Deficient prostaglandin E₂ production by bronchial fibroblasts of asthmatic patients, with special reference to aspirin-induced asthma

Małgorzata Pierzchalska, PhD,^{a*} Zsuzsanna Szabó, MD,^{b*}

Marek Sanak, MD, PhD,^a Jerzy Soja, MD,^a and Andrzej Szczeklik, MD, PhD^a

Krakow, Poland, and Budapest, Hungary

Background: Regulation of prostaglandin synthesis and the activation of human airway fibroblasts associated with the remodeling of the bronchi play an important role in asthma. **Objective:** We sought to assess the cyclooxygenase pathways in airway fibroblasts of patients with bronchial asthma.

Methods: Generation of prostaglandin E₂ (PGE₂) and prostaglandin D₂ (PGD₂) by bronchial fibroblasts was measured by means of mass spectrometry in culture supernatants, and cyclooxygenases expression was estimated by means of RT-PCR and immunoblotting. The cells were isolated from 3 groups of subjects: nonasthmatic patients (n = 10), patients with aspirin-tolerant asthma (ATA, n = 9), and patients with aspirin-intolerant asthma (AIA, n = 7).

Results: The cytotoxic (LPS, 5 µg/mL; IL-1β, 5 ng/mL; and TNF-α, 10 ng/mL; 18 hours) stimulated the production of prostaglandins. Asthmatic patients were characterized by low capacity to produce PGE₂ after cytotoxic stimulation. In the nonasthmatic patient group the mean PGE₂ production was 32 ± 33 ng/mL (35-fold of the basic production), in the ATA group it was 16 ± 18 ng/mL (16-fold), and in the AIA group it was only 5.3 ± 3.6 ng/mL (4-fold). The mean concentration of PGD₂ for nonasthmatic patients, patients with ATA, and patients with AIA was 0.18 ± 0.16 ng/mL (4.7-fold of the basic production), 0.18 ± 0.14 ng/mL (4.2-fold), and 0.235 ± 0.19 ng/mL (1.9-fold), respectively. The observed difference was not due to insufficient cyclooxygenase 2 expression because all groups had similar levels of its mRNA and protein. The patients with AIA had low expression levels of cyclooxygenase 1 protein but not of its mRNA. The PGE₂/PGD₂ concentration ratio increased after cytotoxic stimulation in all groups but was significantly less in patients with AIA than in patients with ATA.

Conclusions: Our results point to a deficient PGE₂ production under proinflammatory conditions in asthmatic airways. This could weaken local defensive mechanisms and promote cysteinyl leukotriene overproduction. (J Allergy Clin Immunol 2003;111:1041-8.)

Key words: Asthma, aspirin-induced asthma, human bronchial fibroblasts, prostaglandins, cyclooxygenase 1, cyclooxygenase 2

Bronchial asthma is a chronic inflammatory disorder characterized by mast cell and eosinophil infiltration of the airways. The inflammation might be related to a possible shift toward an immune response mediated by T_H2 cells and generation of a special subset of cytokines. A link might exist between chronic inflammation of the bronchial wall and its structural remodeling. The latter has been described as thickening of the airway wall and is considered to be a pathomorphological hallmark of asthma.¹ This remodeling is due to profound collagen deposition beneath the basement membrane and subepithelial fibrosis, induction of the myofibroblastic phenotype in airway fibroblasts, smooth muscle hyperplasia, and microvascular congestion.² In the last decade, attention has been focused on the function of bronchial epithelial cells and bronchial fibroblasts. Fibroblast proliferation in asthmatic airways appears to be caused by cytokines released from epithelium in response to its damage by environmental factors.³ Thus the primary defect in asthma could be related to the impaired communication between epithelial and mesenchymal cells (fibroblasts, myofibroblasts, and smooth muscle cells) in the bronchial wall.⁴

Arachidonic acid metabolites have been extensively investigated in the pathogenesis of asthma. Cysteinyl leukotrienes, strong proinflammatory factors generated from arachidonic acid through the 5-lipoxygenase pathway, have been recognized as the key mediators of an asthmatic attack.⁵ The role of cyclooxygenase metabolites⁶ is less clear, but recent results in knockout mice point to an important role of the bronchoconstrictor prostaglandin D₂ (PGD₂) in allergic asthma.⁷ On the contrary, prostaglandin E₂ (PGE₂) protects airways against inflammation and promotes normal airway function.⁸ Because human bronchial fibroblasts (HBFs) produce both PGE₂ and PGD₂, we studied the balance between these prostanoids in asthmatic patients, with

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From ^athe Department of Medicine, Jagellonian University Medical School, Krakow, and ^bKorányi National Institute of Pulmonology, Budapest.

*Both authors contributed to the work equally.

Supported by the Polish State Committee for Scientific Research grant no. 6 P05B 140 21. MP's visit to Southampton was supported by a travel award from the Wellcome Trust. Z.S.'s stay at the University Department of Medicine in Krakow was supported by a scholarship from the Hungarian Pulmonological Foundation.

Received for publication October 30, 2002; revised February 21, 2003; accepted for publication February 27, 2003.

Reprint requests: Andrzej Szczeklik, MD, Department of Medicine, Jagellonian University Medical School, 8. Skawińska Str, 31-066 Cracow, Poland.

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0091-6749/2003 \$30.00 + 0

doi:10.1067/mai.2003.1491

TABLE I. Characteristics of the patient groups studied

	Nonasthmatic patients	Patients with ATA	Patients with AIA
No. of subjects	10	9	7
Sex (M/F)	4/6	4/5	1/6
Mean age, y (range)	46 (20-69)	46 (22-65)	46 (22-63)
Mean duration of asthma, y (range)	—	12 (1-26)	6 (1-9)
Mean FEV ₁ initial value, % predicted (range)	115 (96-139)	79 (33-102)	90 (61-118)
Usage of inhaled steroids: no. of subjects/all subjects (dose, 800 μ g/d)	10/10	9/9	7/7
Use of systemic steroids: no. of subjects/all subjects (mean dose in mg prednisone/d)	1/10 (20)	7/9 (14.5)	4/7 (12.5)

Abbreviations used

AIA: Aspirin-intolerant asthma
ATA: Aspirin-tolerant asthma
BAL: Bronchoalveolar lavage
C _T : Cycle threshold
DMEM: Dulbecco modified Eagle medium
HBF: Human bronchial fibroblast
mPGES: Membrane-bound prostaglandin E ₂ synthase
NSAID: Nonsteroidal anti-inflammatory drug
PGD ₂ : Prostaglandin D ₂
PGE ₂ : Prostaglandin E ₂

special reference to aspirin-induced asthma. In this distinct clinical syndrome, which affects about 10% of adult asthmatic patients,^{9,10} acute asthma attacks are precipitated by cyclooxygenase (COX) 1 inhibitors and are accompanied by massive release of cysteinyl leukotrienes. Deficiency of protective PGE₂ has been considered as a triggering factor.¹⁰ It was also suggested that reduced expression of COX-2, resulting in insufficient PGE₂ production, makes these patients more susceptible to the effect of aspirin.^{11,12}

The cellular source of biologically active eicosanoids *in vivo* remains to be determined. It is known that the HBFs express both COX-1 and COX-2 and produce prostaglandins. HBFs were reported to influence inflammatory reactions in the airways not only by generating prostaglandins but also by secreting cytokines and growth factors or by making direct cell-to-cell contact with infiltrating inflammatory cells.³ Studies of the proliferation rate and collagen synthesis by fibroblasts showed a more aggressive phenotype of asthmatic than normal fibroblasts.¹³ Diminished production of PGE₂ by fibroblasts isolated from fibrotic lung tissue has been also reported,¹⁴ but no analysis of prostaglandin production by fibroblasts from asthmatic subjects has been carried out thus far. We studied prostaglandin production through the COX-1 and COX-2 pathways in fibroblasts of asthmatic patients and those of patients without asthma.

METHODS

Subjects

The study was performed in 3 groups of subjects with a total of 26 individuals. The first group consisted of 10 nonasthmatic patients, the second group of 9 patients with aspirin-tolerant asthma (ATA), and the third group of 7 patients with aspirin-intolerant asthma (AIA).

The latter patients had a clear history of bronchospasm after ingestion of aspirin or other nonsteroidal anti-inflammatory drugs (NSAIDs). The clinical characteristics of the patient groups are given in Table I. All the patients were treated in the Department of Medicine of the Jagellonian University Medical School and were in stable clinical condition. Within the 6 months preceding the study, all asthmatic patients underwent oral aspirin challenge testing, as previously described.¹⁵ The results were positive in patients with AIA and negative in patients with ATA. The nonasthmatic patient group included patients in whom diagnostic bronchoscopy ruled out serious airway pathology, including asthma, fibrotic lung disease, sarcoidosis, or cancer. All nonasthmatic patients, when asked specifically, responded that they used aspirin or other NSAIDs without any adverse events. The study was approved by the university ethics committee, and all patients provided informed consent to participate.

Isolation and culture of HBFs

The bronchial biopsy specimens were obtained from the segmental bronchi during bronchoscopy by using a fiberoptic bronchoscope (Olympus). Three submucosal biopsy specimens from each subject were collected with alligator forceps. The specimens were immediately transferred into a tube containing cold PBS (Sigma) and put on ice. Within the next 20 minutes, the biopsy specimens were transferred into Dulbecco modified Eagle medium (DMEM; Sigma) with 10% FCS (Gibco BRL) containing collagenase type I (Gibco BRL) in a concentration of 1 mg/mL. After 4 to 6 hours of incubation at 37°C, the digested parts of the biopsy specimens and the liberated cells were harvested by means of centrifugation (5 minutes at 90g) and seeded in 6-well plates (Falcon, Primaria, Becton Dickinson). The primary cultures were kept in DMEM and supplemented with 20% FCS and antibiotics and antimycotics (100 U of penicillin, 100 μ g/mL streptomycin, and 250 ng/mL amphotericin B; Sigma). The medium was changed after 24 hours and then every other day. In 2 to 3 weeks, the primary cultures of the fibroblasts, which reached about 80% of confluence, were passaged into new flasks by using the typical trypsinization protocol. For feeding of secondary culture, DMEM with 10% FCS was used. The cells were assessed microscopically to have fibroblast-like morphology. Additionally, the level of expression of α -smooth muscle actin was checked by means of cytoimmunologic staining. Monoclonal antibody against α -smooth muscle actin (clone 1A4 from SIGMA) and a StreptABComplex/HRP from DAKO were used to develop the signal. In all cell lines, less than 5% of cells were stained. This was considered as proof that the isolated cell lines were not smooth muscle cells but fibroblasts. The experiments were done on cells after 2 to 6 passages.

PGE₂ and PGD₂ measurements

PGE₂ and PGD₂ levels were measured in supernatants (after the extraction step, derivation to pentafluorobenzyl ester, thin-layer chromatography purification, and derivation with methoxyamine

and trimethylsilylation) by means of gas chromatography–negative ion chemical ionization–mass spectrometry (Hewlett Packard 5989 B). Deuterium-labeled prostaglandins ($[^2\text{H}_4]$ -PGD₂ and $[^2\text{H}_4]$ -PGE₂, Cayman Chemicals) added immediately to collected supernatants were used as internal standards. For quantification, 2 ions were recorded, m/z 524 for prostaglandin and m/z 528 for the standard.

Experimental design

HBFs were seeded in 12-well culture plates at 5×10^4 cells per well and were allowed to grow in media containing 10% FCS. After reaching confluency (3–5 days), the cells were washed with HBSS, and the medium was changed to a serum-free medium (DMEM with 0.1% BSA). The cells were silenced in this medium for 24 hours. Then growth-arrested cells were stimulated with the cytomix for 18 hours or incubated without any additives in serum-free medium (unstimulated control). The cytomix consisted of 5 ng/mL IL-1 β (human recombinant, BD-Pharmingen), 10 ng/mL TNF- α (Calbiochem), and 5 μ g/mL LPS (LPS from *Pseudomonas aeruginosa*, Calbiochem). The media were changed for DMEM, and exogenous arachidonic acid (Sigma) was added into each well to a concentration of 5 μ mol/L. After 30 minutes of incubation, the cells were washed twice with HBSS and then incubated in 1 mL of serum-free DMEM (without albumin). Indomethacin (Sigma) was added to half of the wells to a final concentration of 10 μ mol/L from a stock solution in methanol. Simultaneously, methanol was added to the other wells to a final concentration of 0.01%. After 90 minutes of incubation, the supernatants were harvested into tubes already containing the standards of PGE₂ and PGD₂ and immediately frozen at -20°C . All the above experimental steps were carried out in duplicate, and prostaglandin levels were measured separately in each supernatant.

Preparation of cell extracts and Western blotting

Cells were cultured in 10-cm-diameter cell-culture Petri dishes until confluency. Half of the dishes were stimulated with cytomix for 18 hours, as described above. Fibroblasts were lysed with the lysis buffer (0.1 mol/L Tris-HCl, 15% glycerol, 2 mmol/L EDTA, 2% SDS, 10 mmol/L phenylmethylsulfonyl fluoride, and 10 μ g/mL aprotinin, pH 7.4), and 40 μ g of total cellular protein was subjected to SDS-PAGE in 12% acrylamide gels under reducing conditions and transferred into polyvinylidene difluoride membrane (Hybond-P, Amersham Pharmacia Biotech). After blocking in PBS-T (0.1% Tween 80 in PBS) containing 5% skimmed milk overnight at 4°C , the membrane was incubated for 1 hour with goat polyclonal anti-COX-2 or anti-COX-1 antibody at 37°C (1:1000 in 5% skimmed milk in PBS-T). After washing 3 times, the membrane was incubated for 1.5 hours with anti-goat IgG horseradish peroxidase–conjugated antibody (1:1000 in PBS-T containing 5% skimmed milk; all antibodies were from Santa Cruz Biotechnology). The extensively washed membranes were incubated with chemiluminescent reagent (ECL–Western blotting analysis system, Amersham) for 60 seconds and exposed to Kodak X-Omat film. The films were digitalized and analyzed with an imaging system (ImageMaster VDS software, Pharmacia). The membranes were stained with Coomassie Brilliant Blue to allow the measurement of protein loading. The pictures of the stained membranes were also analyzed with the imaging system. The values obtained for each specific band were corrected by total protein loading.

Real-time RT-PCR analysis of COX-2 and COX-1 mRNA

In preliminary experiments (done on HBFs isolated from 2 nonasthmatic patients) the expression of COX-1 and COX-2 mRNA was analyzed 0, 1, 2, 6, 12, and 24 hours after the addition of cytomix by means of RT-PCR. On stimulation with cytomix, the

amount of mRNA for COX-1 remained constant; COX-2 mRNA was not detected in the control condition but was present in stimulated cells. The highest amount of COX-2 mRNA was observed in cells stimulated with cytomix for 2 hours (data not shown). All other cell lines were stimulated for 2 hours. Total cellular RNA was extracted from approximately 10^6 unstimulated cells or cells incubated with cytomix (the confluent monolayer culture, 2 wells from 6-well cell plates) by using 1 mL of Trizol (Life Technologies). Reverse transcription of 0.5 μ g of total RNA was performed by using MMLV (Promega) and random primers (Promega). The PCR reaction was performed by using iCycler Real Time PCR equipment (Biorad). Sybr Green (Amresco) was added into each sample (0.3 μ). For each cell line tested, the reaction was performed with specific COX-2 primers (5'-AATGATTCATAGGGCTTCAG-3', 5'-ATTGACCCAGAACTACTTT-3'), COX-1 primers (5'-GAGCTGCTGTTCGGTGTC-3', 5'-CTCTTGCGGTACTCATGAAG-3'), and primers to human β -actin (5'-AGCGGGAATCGTGCGTG-3', 5'-GGGTACATGGTGGTGCCG-3'). The relative abundance of the specific mRNA transcripts was referred to as a cycle threshold value (C_T), a PCR cycle leading to a significant (10 $\times$ SD) increase of the product fluorescence. The comparison between cell lines was performed with the gene encoding β -actin as a normalizer. ΔC_T value was calculated for each sample as follows:

$$\Delta C_T = C_{T\beta\text{-actin}} - C_{T\text{COX}}$$

All samples were run in duplicate.

Statistical analysis

Duplicate results from each individual patient under each particular experimental condition were averaged. The change in PGE₂ and PGD₂ release after cytomix and indomethacin was expressed as a percentage of the production of unstimulated cells (percentage of control). The Wilcoxon paired test was used to compare means inside the group (control vs indomethacin and control vs cytomix), and the Mann-Whitney U test was used for unpaired data to compare groups of patients (nonasthmatic patients vs patients with ATA, nonasthmatic patients vs patients with AIA, and patients with ATA vs patients with AIA). A P value of less than .05 was considered significant.

RESULTS

Release of PGE₂ and PGD₂ by HBFs in culture

Under control culture conditions (cells not stimulated with cytomix and incubated in medium without indomethacin), all cell lines produced detectable amounts of both PGE₂ and PGD₂. The mean concentrations of PGE₂ in the nonasthmatic patients, patients with ATA, and patients with AIA were 1.01 ± 0.95 , 2.17 ± 2.67 , and 1.29 ± 0.77 ng/mL, respectively (Fig 1, A). In all the cell lines, the PGE₂ concentration in supernatants was at least 5 times higher than the concentration of PGD₂; the mean values for nonasthmatic patients, patients with ATA, and patients with AIA were 0.18 ± 0.16 , 0.18 ± 0.14 , and 0.235 ± 0.19 ng/mL, respectively (Fig 1, B). The results between the groups did not differ significantly.

Stimulation of PGE₂ and PGD₂ production by cytomix

To imitate the inflammatory conditions in the bronchial wall, we incubated the fibroblasts in media containing IL-1 β , TNF- α , and LPS, factors that induce

FIG 1. Release of PGE₂ (A) or PGD₂ (B) by HBFs in nonasthmatic patients (circles), patients with ATA (triangles), and patients with AIA (squares) under control conditions or after cytomix (IL-1 β , 5 ng/mL; TNF- α , 10 ng/mL; and LPS, 5 μ g/mL) stimulation. Quiescent fibroblasts were kept in control conditions or stimulated with cytomix for 18 hours in 12-well plates. After addition of exogenous arachidonic acid for 30 minutes, cells were incubated in serum-free DMEM for 90 minutes, and prostaglandin concentrations were measured in the supernatants. The values are represented as the individual data for each cell line and are the means of duplicated experimental points.

COX-2 mRNA expression in fibroblasts.^{16,17} In preliminary experiments the optimal concentration of each factor was chosen, and it was observed that IL-1 β , TNF- α , and LPS work synergistically with respect to the stimulation of the prostaglandin production. The 18-hour incubation in cytomix-containing medium dramatically increased the capacity of the cells to release both types of prostaglandins. The effect of cytomix was the strongest in nonasthmatic patients and less pronounced in both groups of asthmatic patients (those with ATA and those with AIA). The mean PGE₂ value in the nonasthmatic patient group reached 32 ± 33 ng/mL, that in patients with ATA reached 16 ± 18 ng/mL, and that in patients with AIA reached 5.3 ± 3.6 ng/mL. In the case of PGD₂, the values were 0.58 ± 0.44 , 0.50 ± 0.45 , and 0.47 ± 0.46 ng/mL, respectively. Because of large SDs, the differences between the groups did not reach statistical significance (Fig 1). When the percentage of stimulation was considered, in the nonasthmatic patient group cytomix-stimulated PGE₂ production was seen to a higher degree than in the ATA group ($P < .05$, Mann-Whitney U test). In the AIA group the stimulation was at least 10 times less than in the nonasthmatic patient group ($P < .01$) and 5 times less than that in the ATA group ($P < .05$). In the

case of PGD₂ production, the same tendency was observed, but only the difference between the nonasthmatic patient and AIA groups reached statistical significance ($P < .05$, Fig 2).

Inhibition of PGE₂ and PGD₂ production by indomethacin

Indomethacin was used at a concentration of 10 μ mol/L, which was shown to effectively inhibit cyclooxygenases in the other cell culture systems.¹⁸ A significant decrease in the release of PGE₂ and PGD₂ was observed in all groups ($P < .01$, Wilcoxon matched-pairs test), both in control cells (the mean inhibitory effect was 44% in the nonasthmatic patient group, 34% in patients with ATA, and 41% in patients with AIA) and in cytomix-stimulated cells (62% in nonasthmatic patients, 61% in patients with ATA, and 61% in patients with AIA). The inhibitory effect of the drug on PGE₂ synthesis was significantly stronger after the cytomix stimulation than in the nonstimulated cell lines, but the groups did not differ in this respect ($P < .05$, Mann-Whitney U test). Neither was a difference between the control condition and cytomix stimulation observed in the case of PGD₂ (data not shown).

FIG 2. Effect of the cytomix on the production of PGE₂ and PGD₂ by HBFs in nonasthmatic patients (white bars), patients with ATA (green bars), and patients with AIA (red bars). The stimulatory effect is expressed as the percentage of the change in the release of prostaglandins. Each bar represents the median $\pm$ lower and upper quartiles of 10 (nonasthmatic patients), 9 (patients with ATA), and 7 (patients with AIA) cell lines.

PGE₂/PGD₂ ratio

In each experimental sample we measured both PGE₂ and PGD₂ levels and calculated the PGE₂/PGD₂ ratio. Because PGE₂ is the predominantly produced prostaglandin in HBFs,¹⁹ the ratio was greater than 5 for all the tested cell lines. In all cases the PGE₂/PGD₂ ratio increased after cytomix stimulation. This result suggests that the stimulation of COX-2 expression triggers PGE₂ production more effectively than PGD₂ production by HBFs in culture. Furthermore, the PGE₂/PGD₂ ratio observed after cytomix stimulation was significantly smaller in patients with AIA than in the ATA group ($P < .05$, Mann-Whitney U test). When the prostaglandins released by cytomix-stimulated cells were measured in the presence of indomethacin, the PGE₂/PGD₂ ratio was lower than in cells stimulated without inhibitor. In this case a statistically significant difference was observed only in the ATA group ($P < .05$). A similar but statistically not significant tendency was observed in the AIA and nonasthmatic patient groups (Fig 3). On the basis of these data, it can be assumed that in the case of stimulated HBFs, indomethacin inhibits PGE₂ production more effectively than PGD₂.

Level of cyclooxygenase expression

In all tested cell lines, the level of COX-2 protein was undetectable in the extracts from cells maintained under control conditions. After cytomix stimulation, all the cell lines expressed a detectable amount of COX-2 mRNA and protein. The level of induction was variable among individuals, but we did not observe any statistically significant differences among the groups. Unexpectedly, in the AIA group we noticed a lower level of COX-1 protein expression in nonstimulated cells in comparison with that seen in the ATA group ($P < .05$). At the level of mRNA, we could not confirm the existence of COX-1

deficiency in patients with AIA. The mean $\square C_{T(\text{actin-COX1})}$ value was 9.3 ± 1.4 in patients with AIA and 8.7 ± 2.8 in patients with ATA. The mean $\square C_{T(\text{actin-COX2})}$ value was 7.5 ± 2.3 in patients with AIA and 10.9 ± 2.0 in patients with ATA, but the difference was not statistically significant (Fig 4).

DISCUSSION

Our results demonstrate that HBFs isolated from 2 distinct groups of asthmatic patients and control subjects produce both PGE₂ and PGD₂, and their release increases after the addition of proinflammatory factors. As reported by others,²⁰ in HBFs PGE₂ was the predominantly produced prostaglandin, but a smaller amount of PGD₂ was also generated.

We did not observe significant differences among the groups in the basal production of PGE₂. Cytomix stimulated the generation of PGE₂ by fibroblasts in all the cell lines. The highest increase occurred in the nonasthmatic patient group. PGE₂, produced in the airway mainly by smooth muscle cells, fibroblasts, and epithelial cells,^{8,21,22} protects against inflammation and inhibits its propagation.⁸ Inhaled PGE₂ effectively prevents bronchoconstriction induced by allergen, aspirin, exercise, or other nonspecific stimuli in sensitive individuals.²³ Endogenous PGE₂ blocks collagen synthesis by fibroblasts, airway smooth muscle proliferation, and liberation of acetylcholine from parasympathetic nerve endings and attenuates inflammatory cell activation.²³ Thus an intrinsic deficiency in the production of PGE₂ observed by us in cultured fibroblasts obtained from the ATA and AIA groups could contribute to the development of chronic inflammation in asthma. Our results indicate that fibroblasts should be implicated as a source of bioactive eicosanoids in the pathophysiology of asthma in addition to inflammatory cells. This is consistent

FIG 3. Comparison of the PGE₂/PGD₂ ratios produced by HBFs derived from nonasthmatic patients, patients with ATA, and patients with AIA. The prostaglandins were measured in supernatants from cells kept under control conditions in the absence (white bars) or presence (red bars) of indomethacin or after stimulation with cytomix without (green bars) or with (blue bars) indomethacin. Each bar represents the median $\pm$ lower and upper quartiles of 10 (nonasthmatic patients), 9 (patients with ATA), and 7 (patients with AIA) cell lines.

FIG 4. The typical result of Western blotting of protein extracts from nonstimulated cells (COX-1) and from cells stimulated with cytomix (COX-2). Three patients from each group were analyzed in each run. The mean value of the nonasthmatic patient group in each film was considered 100%, and the values in the ATA and AIA groups were calculated in comparison with it. The bars on the accompanying graph presents the relative amount of COX-1 (open bars) and COX-2 (filled bars) proteins. The results were obtained in 3 independent measurements (mean $\pm$ SD of the proteins extracted from at least 6 different patients' fibroblasts in each group analyzed in 3 independent measurements). The amount of COX-1 was lower in the AIA group than in the ATA group ($P < .05$, Mann-Whitney U test). Other differences were not significant.

with studies by Pavord et al,²⁴ who showed a negative correlation between induced sputum PGE₂ concentration and the percentage of sputum eosinophil count, which strongly suggests that some other cell type is a main source of this metabolite in the airways. Vincheri et al¹⁴ and Wilborn et al²⁵ reported a diminished capacity of fibrotic lung fibroblasts to generate PGE₂ as a consequence of decreased COX-2 activity, resulting from reduced expression of TNF- α receptor on the cell surface. Subsequently, the fibroblast proliferation is perpetuated in the inflammatory process. Airway smooth muscle cell proliferation is also increased in asthma.²⁶

The other prostaglandin measured by us was PGD₂, a proinflammatory factor and a bronchoconstrictor produced mainly by mast cells. The role of PGD₂ generated by residual cells of the bronchial wall is less clear. In bronchoalveolar lavage (BAL) fluid of asthmatic patients, the PGD₂ level was shown to be increased, as was its urinary metabolite.²⁷ On the contrary, our *in vitro* experiments did not indicate significant differences among the groups in terms of the basal production of PGD₂. After cytomix stimulation, the cultured HBFs generated increased the amount of PGD₂ in all patients, and the more pronounced increase was detected in the nonasthmatic patient group. We can conclude that the enhanced PGD₂ production in asthmatic patients might be connected mostly with the presence of activated mast cells but not with activated fibroblasts.

PGE₂ and PGD₂ are synthesized from arachidonic acid through the cyclooxygenase pathway. Recent studies demonstrated that COX-2, but not COX-1, can be induced by proinflammatory agents in a number of cultured airway cells.²⁸ In our system COX-2 was upregulated by the cytomix, and in consequence, production of both prostaglandins increased. In a series of preliminary experiments, we checked whether in HBFs the mixture of IL-1 α , TNF- α , and LPS stimulated PGE₂ production to a higher extent than any of these factors used separately. Moreover, COX-2 mRNA was strongly induced after 2 hours, but the increase in its protein expression was most pronounced after 18 hours (data not shown).

The final step of PGE₂ generation depends on the enzyme called PGE₂ synthase. Two forms exist in cells. One is a membrane-bound PGE₂ synthase (mPGES) functionally coupled with COX-2 and taking part in inflammation and delayed PGE₂ synthesis; the other is the cytosolic PGE₂ synthase, which is coregulated with COX-1 and plays a role in immediate PGE₂ synthesis and tissue homeostasis.^{29,30} IL-1 α or TNF- α treatment induced the mPGES mRNA expression in primary synovial cells.³¹ We observed a pronounced increase in the PGE₂/PGD₂ ratio after cytomix stimulation in all the groups studied. Changes in the bronchoconstrictor/bronchodilator ratio was also observed *in vivo* by others. For example, the ratio of combined bronchoconstrictors (PGD₂ and thromboxane) to combined bronchodilators (PGE₂ and 6-keto-PGF_{1 α}) in BAL fluid increased more than 5-fold in atopic asthmatic patients after allergen challenge.³² The change in the PGE₂/PGD₂ ratio would

suggest that mPGES is also upregulated by the cytokines in our system, which is in contrast to the enzymes responsible for PGD₂ synthesis.

In this study we also investigated fibroblasts obtained from patients with AIA. Aspirin bronchial challenge leads to a decrease in PGE₂ concentration and to a simultaneous increase in cysteinyl leukotrienes in the BAL fluid of patients with AIA.³³ According to a current model, NSAIDs trigger adverse reactions by freeing the cysteinyl leukotriene synthetic pathway from the control of endogenous PGE₂.³⁴ Yet it remains obscure why only patients with AIA react with bronchospasm to the administration of aspirin. In our study we did not want to block the prostaglandin production totally. Indomethacin was added at the time of changing of the medium without any preincubation. Under such conditions, indomethacin inhibited approximately by half the synthesis of prostaglandins (because the inhibitor needs some time to reach all COX-1 and COX-2 molecules inside the cells and to inhibit all their enzymatic activity), and we expected that this might reveal possible differences between the studied groups. However, no such differences were detected. This might suggest that the inhibitory potential of indomethacin is not stronger in patients with AIA than in aspirin-tolerant individuals, but dose-response studies would be necessary to support this concept.

In our experiment the ability of cytomix to induce PGE₂ production by HBFs was lowest in the AIA group. These findings corroborate the results of Kowalski et al¹¹ on lower PGE₂ production by nasal polyp epithelial cells isolated from aspirin-sensitive patients in comparison with those harvested from aspirin-tolerant patients. Picado et al¹² suggested that this PGE₂ deficiency might be due to a lower expression of COX-2 in the nasal mucosa of aspirin-intolerant patients, although this has not been uniformly observed by others in bronchial biopsy specimens. Our results point to the similar expression of COX-2 in HBFs from all groups of patients both at the level of mRNA and protein. We observed also that the COX-1 protein level is lower in the AIA group than in the ATA group, which suggests that in the former group NSAIDs can more easily block prostaglandin production driven by COX-1. This finding is in agreement with those of recent clinical studies demonstrating that selective COX-2 inhibitors, contrary to COX-1 inhibitors, are very well tolerated by aspirin-sensitive asthmatic patients and evoke no alterations in their eicosanoid metabolism.^{37,38} However, it remains to be explained why the individuals not sensitive to NSAIDs tolerate well even very high doses of the drug. We can speculate that the impairment in arachidonic acid metabolism in patients with AIA is not directly and solely connected with the COX-2 expression level but is located downstream or upstream in the pathway of prostaglandin production. In this study we observed a lower PGE₂/PGD₂ ratio in the AIA group in comparison with that in the ATA group, which suggests that the coupling between cyclooxygenases and specific prostaglandin synthases can be important in the pathogenesis of AIA.

In conclusion, we have shown, for the first time, that prostaglandin synthesis is altered in fibroblasts cultured from asthmatic patients, and this phenomenon is particularly pronounced in fibroblasts derived from patients with AIA. It can contribute to the mechanisms operating in bronchial asthma because locally produced prostaglandins are known to affect the function of both residual and infiltrating cells of the airways.

We thank Dr Krzysztof Nagraba and Ms Anna Gielicz for measurement of prostaglandins. MP wishes to thank Dr Donna E. Davies and her colleagues from the Division of Respiratory Cell and Molecular Biology, Southampton University School of Medicine, for the introduction into the techniques of primary bronchial cell culture.

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