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Moraxella catarrhalis induces ERK- and NF-κB-dependent COX-2 and prostaglandin E_2 in lung epithelium

P.D. N'Guessan, B. Temmesfeld-Wollbrück, J. Zahlten, J. Eitel, S. Zabel, B. Schmeck, B. Opitz, S. Hippenstiel, N. Suttorp and H. Slevogt

ABSTRACT: ***Moraxella catarrhalis*** **is a major cause of infectious exacerbations of chronic obstructive lung disease. Cyclooxygenase (COX)-derived prostaglandins, such as prostaglandin E_2 (PGE_2), are considered to be important regulators of lung function. The present authors tested the hypothesis that *M. catarrhalis* induces COX-2-dependent PGE_2 production in pulmonary epithelial cells.**

In the present study, the authors demonstrate that *M. catarrhalis* specifically induces COX-2 expression and subsequent PGE_2 release in pulmonary epithelial cells. Furthermore, the prostanoid receptor subtypes EP2 and EP4 were also upregulated in these cells.

The *M. catarrhalis*-specific ubiquitous cell surface protein A1 was important for the induction of COX-2 and PGE_2. Moreover, *M. catarrhalis*-induced COX-2 and PGE_2 expression was dependent on extracellular signal-regulated kinase 1/2-driven activation of nuclear factor-κB, but not on the activation of p38 mitogen-activated protein kinase.

In conclusion, the present data suggest that ubiquitous cell surface protein A1 of *Moraxella catarrhalis*, extracellular signal-regulated kinase 1/2 and nuclear factor-κB control cyclooxygenase-2 expression and subsequent prostaglandin E_2 release by lung epithelial cells. *Moraxella catarrhalis*-induced prostaglandin E_2 expression might counteract lung inflammation promoting colonisation of the respiratory tract in chronic obstructive pulmonary disease patients.

KEYWORDS: **Cyclooxygenase 2, extracellular signal-regulated kinase 1/2, *Moraxella catarrhalis*, nuclear factor-κB, prostaglandin E_2, ubiquitous cell surface protein A1**

Chronic obstructive pulmonary disease (COPD) is a leading cause of morbidity and mortality worldwide. Disease progression is characterised by frequent acute exacerbations caused by bacterial or viral infection [1–3]. Although many studies support the causative role of *Haemophilus influenzae* and *Streptococcus pneumoniae* in the pathogenesis of COPD [2, 4], *Moraxella catarrhalis* was widely ignored for decades because this pathogen was considered to be an irrelevant saprophyte of the upper respiratory tract [5]. However, increasing evidence underlines the importance of *M. catarrhalis* for acute exacerbations and disease progression of COPD [5].

The outer membrane protein ubiquitous cell surface protein (Usp)A1 of *M. catarrhalis*, an antigenically conserved high molecular weight adhesin, is expressed by a majority of Moraxella isolates from COPD patients [6]. This virulence factor has been implicated in the targeting of epithelial cell-associated fibronectin and laminin, as well as the human carcinoembryonic antigen-related cell adhesion molecule (CEACAM1) [7–9]. However, little is known about the *M. catarrhalis*–bronchial epithelium interaction.

Lipid metabolites of arachidonic acid, including prostaglandins and leukotrienes, have emerged as potent endogenous mediators and modulators of innate immunity in the lung [10, 11]. There is growing evidence that increased concentration of prostaglandin (PG)E_2 in the lung of patients is a key event in the pathogenesis of COPD [12]. Increased PGE_2 in the lung has been shown to stimulate the secretion of surfactant by alveolar type II cells and wound closure in airway epithelium [11]. It has also been reported that PGE_2 downregulates the production of important inflammatory cytokines, such as interleukin (IL)-8, IL-12, monocyte chemotactic protein (MCP)-1 and granulocyte-macrophage colony-stimulating factor (GM-CSF), which are essential for leukocyte

AFFILIATIONS
Dept of Internal Medicine/Infectious Diseases and Pulmonary Medicine, Charité-Universitätsmedizin Berlin, Berlin, Germany.

CORRESPONDENCE
H. Slevogt
Dept of Internal Medicine/Infectious Diseases and Pulmonary Medicine
Charité-Universitätsmedizin Berlin
Augustenburger Platz 1
13353 Berlin
Germany
Fax: 49 30450553906
E-mail: hortense.slevogt@charite.de

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migration [11]. In particular, PGE_2 produced at sites of infection was shown to modulate immune and inflammatory responses [10, 11] and is liberated by lung epithelial cells [13, 14]. The activity of PGE_2 is mediated by four receptors, termed E prostanoid receptors (EP1–EP4) [10, 11]. Although the exact roles of each receptor type are not definitively established, it is plausible that cAMP accumulation, promoted by the EP2 and EP4 receptors, is associated with inhibition of effector cell functions. However, the EP1 and EP3 receptors are known to increase intracellular calcium and to promote cellular activation [10, 11]. The existence of four subtypes of receptors and the possible expression of multiple receptors in a single cell can explain the multiplicity of biological responses elicited by PGE_2 and how these responses might be diverse in different cells and tissues [10, 11]. It is also probable that during inflammation the repertoire of receptors expressed changes, leading to a wide array of effects.

PGE_2 is a product of the cyclooxygenase (COX)/prostaglandin H synthase pathway, which includes two distinct isoforms of COX: the constitutively expressed COX-1 and the (generally) inducible COX-2 [15]. The regulation of the *cox2* promoter is subjected to a tight regulatory network involving nuclear factor (NF)-κB, which can be activated by complex kinase pathways centred around p38 and extracellular signal-regulated kinase (ERK)1/2 mitogen-activated protein kinase (MAPK) [13–15].

The MAPK family is involved in multiple cell functions, including inflammation, proliferation and apoptosis [13–15]. Five distinguishable MAPK subfamilies have been identified in mammalian systems; the best described of these are the ERK1/2 (p42/p44), p38 and c-Jun N-terminal kinase pathways [16].

Activation of pro-inflammatory signalling pathways in lung epithelial cells by bacterial infection, including the p38-, ERK1/2-MAPK and NF-κB pathways are suggested to contribute significantly to disease process in COPD and pneumonia [17–19]. Although *M. catarrhalis* efficiently infects and activates lung epithelial cells [19–22], mechanisms of *M. catarrhalis*-induced activation of COX-2 and PGE_2 release in lung epithelial cells are widely unknown.

In the present study, the hypothesis that *M. catarrhalis* induces COX-2 expression and subsequent PGE_2 synthesis by stimulation of MAPK pathways and NF-κB in lung epithelial cells was tested. The present authors report herein that *M. catarrhalis* was found to induce COX-2 expression and a subsequent PGE_2 release. In addition, the prostanoid receptors EP2 and EP4 were also upregulated in *M. catarrhalis*-infected cells. The *M. catarrhalis* outer membrane protein UspA1 was found to be important for COX-2 expression and PGE_2 release in bronchial epithelial cells. Furthermore, it was found that PGE_2 release and COX-2 expression was dependent on the activation of ERK1/2 MAPK driven activation of NF-κB, but not on the activation of p38 MAPK. Therefore, *M. catarrhalis*-induced, COX-2-dependent PGE_2 liberation by lung epithelial cells may contribute significantly to the pathogenesis of COPD.

MATERIAL AND METHODS

Bacterial strains

M. catarrhalis wild-type strain O35E (serotype A) and the isogenic *UspA*1-deficient mutant of O35E (O35E.1) were kindly provided by E. Hansen (University of Texas Southwestern Medical Center, Dallas, TX, USA). Antimicrobial supplementation for the *M. catarrhalis* mutant O35E.1 involved kanamycin (15 $\mu g \cdot mL^{-1}$). *M. catarrhalis* strain was grown overnight at 37°C on brain–heart infusion (BHI) agar (Difco Laboratories, BD Heidelberg, Germany) supplemented with 5% heated sheep blood. For infection experiments, single colonies of bacterial overnight cultures were expanded by resuspension in BHI broth and incubation at 37°C for 2–3 h to midlog phase (A_{405} 0.4–0.6). Subsequently, bacteria were harvested by centrifugation, resuspended in cell culture medium without antibiotics and adjusted to an optical density at 405 nm of 0.3 ($\approx 1 \times 10^6$ colony-forming units (cfu)$\cdot mL^{-1}$) and used for epithelial cell infection at the indicated multiplicity of infection (MOI). To confirm the viability of *M. catarrhalis* in cell culture medium, bacteria were resuspended and optical density was measured over time. Data were verified by different cfu countings at different optical densities of *M. catarrhalis* suspensions.

Cell lines

Human bronchial epithelial cell line BEAS-2B was a kind gift from C. Harris (National Institutes of Health, Bethesda, MD, USA) [23]. Each well was seeded with 4×10^5 BEAS-2B cells per millilitre and grown in Keratinocyte-SFM (Gibco BRL, Life Technologies, Paisley, UK) supplemented with 2 mM L-glutamine, 100 $U \cdot mL^{-1}$ penicillin and 100 $\mu g \cdot mL^{-1}$ streptomycin. Cells were grown to confluence in 75-cm^2 flasks and subsequently cultured in different well plates (both Falcon; Corning Star, Wiesbaden, Germany). Twelve hours before the experiment, cells were grown in medium without antibiotic supplements. Human embryonic kidney cells (HEK)-293 were purchased from ATCC (Manassas, VA, USA) and cultured according to the manufacturer's instructions.

Materials

Keratinocyte-SFM culture medium was purchased by Gibco BRL Life Technologies. Foetal calf serum, trypsin–EDTA solution, CA-650 and antibiotics were obtained from Life Technologies (Karlsruhe, Germany). Protease inhibitors, Triton X-100, 4-dichloroisocumarin and Tween20 were purchased from Sigma Chemical Co. (Munich, Germany). The MAPK inhibitors U0126 and SB202190, indomethacin, SC-560 and NS-398 were purchased from Calbiochem (Merck, Bad Soden, Germany). Tumour necrosis factor (TNF)-α and IL-lβ were obtained from R&D Systems (Wiesbaden, Germany), and IKK-Nemo binding domain (NBD) from Biomol (Plymouth, UK). All other chemicals used were of analytical grade and obtained from commercial sources.

PGE_2 ELISA

Confluent BEAS-2B cells were infected with *M. catarrhalis* or stimulated with TNF-α and IL-1β as indicated. After incubation, supernatants were collected and processed for PGE_2 quantification by immunoassay, according to the manufacturer's instructions (R&D Systems) [13, 14].

Immunoblot analysis

For determination of COX-1, COX-2, EP1, EP2, EP3 and EP4 expression, and p38 MAPK and ERK1/2 phosphorylation, BEAS-2B cells were infected or incubated with TNF-α and IL-1β as indicated, washed twice in Tris buffer, either with or

without phosphatase inhibitors, and then harvested. Cells were lysed in buffer containing Triton X-100, subjected to sodium dodecyl sulphate–polyacrylamide gel electrophoresis and blotted on Hybond-ECL membrane (Amersham Biosciences, Freiburg, Germany). Immunodetection of target proteins was carried out with the following specific antibodies: COX-2 (Santa Cruz Biotechnologies, Santa Cruz, CA, USA), COX-1 (Upstate Biotechnology, Lake Placid, NY, USA), phosphospecific p38 MAPK antibodies (Cell Signaling, Frankfurt, Germany) [13, 14], phosphospecific ERK1/2 [13, 14] and EP1-4 antibodies (Santa Cruz Biotechnologies). In all experiments, actin, p38 or p42 (all Santa Cruz Biotechnologies) were detected simultaneously to confirm equal protein loading [13, 14, 24]. Detection was performed by visualisation of IRDye800- or Cy5.5-labelled secondary antibodies (Odyssey infrared imaging system; LI-COR Inc., Lincoln, NE, USA).

Reverse transcriptase-ploymerase chain reaction

For analysis of COX-2 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene expression in BEAS-2B cells, total RNA was isolated with the RNeasy Mini kit (Qiagen, Hilden, Germany) and reversely transcribed using avian myeloblastosis virus reverse transcriptase (Promega, Heidelberg, Germany) [13, 14]. Generated cDNA was amplified by polymerase chain reaction (PCR) using intron-spanning specific primers for COX-2 (forward: 5′-TGCTGTGGAGCTGTATCC-3′, reverse: 5′-GACTCCTTTCTCCGCAAC-3′), COX-1 (forward: 5′- TGTTCGGTGTCCAGTTCCAATA-3′, reverse: 5′-ACCTTGAAGGAGTCAGGCATGAG-3′), EP1 (forward: 5′-CACGTGGTGCTTCATCGCCCTGGGTC-3′, reverse: 5′-CACCACCATGATACCGACAAG-3′), EP2 (forward: 5′-TCCAATGACTCCCAGTCTGAGGA-3′, reverse: 5′-TCAAAGGTCAGCCTGTTTAC-3′), EP3 (forward: 5′-CTGGTATGCGAGCCACATGAA-3′, reverse: 5′-TGAAGCCAGGCGAACAGCTAT-3′), EP4 (forward: 5′-TCTGACCTCGGTGTCCAAAAATCG-3′, reverse: 5′-TGGGTACTGCAGCCGCGAGCTA-3′) and GAPDH. All primers were purchased from TIB MOLBIOL (Berlin, Germany). After 35 amplification cycles, PCR products were analysed on 1.5% agarose gels, stained with ethidium bromide and subsequently visualised. To confirm the use of equal amounts of RNA in each experiment, all samples were checked for GAPDH mRNA expression [13, 14].

Electrophoretic mobility shift assay

After stimulation of BEAS-2B cells, nuclear protein was isolated and analysed by electrophoretic mobility shift assay (EMSA) as described previously [14, 18, 19]. IRDye800-labelled consensus NF-κB oligonucleotides were purchased from Metabion (Planegg-Martinsried, Germany). Briefly, EMSA binding reactions were performed by incubating 2 μg of nuclear extract with the annealed oligonucleotides according to the manufacturer's instructions. The reaction mixture was subjected to electrophoresis on a native gel PAGE and analysed by Odyssey infrared imaging system (LI-COR Inc.).

Plasmids and transient transfection procedures

HEK-293 cells were cultured in 12-well plates with Dulbecco's modified Eagle medium supplemented with 10% foetal calf serum. Subconfluent cells were co-transfected by using the calcium phosphate precipitation method according to the manufacturer's instructions (Clontech, Palo Alto, CA, USA) with 0.2 μg of NF-κB-dependent luciferase reporter [18], 0.2 μg of respiratory syncytial virus-galactosidase plasmid, 0.1 μg of human Toll-like receptor 2 (hTLR2; generously provided by Tularik, San Francisco, CA, USA) [25] expression vectors or control vector, respectively. A luciferase reporter-gene assay (Promega, Mannheim, Germany) was used to measure luciferase activity, and results were normalised for transfection efficiency, with values obtained by respiratory syncytial virus-galactosidase as described previously [18].

Statistical analysis

Data are shown as mean±SEM of at least three independent experiments. A one-way ANOVA was used for data of figures 1, 2c, 3, 4b, 5c, 6c and 7. The main effects were then compared by a Newman–Keul's post-test. Statistical significance was accepted at $p<0.01$.

RESULTS

M. catarrhalis *induces COX-2-dependent release of* PGE_2 *in human bronchial epithelial cells*

To study the effect of *M. catarrhalis* on lung epithelium, human bronchial epithelial cells, BEAS-2B, were infected with *M. catarrhalis* strain O35E (MOI 0.1 and 1) or exposed to TNF-α (50 ng·mL^{-1}) and IL-1β (10 ng·mL^{-1}) for 4, 8 and 16 h and PGE_2 release was analysed by ELISA (fig. 1). *M. catarrhalis* infection of lung epithelial cells time- and concentration-dependently induced the release of PGE_2. PGE_2 activities are mediated through its binding to the prostanoid receptors EP1, 2, 3 and 4. Furthermore, a time-dependent induction of EP2 and EP4 in infected cells could be seen (fig. 8). The expression pattern of EP1 and EP3 did not change in these cells (fig. 8).

PGE_2 release is dependent on the expression of COX-1 and/or COX-2. COX-2 expression may be increased after pro-inflammatory stimulation of cells. Therefore, the expression of both iso-enzymes in *M. catarrhalis*-infected lung epithelium

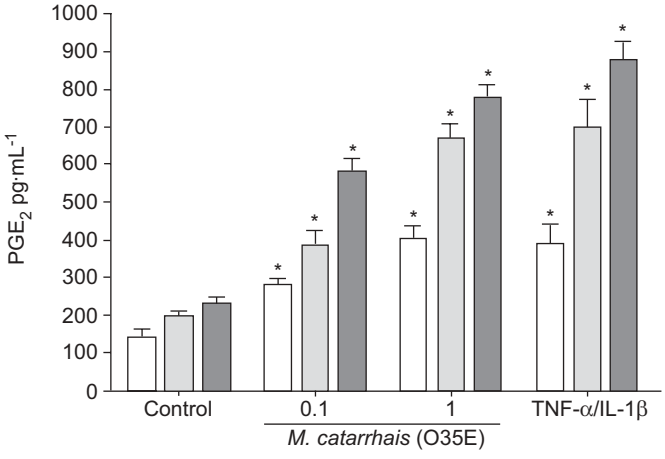

FIGURE 1. *Moraxella catarrhalis*-induced a time- and concentration-dependent prostaglandin (PG)E_2 expression in bronchial epithelial cells. Human bronchial epithelial cell line (BEAS-2B) cells were infected for 4, 8 and 16 h with *M. catarrhalis* (multiplicity of infection 0.1, 1) or stimulated with tumour necrosis factor (TNF)-α (50 ng·mL^{-1}) plus interleukin (IL)-1β (10 ng·mL^{-1}) and PGE_2 release was measured by ELISA. The data represent the mean±SEM of four values. □: 4 h; ▫: 8 h; ■: 16 h. *: $p<0.05$ *versus* infected control.

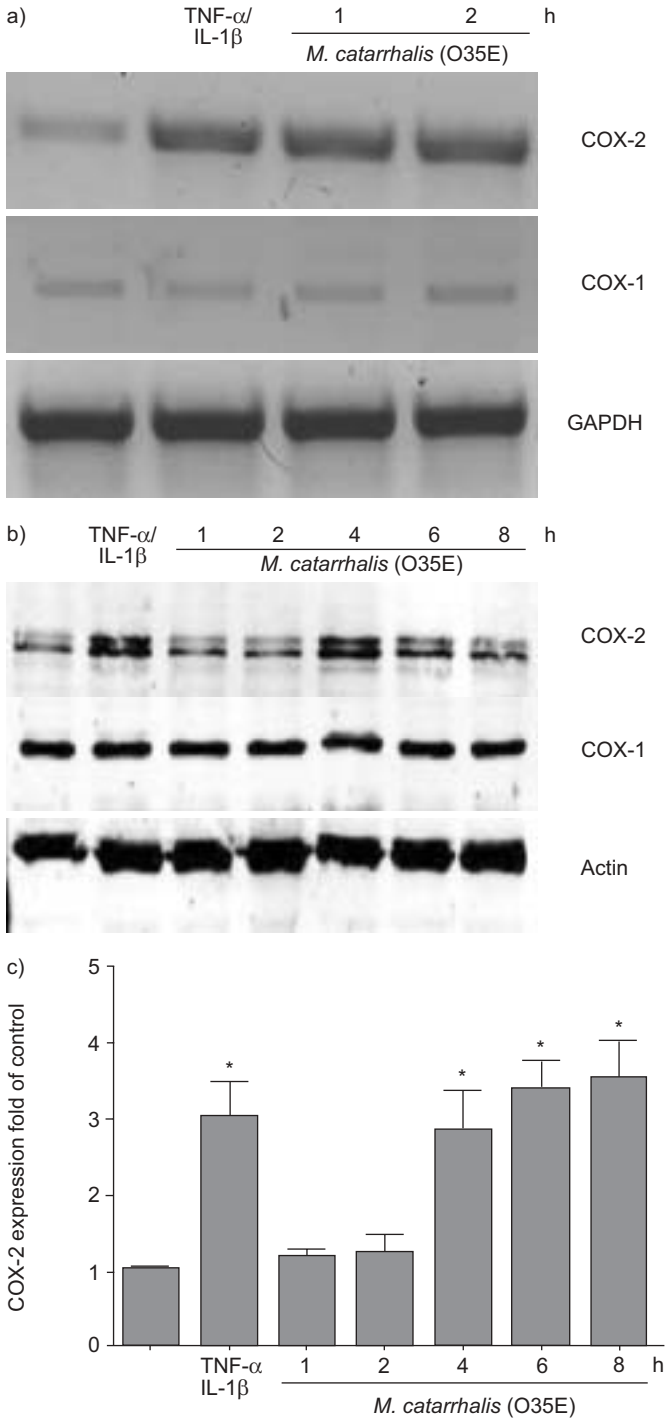

FIGURE 2. *Moraxella catarrhalis*-induced time-dependent expression of cyclooxygenase (COX)-2 in human bronchial epithelial cells. BEAS-2B cells were incubated with *M. catarrhalis* strain O35E (multiplicity of infection 1) or tumour necrosis factor (TNF)-α (50 ng·mL^{-1}) plus interleukin (IL)-1β (10 ng·mL^{-1}) for the indicated time. COX-1 and COX-2 transcription and expression were analysed by a) polymerase chain reaction and b) Western blot. TNF-α (50 ng·mL^{-1}) plus IL-1β (10 ng·mL^{-1}) was used as a positive control by an incubation time of 2 h for PCR and 4 h for Western blot. c) Results of the three responses (Western blots) were graphically analysed. Representative blots or gels of three separate experiments are shown, and data are presented as mean±SEM of the three separate experiments. *: $p<0.05$ *versus* unstimulated control.

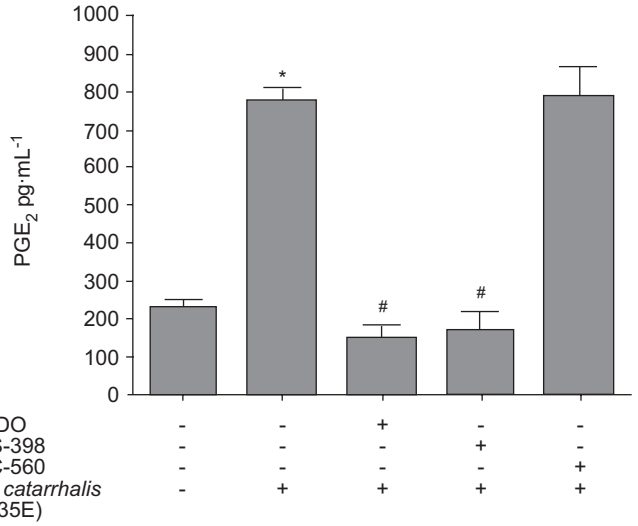

FIGURE 3. Inhibition of cyclooxygenase (COX)-2 but not of COX-1 abrogated *Moraxella catarrhalis*-induced prostaglandin (PG)E_2 release in human bronchial epithelial cells. BEAS-2B cells were pre-treated with the nonselective COX inhibitor indomethacin (INDO; 1 μM), the selective COX-1 inhibitor (SC-560; 1 μM), or with the selective COX-2 inhibitor (NS-398; 1 μM) for 30 min and then infected with *M. catarrhalis* O35E (multiplicity of infection 1) for 16 h. PGE_2 release was measured by ELISA. Data are presented as mean±SEM of the four separate experiments. *: $p<0.05$ *versus* unstimulated control. #: $p<0.05$ either with or without inhibitors.

was analysed. As shown in fig. 2a, *M. catarrhalis* (MOI 1) induced the transcription of COX-2 mRNA after 1 h of infection. Moreover, a time (1–8 h)-dependent increase in the expression of COX-2 protein, but not of COX-1 protein (figs 2b and c), was noted in *M. catarrhalis*-infected BEAS-2B cells.

To test the role of COX-1 and COX-2 in Moraxella-induced PGE_2 synthesis in lung epithelium, cells were infected in the absence or presence of the nonselective COX inhibitor indomethacin (1 μM), the selective COX-2 blocker NS-398 (1 μM), or the selective COX-1 inhibitor SC-560 (1 μM). Cells were pre-incubated with these drugs 30 min prior to infection.

Inhibition of COX-2 but not COX-1 in *M. catarrhalis*-infected BEAS-2B cells blocked PGE_2 release. The nonselective COX inhibitor indomethacin also strongly reduced PGE_2 secretion (fig. 3). Thus, *M. catarrhalis* induced COX-2-dependent release of PGE_2 secretion by cultured lung epithelial cells. The concentration of indomethacin, NS-398 and SC-560 used in the present study did not alter bacterial growth within the time frame tested (data not shown). The inhibitors neither reduced epithelial cell numbers nor induced morphological signs of cytotoxicity (data not shown).

The COX-2-dependent release of PGE_2 is induced by the M. catarrhalis*-specific UspA1*

By infecting bronchial epithelial cells with *M. catarrhalis* strain O35E or its UspA1-deficient mutant strain O35E.1, the impact of this bacterial virulence factor was analysed in more detail. The UspA1-deficient mutant O35E.1 induced COX-2 expression (4 h post-infection) and PGE_2 release (16 h post-infection) to a significantly lower extent (fig. 4) than the wild-type strain O35E, respectively. The present data suggested that UspA1

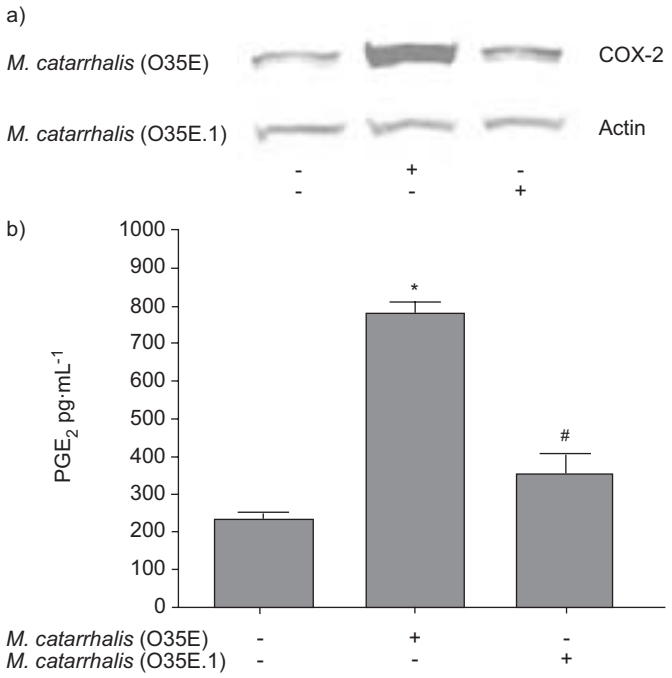

FIGURE 4. The *Moraxella catarrhalis*-specific UspA1 is important for the induction of cyclooxygenase (COX)-2 and prostaglandin (PG)E_2 release in pulmonary epithelial cells. BEAS-2B cells were infected with *M. catarrhalis* strain O35E (multiplicity of infection 1) or with the UspA1-deficient strain O35E.1 for 4 or 16 h. a) COX-2 expression (4 h) was analysed by Western blot and b) PGE_2 (16 h) secretion by ELISA. Representative blots out of three separate experiments are shown. ELISA data are presented as mean±SEM of four separate experiments. *: $p<0.05$ *versus* unstimulated control. #: $p<0.05$ O35E *versus* O35E.1.

plays an important role in the *M. catarrhalis*-induced COX-2 expression and subsequent PGE_2 release in airway epithelial cells.

Inhibition of ERK1/2 and p38 MAPK blocked M. catarrhalis*-induced expression of COX-2 and PGE_2 release in human bronchial epithelial cells*

Since MAPKs are considered to be important regulators of pro-inflammatory gene expression, analysis of MAPK activation and its impact on *M. catarrhalis*-related COX-2 expression and PGE_2 release was carried out. Infection of BEAS-2B cells with *M. catarrhalis* was associated with the activation of the MAPK p38, ERK1/2, as demonstrated by immunoblot analysis of phosphorylated ERK1/2 and p38 (fig. 5a). The degree of MAPK phosphorylation observed was comparable to that seen following TNF-α/IL-1β exposure. The ERK1/2 inhibitor U0126 significantly inhibited COX-2 activation and PGE_2 release by *M. catarrhalis* in BEAS-2B cells, whereas the p38 MAPK inhibitor SB202190 had no effect on either target (figs 5b and c). Similar results were obtained with SB203580, another p38-specific inhibitor (data not shown).

M. catarrhalis*-induced COX-2 expression and PGE_2 release depended on NF-κB activation in bronchial epithelial cells*

Expression of COX-2 and subsequent PGE_2 release in cells is considered to be regulated by NF-κB, which is released of its cytosolic sequestration by phosphorylation of its inhibitor

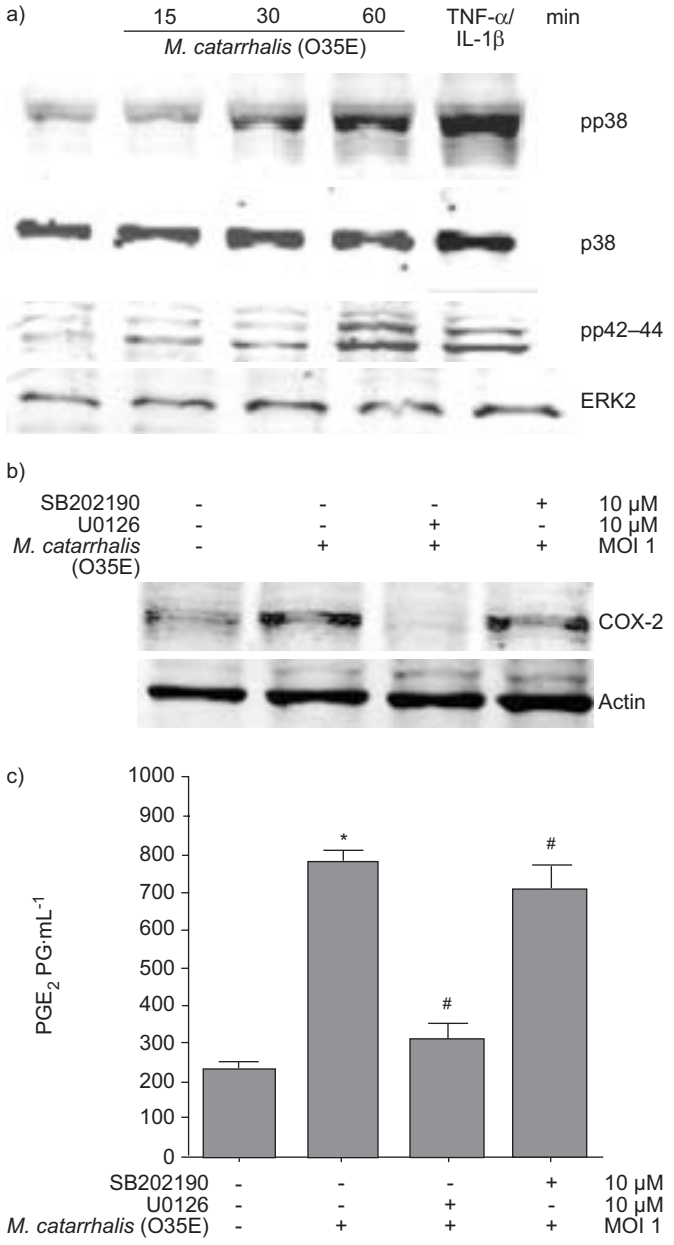

FIGURE 5. *Moraxella catarrhalis* induced cyclooxygenase (COX)-2 expression and prostaglandin (PG)E_2 release are extracellular signal-regulated kinase (ERK)1/2- but not p38 mitogen-activated protein kinase (MAPK)-dependent. BEAS-2B cells were incubated with *M. catarrhalis* for 15, 30 or 60 min, or tumour necrosis factor (TNF)-α (50 ng·mL^{-1}) plus interleukin (IL-1β; 10 ng·mL^{-1}) for 60 min. a) Phosphorylated p38 and ERK1/2 MAPK were detected by Western blot. Expression of p38 or p42 was performed simultaneously to confirm equal protein load. Furthermore, BEAS-2B cells were pre-incubated with the ERK1/2 inhibitor U0126 and the p38 MAPK inhibitor SB202190 for 60 min and then infected with *M. catarrhalis* strain O35E (multiplicity of infection (MOI) 1) for b) 4 h or c) 16 h. Data presented in (c) are mean±SEM of four separate experiments. Representative blots out of three separate experiments are shown. *: $p<0.05$ *versus* unstimulated control. #: $p<0.05$ with or without inhibitors.

(I)κBα by inhibitor of κB kinase (IKK)β and subsequent proteolytic degradation [15]. To assess NF-κB activation, *M. catarrhalis*-infected BEAS-2B cells were examined for different

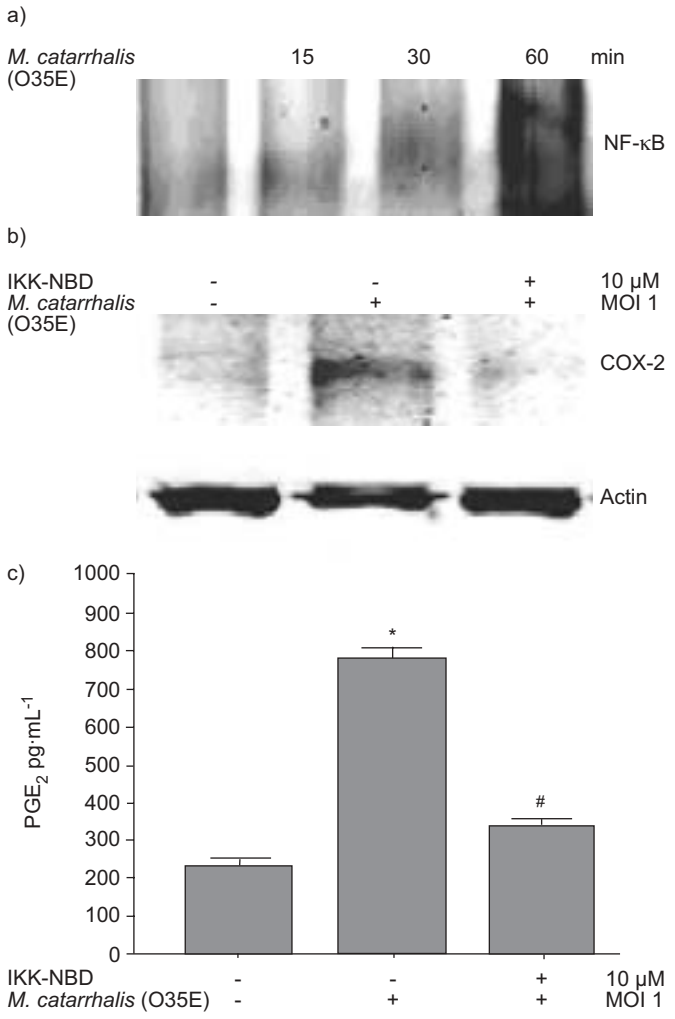

FIGURE 6. *Moraxella catarrhalis*-induced cyclooxygense (COX)-2 expression and prostaglandin (PG)E_2 release is dependent on nuclear factor (NF)-κB activation. BEAS-2B cells were infected with *M. catarrhalis* strain O35E (multiplicity of infection (MOI) 1) for the indicated time periods. An increased DNA binding of NF-κB in nuclear cell extracts of a) Moraxella-exposed cells was shown by electrophoretic mobility shift assay. Furthermore BEAS-2B cells were pre-treated with a specific inhibitor (I)κB kinase, IKK-Nemo binding domain (NBD; 10 μM) for 60 min and infected with *M. catarrhalis* strain O35E for either b) 4 h or c) 16 h. Induction of COX-2 was assessed by Western blot (b). PGE_2 release was measured by ELISA (c). Representative blots or gels out of three experiments are shown in a) and b). Data are presented as mean±SEM of four separate experiments (c). *: $p<0.05$ *versus* unstimulated control. #: $p<0.05$ with or without inhibitor.

time periods by electrophoretic mobility shift assay (EMSA). As shown in figure 6a, Moraxella induced NF-κB activation within 60 min. In the next step, the role of IκB kinase, the central kinase complex of the canonical NF-κB pathway, was analysed. The IκB kinase complex was blocked by pre-incubation of BEAS-2B cells with the cell permeable peptide inhibitor IKK-NBD [26]. IKK-NBD strongly reduced COX-2 protein expression (fig. 6b) and release of PGE_2 (fig. 6c) in Moraxella-infected cells. Overall, the present data demonstrate that activation of the NF-κB signalling pathway by *M. catarrhalis* was necessary for the expression of COX-2 and

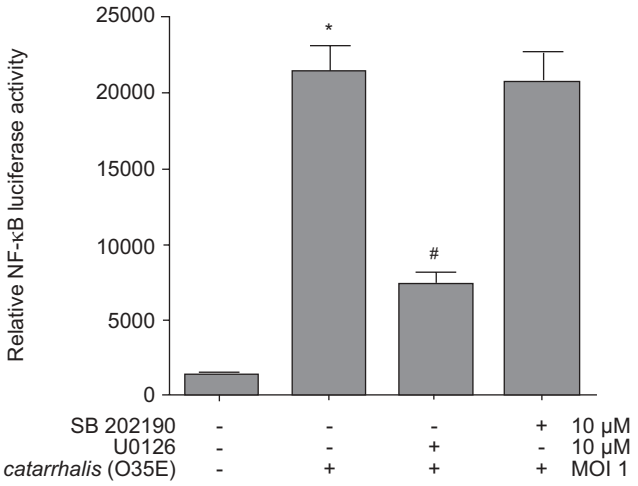

FIGURE 7. *Moraxella catarrhalis* activated nuclear factor (NF)-κB *via* extracellular signal-regulated kinase 1/2 but not *via* p38 mitogen-activated protein kinase. Human embryonic kidney-293 cells were co-transfected with human Toll-like receptor 2, an NF-κB-dependent luciferase reporter plasmid, and a β-galactosidase (β-Gal) construct. Cells pre-treated with U0126 (10 μM) or SB202190 (10 μM) were infected for 6 h with *M. catarrhalis* O35E (multiplicity of infection (MOI) 1), and luciferase and β-Gal activities were determined and normalised. Data are presented as mean±SEM of four separate experiments. *: $p<0.05$ *versus* unstimulated control. #: $p<0.05$ with or without inhibitor.

PGE_2 release in lung epithelium. IKK-NBD did not alter bacterial growth within the concentration and time frame tested (data not shown).

M. catarrhalis *activated NF-κB* via *ERK1/2 but not* via *p38 MAPK*

The present data suggest that activation of ERK1/2 and NF-κB but not p38 MAPK-dependent signalling contributed to *M. catarrhalis*-related expression of COX-2 and subsequent PGE_2 release in BEAS-2B cells. Thus, the present authors hypothesised that ERK1/2 activity is necessary for NF-κB-dependent gene transcription in Moraxella-infected cells. It was found that ERK1/2 pathway inhibitor U0126 (10 μM) but not p38-inhibitor SB202190 (10 μM) blocked *M. catarrhalis*-induced NF-κB activation, as shown by NF-κB luciferase reporter assay (fig. 7). The data shown in figure 7 indicate that ERK1/2 controlled COX-2 expression and PGE_2 secretion *via* NF-κB in *M. catarrhalis*-infected bronchial epithelial cells.

DISCUSSION

An increasing number of epidemiological studies demonstrating an association between *M. catarrhalis* and respiratory morbidity in the course of COPD prompted the present authors to undertake a detailed analysis of the *M. catarrhalis*–bronchial epithelium interaction [2, 4, 5].

In the present study, the authors demonstrated that infection of *M. catarrhalis* induces ERK1/2-dependent NF-κB activation and subsequent COX-2 expression and PGE_2 release in cultured bronchial epithelial cells. The present authors have previously demonstrated that *M. catarrhalis* significantly contributes to the activation of lung tissue cells [19–21]. In

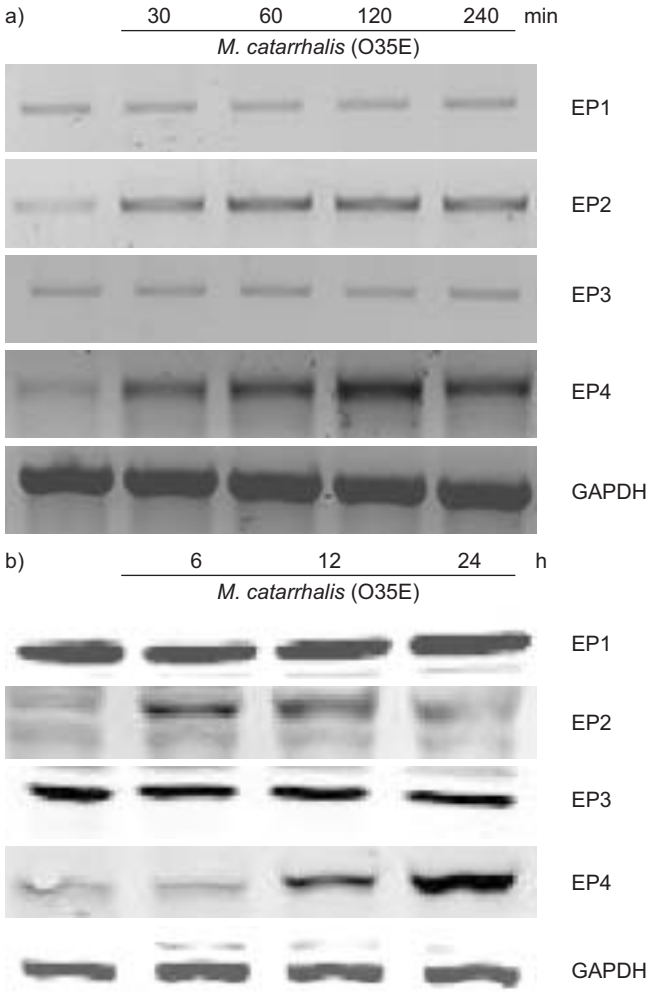

FIGURE 8. *Moraxella catarrhalis*-induced time- and concentration-dependent expression of the E prostanoid receptors EP2 and EP4 in human bronchial epithelial cells. BEAS-2B cells were incubated with *M. catarrhalis* strain O35E (multiplicity of infection 1) for the indicated time. EP2 and EP4 transcription and expression were analysed by a) reverse transcriptase-polymerase chain reaction and b) Western blot. Representative blots or gels from three separate experiments are shown. GAPDH: glyceraldehyde phosphate dehydrogenase.

the present study, it was found that *M. catarrhalis* infection resulted in increased expression of COX-2 in BEAS-2B cells. Increased COX-2 protein expression was followed by PGE_2 liberation, which is known to be the major COX product released by pulmonary epithelial cells [13, 14]. The secretion of several cytokines involved in the cellular inflammatory and reparative processes are known to be modulated by PGE_2. Interestingly, PGE_2 has been shown to increase the secretion of G-CSF in human airway smooth muscle cells [27]. Additionally, the ability of PGE_2 to downregulate the production of important cytokines, such as IL-8, MCP-1 and GM-CSF, which are significantly involved in the recruitment of inflammatory cells has also been reported [11, 27]. MONTUSCHI *et al.* [12] demonstrated that exhaled PGE_2 was increased in patients with stable COPD and suggested this to be a mechanism counteracting lung inflammation in COPD. It is reported herein, that COX-2 expression and PGE_2 release was dependent on UspA1 of *M. catarrhalis*. UspA1, an important adhesin, mediating the adherence of *M. catarrhalis* to human respiratory epithelial cells, has been described as being present on the surface of most *M. catarrhalis* disease isolates examined to date [6, 9, 22, 28]. UspA1 is known to adhere to the epithelial cell-associated laminin and fibronectin [9]. In addition, UspA1 targets the human CEACAM1 a member of the carcinoembryonic antigen family and the immunglobulin superfamily [29]. Recently, the present authors demonstrated that adhesion of *M. catarrhalis* wild-type strain O35E to BEAS-2B cells did not differ compared with the UspA1-deficient mutant [22]. Thus, the present findings suggest that the UspA1-dependent interaction to epithelial cells is essential for COX-2 expression and PGE_2 release. Taking into account that *M. catarrhalis* colonises the lower respiratory tract of up to 32% of adults with COPD [5], it is likely that the UspA1-dependent induction of PGE_2 release might promote the ability of *M. catarrhalis* to colonise the bronchial epithelium in COPD patients. A significant UspA1-independent induction of COX-2 expression and PGE_2 release in *M. catarrhalis*-infected lung epithelial cells could also be observed. These results suggest that other receptors, such as TLR2 and TLR4, may partly mediate COX-2-dependent [30] and Moraxella-related signalling, as published previously [19, 21].

The activity of PGE_2 is mediated by four receptors, termed E prostanoid receptors (EP1–EP4) [11]. In the present study, the authors demonstrated that infection of bronchial epithelial cells with *M. catarrhalis* increased the transcription and expression of the prostanoid receptors EP2 and EP4.

Activation of EP2 and EP4 increases intracellular cyclic adenosine monophosphate concentrations, which is associated with inhibition of effector cell functions [10, 11]. Human tracheobronchial epithelial cells express all four EP subtypes, but only activation of EP2 or EP4 mediates respiratory mucin MUC5AC expression [31]. Respiratory mucins protect the airway epithelium against exogenous insults. In chronic airway diseases, such as COPD, mucin hyperproduction contributes to airway obstruction, accelerated decline of lung function, morbidity and mortality [32]. Their hyperproduction is evoked by a variety of pro-inflammatory stimuli as a part of the inflammatory response in airways in COPD [31]. Therefore, *M. catarrhalis*-induced expression of EP2 and EP4 may also be important for the pathogenesis of chronic airway diseases.

In contrast to the constitutively expressed COX-1, a complex signalling network regulates the expression of inducible COX-2 [15]. In Moraxella-infected lung epithelial cells, the present authors demonstrated the activation of ERK1/2 and p38 MAPK. These kinases were considered to be important regulators of COX-2 and other pro-inflammatory signalling pathways [13, 14, 17, 19]. Interestingly, it was found that inhibition of ERK1/2 but not of p38 MAPK reduced Moraxella-related expression of COX-2 and PGE_2 liberation. The present authors recently reported that *S. pneumoniae*-induced COX-2 expression was dominantly mediated by p38 MAPK and Jun N-terminal kinase but not by ERK1/2 [14]. The results of the present study suggest a possible pathogen-specific regulation of COX-2 expression in lung tissue.

NF-κB mediates multiple aspects of host response to bacterial infection [13, 14, 17–19] and activation of the transcription factor NF-κB is considered to contribute significantly to COX-2 expression and PGE_2 liberation [13, 14]. In resting cells, IκB molecules sequester NF-κB in the cytosol. After cell activation, the signalling cascade containing IKK complex results in degradation of IκBα, thus allowing NF-κB transfer into the nucleus [26]. In concurrence with these findings, *M. catarrhalis*-infected cells showed an increased NF-κB activation. Moreover, the highly specific cell permeable inhibitor of IKK, IKK-NBD [26] abolished Moraxella-related COX-2 protein expression and subsequent PGE_2 release. Recently, DI STEFANO *et al.* [33] observed a marked increase in the expression of p65 protein, the major subunit of NF-κB, in bronchial biopsies of COPD patients. This finding was significantly correlated with the degree of airflow limitation and with increasing severity of the disease [33]. Overall, the present results emphasise a crucial involvement of NF-κB in Moraxella-induced COX-2 and PGE_2 induction.

As both ERK1/2 and NF-κB pathways seem to be essentially involved in COX-2 and PGE_2 expression in Moraxella-infected lung epithelium, the impact of ERK1/2 on NF-κB activation was analysed in more detail. Since BEAS-2B cells could only be poorly transfected, use was made of TLR2-overexpressing HEK-293 epithelial cells as a model that has been applied successfully in earlier studies investigating Moraxella-related cell activation [18, 19]. A chemical inhibitor of ERK1/2, but not an inhibitor of p38 MAPK, blocked Moraxella-driven NF-κB-dependent reporter-gene expression in HEK-293 epithelial cells. Thus, the data confirmed an important role of ERK1/2 for Moraxella-induced COX-2 and PGE_2 induction.

In conclusion, the present data suggest that *M. catarrhalis* contributes to COX-2 dependent PGE_2 release of bronchial epithelium. Moreover, this requires an ERK1/2-dependent activation of NF-κB, as well as an increased expression of the E prostanoid receptors EP2 and EP4.

Moraxella catarrhalis-induced prostaglandin E_2 expression might counteract lung inflammation, promoting colonisation of the respiratory tract in chronic obstructive pulmonary disease patients, and may thus play an important role in the pathogenesis of this disease. Additional studies are required to follow up this observation in an *in vivo* model.

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