**Objective:** To evaluate the lipopolysaccharide induction of cyclooxygenase-2 (COX-2) in long-term epithelial cultures from nasal polyp tissue of patients with cystic fibrosis (CF) and severe nasal polyposis.

**Design:** Experimental and histologic study.

**Setting:** Department of Otorhinolaryngology, University of Ulm, Ulm, Germany.

**Participants:** Nasal polyp tissue was evaluated from 9 patients with CF and obstructing nasal polyps undergoing elective sinus surgery. Nasal mucosa from the hypertrophic inferior turbinate of 9 patients without a history of CF or aspirin intolerance undergoing nasal corrective surgery served as control specimens.

**Interventions:** Tissue culturing, Western blotting, and tissue staining with hematoxylin-eosin.

**Main Outcome Measures:** The expression and lipopolysaccharide induction of COX-2 was detected and compared with those of the control group. Tissue was analyzed for the presence of inflammatory cells such as neutrophils, eosinophils, mast cells, plasma cells, lymphocytes, and monocytes.

**Results:** COX-2 was detectable in tissue specimens from all of the patients with CF and control subjects. In patients with CF, however, COX-2 expression was significantly lower in lipopolysaccharide-stimulated, long-term cultured epithelial cells compared with control tissue. Polyps from patients with CF contained markedly more neutrophils, macrophages, and plasma cells than did nasal mucosa from hypertrophic inferior turbinates of controls.

**Conclusions:** COX-2 expression in lipopolysaccharide-stimulated, long-term cultured epithelial cells in patients with CF is decreased compared with that in hypertrophic turbinate mucosa. The estimated defect in lipopolysaccharide responsiveness and the reduced induction of COX-2 needs further investigation.
Modulation of COX activity may be associated with shunting of AAs from biosynthesis of prostaglandins to leukotrienes, that is, the 5-lipoxygenase pathway, as it is known in aspirin-intolerant asthma.4 Whereas similar mechanisms in patients with CF and NPs have not yet been investigated, the release of leukotriene B4 in the bronchopulmonary airways has been examined.8-10 Leukotriene B4 and other mediators of the 5-lipoxygenase pathway are proinflammatory mediators that are markedly elevated in chronic neutrophilic inflammation.11 Neutrophilic epithelial invasion is also typically found in CF airway mucosa.3,12

Various investigations on COX regulation in nasal mucosa (NM) and NPs have been reported in recent years. Mullol et al13 found spontaneous but delayed upregulation of COX-2 messenger RNA in NPs of aspirin-intolerant patients after 24 hours of incubation compared with that in NM of aspirin-tolerant patients without nasal polyposis after 6 hours of incubation. Pujols et al14 showed low COX-2 expression in aspirin-tolerant and aspirin-intolerant patients with NPs compared with that in healthy NM. Patients with chronic rhinosinusitis without polyposis had high expression of COX-2, whereas patients with NPs showed substantially lower COX-2 expression in nasal polypous epithelial cells.15 Whereas Pérez-Novo et al16 also demonstrated downregulation of COX-2, especially in aspirin-intolerant patients with chronic rhinosinusitis and NPs, Liu et al17 showed up-regulation of COX-2 in aspirin-tolerant patients and Owens et al18 in aspirin-tolerant and aspirin-intolerant patients.

Roca-Ferrer et al19 studied the expression of COX-2 in NPs of patients with CF and observed significant up-regulation of COX-2 protein levels compared with that in non-CF polyp tissue or NM. Owens et al20 recently also studied the expression of COX-2 in the sinonasal mucosa of patients with CF. Expression of COX-2 in columnar epithelium was noted in all of the CF specimens. COX-2 was expressed in the submucosal glands and in the cytoplasm in 86% of the CF specimens.20

The invasion of the respiratory epithelium by neutrophils is a typical finding in CF airway mucosa, indicating bacterial colonization or mucosal infection.3 Because the expression of COX-2 can be induced by various substances, such as lipopolysaccharides, the objective of this study was to investigate the expression and lipopolysaccharide induction of COX-2 protein in cultured human NP explants from patients with CF. In addition, we examined the morphologic features of the nasal epithelium and the presence of inflammatory cells in CF polyp sections and in hyperplastic NM of control subjects.

**METHODS**

**PARTICIPANTS**

Patients were recruited from the Department of Otorhinolaryngology at the University of Ulm during a 31/2-year period. After an initial 1-year pilot study including 4 patients with CF, an additional 5 patients with CF undergoing functional endoscopic sinus surgery for nasal polyposis were included in the study. Cystic fibrosis was diagnosed by positive sweat chloride test results and characteristic genotype abnormalities, as reported elsewhere by the same working group.21 The mean age of the 9 patients with CF (3 males and 6 females) was 21 years (age range, 12-35 years; median NP score, 2).22 Nine patients undergoing septoplasty or septrhinoplasty because of anatomical variations and not from having any sinus disease were considered controls (NM group). None of the patients in the NM group had bronchial asthma or evidence of CF or aspirin-intolerance syndrome. The mean age of the controls (4 males and 5 females) was 22 years (age range, 18-42 years; no nasal polyposis). Patients with known immunodeficiency disorders or use of topical or systemic corticosteroids were excluded from the study.

Tissue samples of NPs protruding from the ethmoid of patients with CF and NM from the anterior/inferior part of the hypertrophic inferior turbinate of patients without CF were obtained during nasal surgery. At the time of surgery, patients with CF and controls were not taking corticosteroids or nonsteroidal anti-inflammatory drugs. All of the participants agreed to participate in the study, which was approved by the institutional ethics committee of the University of Ulm.

**TISSUE HANDLING**

All nasal samples for histopathologic examination were immediately fixed in formaldehyde and embedded in paraffin. For each NP and NM sample, a section was systematically stained with hematoxylin-eosin for standard histomorphologic analysis and morphologic evaluation according to the method of Lesprit et al.23

Immediately after removal, NPs and NM for COX detection were cut into approximately 1-mm3 cubes, and these fragments were placed in 10-mL test tubes with 5 mL of Dulbecco modified eagle medium, on room temperature, containing 0.1% Accutase, penicillin (105 U/L), streptomycin sulfate (100 mg/L), and gentamicin sulfate (50 mg/L). After 1 hour of gentle shaking, fetal bovine serum (FBS) was added to neutralize the Accutase. The solid parts of the polyp were discarded, and the remaining epithelial suspension was washed twice with Dulbecco modified eagle medium/F12 1:1 culture medium containing 10% FBS and antibiotics as described previously herein. The pellet was resuspended in 5 mL of culture medium in a small flask and was incubated on a controlled atmosphere at 37°C with 5% carbon dioxide. During the first 3 to 4 hours, the flask was gently moved to reduce attachment of cell sheets to the bottom of the flask. The medium was changed daily for the first 2 days and then 3 times weekly. From the third day, gentamicin was omitted from the medium.24 Cells were then seeded in a 6-well culture plate. After a minimum of 3 hours, the medium was changed to a serum-free medium (culturing medium without FBS), and cells were grown to confluence.

**WESTERN BLOTTING**

On the basis of a preliminary study on NP immortalized cells from patients with CF, COX-2 detection in this type of cell was established. After 72 hours in culture and the cells growing to confluence (approximately 106 cells per sample), the medium was changed to a serum-free medium (culturing medium without FBS) with or without lipopolysaccharide supplementation. We used lipopolysaccharide, 10 µg/mL, for stimulation for 20 hours.

After 20 hours of incubation at 37°C with 5% carbon dioxide, the cell medium was discarded and the cells were trypsin-
ized with 500 µL of trypsin-EDTA solution for 5 minutes. Tryp- 
sinized cells were transferred into labeled Eppendorfs vials 
containing 500 µL of culture medium supplemented with 10% 
FBS to stop the trypsin effect. The cell suspension was then cen- 
trifuged at 4°C for 5 minutes. Supernatant was care- 
fully discarded. Cells were resuspended in lysate buffer (100 
µL of 1% Triton-X-100 in phosphate-buffered saline [PBS] 
supplemented with a Complete protease inhibitor cocktail tablet 
[Roche, Basel, Switzerland]) and were left on ice for 30 min- 
utes at 4°C. Cell lysates were sonicated twice for 20 seconds. 
As a next step, cells were centrifuged for 15 minutes at 4°C with 
10 000. 
Ten microliters of sample-loading buffer (4X NuPAGE LDS; 
Invitrogen, Carlsbad, California) was added to 10 µL of the su-
pernatant. All the samples were heated in a 70°C water bath for 
ten minutes. Rainbow molecular weight marker (15 µL) (Am-
ersham Biosciences, San Francisco, California), positive con-
trol for COX-2 (7 µL) (BD Transduction Laboratories, BD Bio-
sciences, Mississsauga, Ontario, Canada), and samples were 
loaded on a 4% to 12%, 15-well NuPAGE Bis-Tris ready gel (In-
vitrogen) and run at constant 200 V at 120 mA in an electro-
phoresis cell (XCell SureLock; Invitrogen) with running buffer 
(NuPAGE MOPS SDS; Invitrogen).
After electrophoresis, proteins were transferred to a poly-
vinyl difluoride membrane (Bio-Rad Laboratories, Hercules, 
California). Blot was run at 0.8 mA/cm² (2 blots: 2/7.5 
cm=60 mA for 2 gels), minimal voltage (10 V), for ap- 
proximately 1 hour. We used the semidyblotting technique 
(Pegasus; Phase GmbH, Lubeck, Germany). After blotting, 
polyvinyl difluoride membranes were washed with aqua dest 
and were placed in blocking buffer (10% skim milk–PBS solu-
tion) overnight at 4°C to block nonspecific binding sites. 
Membranes were washed in PBS and were incubated with the 
primary antibody solution (antibody dilution buffer; In-
vitrogen; anti–COX-2 antibody, 1:500 mouse IgG1; BD Trans-
duction Laboratories, BD Biosciences). As a next step, mem-
branes were washed 3 times with 0.05% PBS-polyborate 
solution, and then the secondary antibody solution was ap-
plied (1:1000, peroxidase-linked anti–mouse IgG from sheep; 
Amersham Biosciences).
After a new series of washes, COX-2 immunoreactive bands 
were visualized using a light-emitting chemiluminescent method 
(SuperSignal Femto Maximum; Invitrogen). Light emission 
was detected by exposure to blue light–sensitive autoradiography 
film (Hyperfilm ECL; Amersham Biosciences).
Chemiluminescent intensity was semiquantitatively 
assessed and was graded as none, low, medium, or high for 
each sample. Evaluations were performed by the same person 
(A.R.) for all samples to reduce rater-related variability. Statis-
tical analysis was performed to compare the chemilumines-
cent intensities in samples from patients with and without CF 
using the Fisher exact test. Statistical significance was defined 
as $P<.05$.

RESULTS

SPONTANEOUS REGULATION 
OF COX-2 EXPRESSION

In all of the cell lines, spontaneous but low-level expression 
of COX-2 in long-term cultured epithelial cells could be seen. Comparing the 2 different groups, cultured cells 
from the NPs of patients with CF without lipopolysaccha-
ride stimulation had a nonsignificantly higher level 
of COX-2 content than did NM cells from patients without 
CF ($P>.05$) (Figure 1).

EFFECT OF LIPOPOLYSACCHARIDE 
ON COX-2 EXPRESSION

COX-2 was only slightly upregulated when stimulated 
with lipopolysaccharide in cultured cells from CF NPs 
($P>.05$) (Figure 2). Cultured cells from NM of infe-
rior turbimates of controls showed stronger upregula-
tion of COX-2 after stimulation with lipopolysaccha-
ride ($P<.05$) (Figure 2).
The NPs of patients with CF showed various patterns of acute inflammation (ie, only a few or missing glands in the stroma) and chronic inflammation (ie, seromucinous glands present in the stroma) (Figure 3). Neutrophils were numerous in CF polyp tissue, infiltrating intraepithelially and subepithelially. Only low numbers of eosinophils were seen in CF NPs. In non-CF NM, relatively few macrophages, lymphocytes, and granulocytes were found.

HISTOLOGIC EXAMINATION

The etiology and pathogenesis of nasal polyposis in CF are poorly understood. Chronic neutrophilic airway inflammation is a main characteristic of CF. Previous studies on proinflammatory leukotrienes and the COX and 5-lipoxygenase pathways of the respiratory epithelium have shown an impact of the AA cascade on respiratory inflammation in CF.

The objective of this study was to investigate whether there is an abnormality in the AA cascade, especially of the COX pathway similar to that in aspirin-intolerant asthma, in the pathogenesis of NPs in CF. For this purpose, the presence of COX-2 in NP tissue in patients with CF and severe nasal polyposis and its induction by incubation with lipopolysaccharide was evaluated. In addition, histologic examinations of NPs and evaluation of neutrophilic infiltration of the epithelium were performed.

Nasal polyp tissue was evaluated from 9 patients with CF undergoing sinus surgery. Tissue was analyzed for the presence of neutrophils, indicating neutrophilic inflammation. Additional NP tissue was obtained for COX-2 determination. Conchal mucosa from the inferior turbinate of 9 controls undergoing nasal corrective surgery served as control tissue. Polyps from patients with CF contained markedly more neutrophils than did NM from controls. COX-2 was detectable in all patients with and without CF. Cultured cells from the NPs of patients with CF without lipopolysaccharide stimulation had a non-significantly higher level of COX-2 content than did nasal mucosal cells from controls. In patients with CF, however, COX-2 expression was significantly lower in lipopolysaccharide-stimulated long-term cultured epithelial cells compared with that in control tissue.

These data indicate that in patients with CF and NPs, a feasible defect in lipopolysaccharide responsiveness seems to be responsible for significant downregulation of COX-2 after lipopolysaccharide stimulation compared with control tissue from the inferior turbinate.

Another study has reported spontaneous upregulation of COX-2 with the time in cultured NP explants from aspirin-tolerant patients without CF and in normal NM. According to the time curve related to the level of COX-2 content in a certain amount of cells, spontaneous upregulation of COX-2 in normal NM can be seen starting from the zero point at the time of nasal surgery, reaching its peak 24 hours after surgery. A lower and delayed peak could be seen in NPs from aspirin-tolerant patients 72 hours after putting polyp explants into culture. Under stimulation with proinflammatory cytokines, normal NM showed upregulation of COX-2 expression, whereas in NPs, delayed upregulation of COX-2 was seen. The present findings of weaker spontaneous upregulation of COX-2 in CF NPs are in contrast to these results in non-CF NPs and underline the possible different etiology of NPs in CF disease.

Neutrophilic inflammation was histologically found in polyp specimens from all the patients with CF. Expression of COX-2 is induced by bacterial products from Pseudomonas aeruginosa, a bacteria often found in patients with CF and NPs.25 This observation may explain the finding of an upregulated COX-2 protein level in NPs of CF detected by our and other work groups.19,20 However, the mechanism of induction and suppression of COX-2 in CF NPs is not yet completely understood.26

In summary, COX-2 expression in lipopolysaccharide-stimulated, long-term cultured epithelial cells in patients with CF is decreased compared with expression in hypertrophic turbinate mucosa. The estimated defect in lipopolysaccharide responsiveness and the reduced induction of COX-2 needs further investigation.

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Author Contributions: Both authors had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Rozsasi and Keck. Acquisition of data: Rozsasi and Keck. Analysis and interpretation of data: Rozsasi and Keck. Drafting of the manuscript: Rozsasi and Keck. Critical revision of the manuscript for

Figure 3. Inflammatory polyoid nasal tissue from a patient with cystic fibrosis (hematoxylin-eosin, original magnification ×360). The epithelium (upper part of the figure) is broad and edematous. Note the neutrophils infiltrating the subepithelial connective tissue and lamina propria (residual neutrophils) (a) and the epithelial layer of the nasal polyp (b). The subepithelial connective tissue and lamina propria are dominated by lymphoid cells (c), plasma cells (d), and macrophages (e). Eosinophils were not regularly seen. f Indicates stromal cell.


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