosamine-6-O-sulfotransferase (Fig. 1).

Although bronchial mucins secreted by patients suffering from cystic fibrosis or chronic bronchitis share various epitopes such as the sialyl-Lex1 and sulfo-Lex determinants (4–8), the sulfo-sialyl-Lex epitope has been mostly characterized in mucins secreted by patients suffering from cystic fibrosis (8), in agreement with the oversialylation of mucins observed in this disease.

Recently, differences have been observed in the glycosylation of bronchial mucins secreted by patients suffering from bronchial diseases according to the severity of bacterial infection (9). These observations suggest that chronic and severe inflammation of the airway mucosa may be responsible for increased sialylation and expression of sialyl-Leα epitope on the carbohydrate chains of these mucins.

There is increasing evidence for a link between inflammation and glycosylation. Since the original work describing increased activity of several glycosyltransferases of the rat liver during turpentine-induced inflammation (10), many modifications in the glycosylation of acute-phase glycoproteins have been ob-

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1 The abbreviations used are: Leα, Lewis x; CF, cystic fibrosis; HPAEC, high performance anion-exchange chromatography; PAD, pulsed amperometric detection; ST3Gal, CMP-NeuAc:Galα2,3-NeuAc transferase; FUT, GDP-Fuc:Galα1,2 (or 3)-Fuc transferase; TNF-α, tumor necrosis factor α; GlcNAc, N-acetylgalactosamine; IL, interleukin; Th, T helper; TBE, Tris-borate electrophoresis buffer.
served during acute and chronic inflammation. In humans, inflammation induces modifications of the N-glycan chains of different acute-phase glycoproteins, such as α1-acid glycoprotein (11–13), haptoglobin (14, 15), or α1-antitrypsin (16). These modifications may differ according to the early or late phase of acute inflammatory response (17) or the stage of the disease (18). Increased fucosylation and increased expression of sialyl-Le^a^ epitopes have been observed in α1-acid glycoprotein (11, 17, 19): these modifications seem to be related to an induction of the fucosyltransferase FUT6 in the liver (20). Monokines may regulate the glycosylation of acute-phase proteins (21). As a matter of fact, IL-1β and TNF-α affect the glycosylation of rat α1-acid glycoprotein. IL-1 and IL-6 change the glycosylation of human α1-acid glycoprotein produced by hepatocytes (22, 23), whereas transforming growth factor β has an effect opposite to that produced by IL-6 (24).

Other cells, such as neutrophils, T cells, endothelial cells involved in inflammation, and several epithelial tumor cells, have the machinery to synthesize the sialyl-Le^a^ determinant, a selectin ligand (25–27). Activated Th1 CD4 T cells, which bind to P-selectin and migrate to inflamed tissue, up-regulate α2,3-sialyltransferase (ST3Gal-IV) and α1,3-fucosyltransferase FUT7 (26). Endothelial cells from human umbilical vein express several α2,3-sialyltransferases and α1,3-fucosyltransferases, allowing the synthesis of sialyl-Le^a^ determinants, and the expression of these transferases is enhanced by TNF-α (28).

TNF-α is also able to enhance the expression of sialyl-Le^a^ in some lung cancer cell lines (29) and of α2,3-sialyltransferase and α1,3/4-fucosyltransferase in colon carcinoma cell lines that synthesize sialyl-Le^a^ (30).

TNF-α is also an important factor in airway mucosa inflammation, acting as an initial inflammatory cytokine that subsequently regulates both early neutrophil infiltration and eosinophil recruitment into the lung and airspace (31). TNF-α, as other cytokines, is found in the airways of patients suffering from bronchial diseases such as chronic bronchitis or cystic fibrosis (32, 33) and might affect the glycosylation of airway mucins.

The present work was designed to determine the effect of TNF-α on (i) the α2,3-sialyltransferases, α1,3-fucosyltransferases, galactose-3-O-sulfotransferase, and N-acetylgalactosamine-6-O-sulfotransferase activities involved in the biosynthesis of Le^a^, sialyl-Le^a^, sulfo-Le^a^, and sulfo-sialyl-Le^a^ determinants by the human bronchial mucosa and (ii) the expression of the different genes possibly encoding these enzymes.

**EXPERIMENTAL PROCEDURES**

**Explant Culture**—Tissues were collected in macroscopically healthy areas of the bronchial tree from patients undergoing surgery for bronchial carcinoma. They were immersed in Leibovitz L15 medium (In vitrogen), immediately transported on ice to the laboratory, and then processed to isolate the mucosa. Mucosa (2–3 cm^2^) were cut into 1-mm^2^ pieces and suspended in CMRL-1066 medium (In vitrogen) complemented with 0.2 ml 1-glutamine (34). They were maintained at 37 °C for 4 or 16 h in the presence or absence (controls) of 20 ng/ml TNF-α (Prepro Tech, London, United Kingdom).

**Enzyme Preparation**—After incubation with or without TNF-α, explants were disintegrated with a glass-Teflon homogenizer (1,400 rpm, five strokes) in 50 ml Tris-HCl buffer, pH 7.4, containing 25 m M potassium chloride, 250 mM saccharose, 5 mM β-mercaptoethanol, and 5 mM magnesium acetate (34). The mixtures obtained were subjected to ultracentrifugation further at 180,000 g for 1 or 16 h at 4 °C. The supernatants were ultracentrifuged further at 180,000 × g for 1 h at 10 °C. The resulting pellets containing microsomal fractions were stored at −80 °C until use (34), as were the 180,000 × g supernatants.

**Genotyping of Fucosyltransferases FUT2 and FUT3**—Before assaying α1,2- or α1,3-fucosyltransferase activities, blood samples were obtained from patients to define their secretor and Lewis status. Methods based upon polymerase chain reaction and restriction fragment length polymorphism were used to detect mutations of the FUT2 and FUT3 genes (35, 36).

**GlcNAc-6-O-sulfotransferase and Galactose-3-O-sulfotransferase Assays**—The GlcNAc-6-O-sulfotransferase assay was performed as described previously (37). The incubation mixture contained 50–100 μg of microsomal protein, 0.5 μCi of [35S]adenosine 3′-phosphate 5′-phosphosulfate (2.25–2.50 Ci/mmol, PerkinElmer Life Sciences), and 5 μM of the substrate GlcNAc-β1-OMe (Sigma) in a 2-(N-morpholino)propanesulfonic acid/NaOH buffer, pH 6.7, containing 0.1% (w/v) Triton X-100, 20 mM MnCl_2_, 30 mM NaF, 5 mM AMP, and 1 mM 4-(2-aminoethyl)benzenesulfonyl fluoride (Sigma).
Effect of TNF-\(\alpha\) on Glycosyl- and Sulfotransferases

Table I

| mRNA detected | Primers used | Time (C) | Product size (bp) | Ref. no. |
|---------------|--------------|----------|-------------------|----------|
| \(\beta\)-Actin | S | 58 | 228 | (42) |
| FUT1 | S | 72 | 374 | (35) |
| FUT2 | S | 72 | 368 | (35) |
| FUT3/5 | S | 72 | 447/486 | (43) |
| FUT4 | S | 72 | 319 | (43) |
| FUT6 | S | 72 | 404 | (44) |
| ST3Gal-III | S | 58 | 300 | (46) |
| ST3Gal-IV | S | 58 | 458 | (46) |

\(a\), sense; \(AS\), antisense.

\(b\), Time, temperature.

For the galactose-3-O-sulfotransferase assay, the incubation mixture contained 50–100 \(\mu\)g of microsomal proteins, 0.5 \(\mu\)M of \(1^{\text{35S}}\)-adenosine 3'-phosphate 5'-phosphosulfate, and 5 \(\mu\)M of the substrate Gal\(\beta\)-O-Met (Sigma) in a 2-(\(N\)-morpholino)ethanesulfonic acid/NaOH buffer, pH 6.1, containing 0.1% (w/v) Triton X-100, 20 \(\mu\)M MnCl\(_2\), 30 \(\mu\)M NaF, 10 mM AMP, and 1 mM 4-(2-aminomethyl)benzenesulfonfonyl fluoride (34). After incubation for 1 h at 30 °C, the reactions were stopped by the addition of ice-cold methanol. The mixtures were kept at 4 °C overnight, and the resulting precipitates were eliminated by centrifugation at 10,000 \(\times\) \(g\) for 20 min. The pellets were washed twice with ice-cold methanol and centrifuged. The supernatants were pooled, evaporated to dryness, and then directly subjected to HPAEC-PAD (34).

Table I. Oligonucleotide primers for amplifying glycosyltransferases and \(\beta\)-actin mRNAs

| mRNA detected | Primers used | Time (C) | Product size (bp) | Ref. no. |
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\(a\), sense; \(AS\), antisense.

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Effect of TNF-α on Glycosyl- and Sulfotransferases

TABLE II
Effect of TNF-α at 20 ng/ml on glycosyl- and sulfotransferases activities

| Enzyme  | Incubation time (h) | n | Controlsa | + TNF-α | p² |
|---------|---------------------|---|-----------|---------|----|
|         | Range | Median |       | Range | Median |   |
| α2-FUTb | 4     | 5      | 121–193.1 | 162 | 136–191.3 | 169 | n.s.² |
| α3-FUT  | 4     | 5      | 45.3–111 | 98 | 86.1–177 | 151 | 0.0431 |
| ST3Gal  | 4     | 5      | 68.9–116 | 84.5 | 205–588 | 263.2 | 0.0277 |
| 3-sulfoT | 16    | 5      | 24.6–55 | 38.6 | 25.6–130 | 63.1 | 0.0431 |
| 6-sulfoT | 16    | 5      | 85.6–122 | 105.7 | 390.6–501 | 453.4 | 0.0431 |

a, number of individual mucous samples analyzed.
b, The activity of the different enzymes was expressed as femtomol radiolabeled fucose transferred/min/mg protein for α1,2- and α1,3-fucosyltransferases and as picomol radiolabeled N-acetylneuraminic acid or sulfate transferred/min/mg protein for α2,3-sialyltransferases and sulfotransferases.

The p value was calculated using the Wilcoxon signed-rank test.

d, α2-FUT, α1,2-fucosyltransferase; α3-FUT, α1,3-fucosyltransferase; ST3Gal, α2,3-sialyltransferase; 3-sulfoT, galactose-3-O-sulfotransferase; 6-sulfoT, GlcNAc-6-O-sulfotransferase.

n.s., nonsignificant (p < 0.05).

sion of the β-actin gene. β-Actin was expressed equally in all samples, indicating that the glycosyltransferase mRNAs were obtained from similar quantities of total RNA. The amplified fragments were verified by digestion with restriction enzymes from Invitrogen (43). To determine whether the amplified fragments were FUT3 or FUT5 products, a digestion was performed with NoI, which cleaves the fragments from FUT5 but not those from FUT3.

Eleven μl of PCR products were subjected to electrophoresis (220 V, constant-voltage field) on a 2% agarose gel equilibrated in Tris-borate electrophoresis buffer containing ethidium bromide (1 μg/ml). Gels were photographed under UV light and analyzed by computerized scanning of the image using the Gel Analyst 3.01 program. Sizes of the amplified fragments were checked according to the migration of DNA ladders (Roche Molecular Biochemicals).

RESULTS

TNF-α has a concentration-dependent effect on the secretion of mucins by human airway organ explant cultures (48). At a concentration of 20 ng/ml in these cultures, it increases MUC2 mRNA levels and, within 8 h, more than doubles the airway mucin secretion. TNF-α also increases MUC2 and MUC5B expression by cancer cell line LS180 (49) and MUC2 expression in middle ear epithelium (50).

The effect of increasing concentrations of TNF-α on the α1,3-fucosyltransferase activity of a bronchial mucosa explant was checked. After a 4-h incubation, this activity (using 5 and 10 fucosyltransferase activity of a bronchial mucosa explant was maintained for 16 h at 37 °C, the neosynthesized fucosylated product co-eluted exactly with this standard, showing the presence of the α1,3-fucosyltransferase activity in the microsomal fractions. A similar elution profile was observed at 13 min, which was absent when the incubations were performed without Galβ1-4GlcNAc. When the radiolabeled products were injected with cold Galβ1-4GlcNAc, the neosynthesized fucosylated product co-eluted exactly with this standard, demonstrating the presence of the α1,3-fucosyltransferase activity in the microsomal fractions (Fig. 2).

A similar elution profile was obtained when incubations were performed with microsomes from bronchial mucosa explants treated with TNF-α. However, the incorporation of radiolabeled fucose from [3H]GDP-Fuc in Galβ1-4GlcNAc was increased in microsomes from explants treated for 4 h with TNF-α as compared with control (Table II). When the incubation of explants with TNF-α was extended to 16 h, the incorporation of radiolabeled fucose was even higher as compared with the control (Table II). After incubation with TNF-α, no α1,3-fucosyltransferase activity was detected in the 180,000 × g supernatant.

Incubation of explants with TNF-α for 4 h stimulated the expression of FUT3 and FUT4 mRNAs (Fig. 3 and Table III). The primers used to amplify FUT3 cDNA (447 bp) also amplified FUT5 cDNA (486 bp). In our experiments, a single band appeared after migration on an 8% polyacrylamide gel; this reverse transcription-PCR product was not cleaved by NaeI digestion (43), indicating that only FUT3 RNA had been amplified. The expression of the mRNA from FUT3 was also significantly modified after incubation with TNF-α for 4 h (Fig. 3 and Table III). FUT5, FUT6, and FUT7 are not normally expressed in the human bronchial explants, and they were not induced by TNF-α.

Effect of TNF-α on α1,2-Fucosyltransferases—When microsomes from bronchial mucosa explants were incubated with [3H]GDP-Fuc and Galβ1-4GlcNAc, the HPAEC elution profile showed one characteristic peak at 7 min that was absent when incubations were performed without any substrate. When the radiolabeled products were injected with unlabeled Fucα1-2Galβ1-4GlcNAc, the neosynthesized fucosylated product co-eluted exactly with this standard, showing the presence of α1,2-fucosyltransferase activity in the microsomal fractions. A similar elution profile was obtained with microsomes from bronchial mucosa explant treated for 4 h with TNF-α. The incorporation of [3H]GDP-Fuc in Galβ1-4GlcNAc was similar to that of the control, indicating that TNF-α had no effect on α1,2-fucosyltransferase activity (Table II). When the explants were maintained for 16 h at 37 °C, no α1,2-fucosyltransferase activity was recovered in the microsomes of controls or in those of TNF-α-stimulated explants. No α1,2-fucosyltransferase activity was detected in the 180,000 × g supernatant.

The expression of FUT2 mRNA in explants incubated for 4 h was not significantly modified by TNF-α (Table III). The FUT1 mRNA was not detected, even when PCR experiments were performed with a higher number of cycles.

Effect of TNF-α on α1,3-Fucosyltransferases—Control microsomes from bronchial mucosa explants were incubated with Galβ1-4GlcNAc and [3H]GDP-Fuc, and the radiolabeled products were analyzed by HPAEC: one characteristic peak was observed at 13 min, which was absent when the incubations were performed without Galβ1-4GlcNAc. When the radiolabeled products were injected with cold Galβ1-4GlcNAc, the neosynthesized fucosylated product co-eluted exactly with this standard, demonstrating the presence of α1,3-fucosyltransferase activity in the microsomal fractions (Fig. 2).
Effect of TNF-α on Glycosyl- and Sulfotransferases

Table III

Expression of fucosyl- and sialyltransferase mRNAs in human bronchial explants treated with 20 ng/ml TNF-α for 4 h

| Gene   | n* | Range Median | + TNF-α*   |
|--------|----|--------------|------------|
|        |    | Controls     |            |
|        |    |              | Range Median | p* |
| FUT2   | 5  | 0.105–0.092  | 0.161      |
|         |    |              | 0.118–0.232 | n.s. |
| FUT3   | 5  | 0.087–0.26   | 0.112      |
|         |    |              | 0.332–0.508 | 0.0431 |
| FUT4   | 5  | 0–0.131      | 0.041      |
|         |    |              | 0.237–0.666 | 0.0431 |
| ST3Gal III | 5 | 0–0.042 | 0.152 |
|         |    |              | 0.090–0.725 | 0.0431 |
| ST3Gal IV | 5 | 0.018–0.305 | 0.152 |
|         |    |              | 0.102–1.836 | 0.0431 |

a n, number of individual mucosae analyzed.

b Amplified products were quantified in each sample using the Gel Analyst 3.01 software. The densitometric values of cDNA products corresponding to the expression of β-actin and transferase genes were calculated for each reaction. The results were expressed as relative expression units (transferase/β-actin scores) TNF-α. FUT-1, -5, -6, and -7 were not expressed in these experiments, and they were not induced by TNF-α.

c The p value was calculated using the Wilcoxon signed-rank test.

d n.s., nonsignificant (p > 0.05).

FIG. 2. HPAEC elution profile of mixtures containing radiolabeled products synthesized when equal amounts of microsomal proteins from bronchial explants treated with TNF-α (A) or not treated with TNF-α (B) were incubated with [35S]GDP-Fuc and Galβ1-4GlcNAc. The elution was monitored by radioactivity detection (solid line) for the synthesized products and by PAD (dashed line) for the nonlabeled standard, Galβ1-4[Fucα1-3]GlcNAc. Elution was performed with gradient II (described under “Experimental Procedures”).

The sulfotransferase activity was observed in the 180,000 × g supernatants of both control and TNF-α explants (0.79 ± 0.04 and 1.6 ± 0.14 pmol/min/mg protein, respectively).

The expression of the mRNAs from ST3Gal-III and ST3Gal-IV was significantly increased by TNF-α (Fig. 3 and Table III).

Effect of TNF-α on Galactose 3-O-sulfotransferase and N-Acetylgalcosamine 6-O-sulfotransferase—Bronchial microsomes obtained from control explants and explants treated with TNF-α for 4 or 16 h were incubated with [35S]adenosine 3’-phosphate 5’-phosphosulfate and either Galβ1-0-Met or GlcNAcβ1-0-Met. The sulfotransferase activities were measured by the production of 3-sulfated Galβ1-0-Met or 6-sulfated GlcNAcβ1-0-Met (Table III). When microsomes were obtained from bronchial mucosa explant treated with TNF-α for 4 h, both sulfotransferases were significantly increased. These microsomal activities were lost when the explants were maintained for 16 h. After a 16-h incubation, some galactose-3-O-

sulfotransferase and GlcNAc-6-O-sulfotransferase activities were observed in the 180,000 × g supernatants of both controls (0.03 ± 0.01 and 0.06 ± 0.01 pmol/min/mg protein, respectively) and TNF-α explants (0.08 ± 0.1 and 0.17 ± 0.005 pmol/min/mg protein, respectively).

DISCUSSION

TNF-α is a multifunctional proinflammatory cytokine able to activate diverse target genes, and its actions are mediated by several signal transduction pathways.

The present study demonstrates its influence on the expression of different glycosyl- and sulfotransferases involved in the biosynthesis of various Lewis x epitopes by the human bronchial mucosa.

TNF-α may influence the expression of some mucin genes and transferases involved in posttranslational modification of the peptides. At a concentration of 20 ng/ml in human airway organ cultures, it increases the expression of a mucin gene, MUC2, and, within 8 h, more than doubles the airway mucin secretion (48). The present study indicates that, at a similar concentration, TNF-α also influences glycosylation and sulfation. It increases the expression of α2,3-sialyltransferase, α1,3-fucosyltransferases, galactose-3-O-sulfotransferase, and N-acetylgalcosamine-6-O-sulfotransferase, which are involved in the biosynthesis of Lewis x, sialyl-Lewis x, sulf-Lewis x, and sulfo-sialyl-Lewis x determinants by the human bronchial mucosa (Fig. 1), but it does not influence the α1,2-fucosyltransferases.

Human bronchial mucosa expresses α1,2-fucosyltransferase...
activity (Table II). This activity is due to the fucosyltransferase FUT2, or secretor enzyme, responsible for the secretor status of human airway mucins (9). This activity is lost or degraded when the explants are kept for 16 h. TNF-α has no effect on the expression of FUT2 (Table II). Interestingly, the FUT1 enzyme is not expressed in any of the adult mucosa studied thus far, in contrast to cultures of human airway cells, where its expression appears after 3 weeks (47). The unique expression of FUT2 is consistent with the observation that human airway mucins secreted by nonsecretor individuals belonging to blood group O never express H determinants (9).

In addition to the secretor enzyme, human bronchial mucosa expresses an α1,3-fucosyltransferase activity that is increased after 4 h of incubation with TNF-α and increased even more after 16 h of incubation (Table II). A small part of this activity is released in the 180,000 g supernatant during microsome preparation. In the bronchial mucosa, α1,3-fucosyltransferases are considered to be active in the biosynthesis of Lewis x or sialylated Lewis x determinants. Different genes (FUT3, FUT4, FUT5, FUT6, and FUT7) might be responsible for such an activity. As a matter of fact, the human adult bronchial explants only express FUT3 and FUT4 (Fig. 3). This expression of FUT4 may explain the observation that human airway mucins from nonsecretor individuals, which do not express active FUT3, are nevertheless fucosylated (9). Moreover, because FUT7 is expressed in activated Th1 CD4 T cells (26), its lack of expression in the respiratory explants is evidence that the α1,3-fucosyltransferase activity measured in these explants is not due to the few T cells present in this tissue.

The expression of both FUT3 and FUT4 genes in the explants analyzed in the present study was increased by TNF-α. Therefore, the increased α1,3-fucosyltransferase activity by TNF-α in the human airway mucosa is most probably due to an increased expression of FUT3 and FUT4, although an overexpression of some still unknown fucosyltransferase cannot be excluded. The stimulatory effect of TNF-α on the α1,3-fucosyltransferase activity even increases when the incubation is extended to 16 h. These results are most probably related to an indirect effect of TNF-α. TNF-α is considered to be an early marker of inflammation responsible for a cytokinetic cascade. The induction of other cytokines might explain the difference between the increased activity at 4 h and 16 h.

These results are in agreement with the observations that inflammation, and particularly TNF-α, changes α3-fucosylation and induces sialyl-Lewis x expression on several glycoproteins (23, 51, 52). α1,3-Fucosyltransferases are required after α2,3-sialyltransferases in the generation of the sialyl-Leα determinant. However, the enzymes involved in this process differ from one tissue to another: FUT6 in the liver (20), FUT7 in activated Th1 CD4 T cells (24), and FUT3 and FUT4 in the airway mucosa. In the latter case, there is no expression of any other known α1,3-fucosyltransferase. From a general point of view, it is also interesting to note that IL-4, a Th2-polarizing cytokine, is known to inhibit FUT7 expression and binding to vascular selectins (53), suggesting that its action on other α1,3-fucosyltransferases should also be studied.

The human bronchial mucosa also has α2,3-sialyltransferase activity, which increases after incubation with TNF-α for 4 h and increases even more after 16 h of incubation with TNF-α (Table II). There are large differences in the levels of these fucosyl- and sialyltransferase activities that may be due to the fact that the oligosaccharidic substrate Galβ1-4GlcNAc used in the present study is not the natural substrate. Among the human α2,3-sialyltransferases already cloned, ST3Gal-III and ST3Gal-IV use this disaccharide sequence as an acceptor to synthesize sialyl-Lewis x (54). These two enzymes are expressed in the human bronchial mucosa, and their expressions increase after treatment with TNF-α (Table III). The ST3Gal-IV involved in the biosynthesis of sialyl-Leα is also up-regulated in activated Th1 CD4 T cells (26).

This increased activity is in agreement with the hypersialylation and overexpression of sialyl-Leα in mucins from severely infected patients as compared with mucins from noninfected patients (9). However, other cytokines might be overexpressed in severe inflammation of the respiratory mucosa and induce similar effects.

Finally, TNF-α also increases the activities of both galactose-3-O-sulfotransferase and N-acetylgalcosamine-6-O-sulfotransferase, which can lead to various sulfated epitopes of bronchial mucins such as the 3-sulfo-Lewis x or the 6-sulfo-sialyl-Lewis x determinants (Fig. 1). These activities were increased after a 4-h incubation with TNF-α but were not observed after 16 h, probably indicating that, as seen for the α1,2-fucosyltransferase, they were degraded after 16 h of incubation. A cathepsin D-like proteinase has been reported previously to release an α2,6-sialyltransferase during acute-phase response in rat Golgi liver (55).

There are several observations indicating abnormal sulfation in CF. The sulfate content of bronchial mucins secreted by CF patients is increased (56–59). Altered sulfation and glycosylation of glycoproteins secreted by CF cells in culture (60–62), as well as hypersulfation of CF human airway mucins secreted by a xenograft model of CF airway mucosa, have also been reported (63). Because there is no bacterial infection in the xenograft model, a link between hypersulfation of CF mucins and the primary defect of the disease has been envisaged (63).

The airways of patients suffering from CF are usually heavily infected by Pseudomonas aeruginosa, and the relations between infection and inflammation have been questioned by investigators who observed the precocity of lung inflammation in CF patients, possibly before colonization by P. aeruginosa (64–67). Although CF mice have no spontaneous airway infection by P. aeruginosa, they have an excessive inflammatory response of the airways when they are challenged with P. aeruginosa (68).

In a recent work, mucins secreted by patients suffering from either cystic fibrosis or chronic bronchitis, with or without a
severe infection, were compared for their sialic acid and sulfate contents, as well as for sialyl-Lewis x expression (9). As already mentioned, this study described the hypersialylation and over-expression of the sialyl-Lewis x epitope in mucins from severely infected patients and confirmed the higher sulfation already reported in cystic fibrosis (56–58). Interestingly, the sialic content of the mucins from the infected patients was also higher than that of the mucins from the noninfected patients, raising the question of a possible influence of severe inflammation on the sulfation process of the bronchial mucosa (9).

In airway inflammation, the release of TNF-α and other exokins induce the expression of E-selectin and adhesion molecules such as intercellular adhesion molecule 1 on microvascular endothelial cells, allowing the migration of leukocytes through the endothelium. Moreover, TNF-α up-regulates the expression of intercellular adhesion molecule 1 by airway epithelial cells (69) and favors the subsequent migration of leukocytes across the epithelium (70) and their retention in the airways (69).

Simultaneously, the up-regulation of the different fucosyltransferases, sialyltransferases, and sulfotransferases of mucin-secreting cells by TNF-α may increase the synthesis of Lewis x epitopes, especially sialyl-Le x, sulfuro-Le x, and sulfosialyl-Le x, on airway mucins, allowing the attachment of leukocytes to the mucus film covering the airway lumen.

These epitopes are largely expressed on CF mucins (8) and are also possible sites of attachment for *P. aeruginosa* (71, 72). Therefore, by offering a large array of possible ligands for leukocytes and for the adhesions of *P. aeruginosa*, they may contribute to the chronicity of airway infection in cystic fibrosis and in other chronic bronchial diseases with severe inflammation. Moreover, *P. aeruginosa* interacting with mucins may escape opsonophagocytic killing by human polymorphonuclear leukocytes (73).

In conclusion, the action of TNF-α on the glycosylation and sulfation process of the human bronchial mucosa raises the more general question of the influence of inflammation on posttranslational modifications of proteins, such as glycosylation and sulfation. In the future, it will be necessary to find out whether other pro-inflammatory cytokines (IL-1 and more specifically, IL-6 and IL-8, which are secreted in abundance by CF cells (74)) may also alter the glycosylation and sulfation processes in human bronchial mucosa. In human epithelial cells, signaling induced by TNF-α was recently described as occurring through nuclear factor κB (69, 75, 76) and activator protein 1 (76). In the future, it will be important to study the regulatory mechanisms of glycosyltransferase and sulfotransferase genes by TNF-α.

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