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Perfluorocarbons Decrease *Chlamydophila pneumoniae*–Mediated Inflammatory Responses of Rat Type II Pneumocytes *In Vitro*

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ABSTRACT: *Chlamydophila pneumoniae* alter the expression of Toll-like receptor (TLR) 4 in alveolar type II (ATII)-cells. Subsequently nuclear factor kappaB (NF-κB) is activated and tumor necrosis factor-α (TNF-α) and macrophage inflammatory protein 2 (MIP-2) are produced. Perfluorocarbons (PFC) are beneficial in animals with bacterial pneumonia and reduce production of TNF-α. Using isolated ATII-cells, it was studied whether PFC prevent *C. pneumoniae*-induced TNF-α and MIP-2 release and what the underlying pathway is. PF5080 preincubation prevented *C. pneumoniae*-induced secretion of TNF-α (43 ± 10 *versus* 661 ± 41 pg/mL) and MIP-2 (573 ± 41 *versus* 4786 ± 502 pg/mL). The *C. pneumoniae*-induced 2.2-fold increase of TNF-α Receptor 1 expression was reduced by PF5080. *C. pneumoniae* reduced cytoplasmatic IκBα (3.7 ± 0.3 *versus* 14 ± 1) and increased NF-κB p65 (31 ± 7.5 *versus* 3.6 ± 1.1) compared with control. PF5080 prevented NF-κB activation. TLR4 expression was 1.5-fold higher after *C. pneumoniae* incubation, but remained at control levels after PF5080 pretreatment. After 24 h of *C. pneumoniae* incubation, in 88 ± 6% of cells bacteria were found in the perinuclear region and in 50% of these cells bacteria adhered to cellular surface. After PF5080 preincubation, *C. pneumoniae* were in 32 ± 4% attached to and in 5 ± 1% internalized in ATII-cells. Since PF5080 was found in ATII-cell membranes, PF5080 effect could be explained by an alteration of the cellular membrane, preventing activation of the inflammatory cascade. **(*Pediatr Res* 60: 264–269, 2006)**

Chlamydophila pneumoniae are obligate intracellular Gram-negative bacteria that cause respiratory infections in pediatric populations and adults (1,2). Recent studies demonstrated the presence of *C. pneumoniae* in alveolar macrophages and ATII cells in patients with chronic obstructive pulmonary disease (COPD) (3). Whereas most respiratory infections with *C. pneumoniae* are self-limited (4), bacterial colonization can lead to infectious exacerbations and development of COPD (5). Because detection rate of *C. pneumonia* varies significantly (6), the clinical relevance of *C. pneumoniae* is still controversial.

The effect of *C. pneumoniae* on isolated ATII-cells is well characterized. *C. pneumoniae* inhibit the surfactant metabolism of ATII-cells after internalization (7). Many of *C. pneumoniae*-induced effects are mediated by cytokines. *C. pneumoniae* act at the ATII cell membrane to induce the expression of TLR4 (8). TLR signaling is partly mediated by the transcription factor NF-κB, which controls the expression of many genes involved in the inflammatory response (9). *C. pneumoniae*-induced activation of NF-κB leads to a release of the chemokine MIP-2 and the pro-inflammatory cytokine tumor necrosis factor alpha (TNF-α) (8) and its receptor tumor necrosis factor receptor 1 (TNFR1). Together, they play an important role in bacterial infections and modulate the inflammatory process (10).

PFC—administered as liquid (11), vapor (12), or gas (13) into the lung—represent an alternative approach to treat respiratory insufficiency. Intratracheal PFC application prolonged the survival of rats with bacterial pneumonia (14) and reduced the bacterial count in the lungs of newborn rabbits with connatal group B streptococcal pneumonia (15). Furthermore, PFC exhibit anti-inflammatory properties. In isolated cells, PFC prevent LPS-induced production of TNF-α (16,17). A similar effect is found in different animal models of lung injury (18–20). Thus, PFC represent a promising supplementary therapy in patients with pulmonary infections.

The pathway that mediates anti-inflammatory properties of PFC remains unknown. Initially, a "liquid-PEEP" (21–23) or a barrier function that prevents ligand receptor contact (24,25) was proposed. But recent data suggest a direct interaction of PFC with intracellular pathways (16,26) or a stabilizing effect on the cellular membrane (27).

The present study was performed on an established model of *C. pneumoniae*–mediated inflammation in isolated ATII cells. The aim was to test whether preincubation of isolated ATII cells with PFC prevents the *C. pneumoniae*–induced rise in inflammatory mediators. To elucidate mechanisms of PFC activity, intracellular steps that mediate the *C. pneumoniae*—induced TNF-α release were studied in detail.

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Abbreviations: ATII, alveolar type II; **cHSP60,** chlamydial Heat Shock Protein 60; **CLSM,** confocal laser scanning microscopy; **MIP-2,** macrophage inflammatory protein 2; **NF-κB,** nuclear factor kappaB; **PFC,** perfluorocarbons; **TLR4,** Toll-like receptor 4

MATERIALS AND METHODS

Cell culturing. *C. pneumoniae* strain TW183 was cultured and purified as described (28). Isolation of rat ATII cells and incubation with *C. pneumoniae* were described in detail recently (8).

The 3-h adherent cells were preincubated with PF5080 (C6F18, molecular weight 438, density 1.77 g/mL, viscosity 0.75 cSt, surface tension 15 mN/m, vapor pressure 61 torr) that was obtained from 3M (Neuss, Germany) or with modified Eagle's medium (MEM) (PAA Laboratories GmbH, Linz, Austria) for 30 min at 37°C. Thereafter, PFC or MEM was removed and ATII cells were incubated with *C. pneumoniae* at a concentration of 2 inclusion forming units (IFU) per cell. Time of *C. pneumoniae* incubation varied and is specified in the results section.

Viability of isolated ATII cells (assessed by trypan blue staining) was >97% and remained above 92% after cell incubation with PF5080 and *C. pneumoniae*. Cell purity, estimated by hematoxylin staining, was >90%.

Western blot analysis. Nuclear and cytoplasmatic extracts and membrane fractions were prepared from cells as reported previously (29,30). TNFR1 and TLR4 protein were assayed in membrane, IκBα protein, and chlamydial-HSP60 in cytoplasmatic and NF-κB p65 in nuclear extracts of ATII cells by Western blotting. Proteins (50 μg for TNFR1, TLR4, and cHSP60; 40 μg for IκBα; 10 μg for p65) were mixed with 2 × SDS sample buffer, heated at 95°C for 5 min, and separated by 10% (TLR4, p65) and 12.5% (TNFR-1, IκBα, cHSP60) SDS-polyacrylamide gels. Separated proteins were blotted onto nitrocellulose membranes. Mouse IgG anti-cHSP60 (ALX-804-072-R100) antibodies (from ALEXIS Biochemicals, AXXORA GmbH, Grünberg, Germany) and rabbit IgG anti-TNFR1 (H-271) sc-7895, goat IgG anti-TLR4 (L-14) sc-16240, mouse monoclonal IgG anti-IκBα (H-4) sc-1643, and rabbit IgG anti-NF-κB p65 (C-20) sc-372 antibodies (from Santa Cruz Biotechnology, Heidelberg, Germany) were used for primary detection. Peroxidase-conjugated anti-goat IgG, anti-mouse IgG, and anti-rabbit IgG antibodies (Dianova, Hamburg, Germany) were used for secondary detection. Protein bands were visualized by enhanced chemiluminescence (ECL) (Amersham Biosciences, Piscataway, NJ) and quantified by scanning densitometry (GS-710 Imaging Densitometer; Bio-Rad, Hercules, CA).

Immunocytochemistry and CLSM. For immunocytochemistry, isolated ATII cells were plated on glass cover slips for 3 h at 37°C. Cells were preincubated with or without PF5080 for 30 min at 37°C. Thereafter cells were incubated with *C. pneumoniae*, fixed in 1% paraformaldehyde/250 mM HEPES (pH 7.4) and permeabilized with 0.04% saponin as described (8). For staining of TNFR1 and TLR4 cells were incubated with rabbit IgG anti-TNFR1 (H-271) and goat IgG anti-TLR4 (L-14) (Santa Cruz Biotechnology) for 20 h at 4°C. To stain the NF-κB subunits, the following antibodies were used: MAb anti-IκBα (H-4) (Santa Cruz Biotechnology) and MAb anti-NF-κB p65 subunit (3026), recognizing predominantly p65 when IκBα is not bound to p65 (Chemicon, Hofheim, Germany). To stain *C. pneumoniae*, cells were incubated with MAb against major outer membrane protein 1 (MOMP-1) of *C. pneumoniae* (DakoCytomation, Hamburg, Germany). To identify ATII cells, MAb antibody 3C9 was used against the 180-kDa lamellar body-limiting membrane protein (Berkeley Antibody, Richmond, VA).

Thereafter, cells were incubated with one of the secondary antibodies: Alexa 594 anti-goat IgG, anti-mouse IgG labelled with Alexa 488 or Alexa 594, anti-rabbit IgG labeled with Alexa 594 (Molecular Probes Europe BV, Leiden, Netherlands) for 2 h at 22°C. F-actin cytoskeleton, Alexa 488-conjugated phalloidin (Molecular Probes, Eugene, OR) was used (2 h at 22°C). Nuclear DNA was stained with 4′,6-diamidino-2-phenylindole (DAPI) (Molecular Probes) for 20 min.

Staining was analyzed with a Zeiss laser scanning microscope LSM 510 META with Axiovert 200 M (Carl Zeiss Jena GmbH, Jena, Germany) as described (8). Images were taken using a Plan-Apochromat 63×/1.4 oil immersion objective and fluorescence excitation at 488 nm (30 mW Ar laser), 543 nm (1 mW HeNe laser), and 405 nm (diode laser). To minimize signal crosstalk, a sequential scan with fast change of excitation lines was performed.

Semi-quantitative estimation of NF-κB subunits was performed as described (8). Thirty randomly chosen cells from six different regions were analyzed with a Zeiss LSM 510 system. Confocal settings were maintained for all images. Images were recorded with a scanning speed at 8 s/frame with a resolution of 512 × 512 pixels. Serial sections of cells (z stack) at a depth of 0.5 μm were performed. Fluorescence intensity was determined on confocal images using a computer-based image analysis system (MetaView; Visitron Systems GmbH, Puchheim, Germany) by measuring average intensity per pixel within a fixed-area of the relevant cellular area.

TNF-α and MIP-2 ELISA. Three-hour adherent ATII cells on plastic dishes were preincubated with PF5080 followed by incubation with *C. pneumoniae* for 3 or 6 h. Cell culture supernatants were collected and analyzed for TNF-α after 3 h and for MIP-2 after 6 h secretion by ELISA. TNF-α concentration was determined by rat TNF-α Quantikine ELISA (R&D Systems Inc, Minneapolis, MN) and MIP-2 concentration by rat MIP-2 ELISA kit (Biosource Europe, Nivelles, Belgium).

Intratracheal administration of PF5080. To study intracellular PFC-distribution, PF5080 was labeled with 4,4-difluoro-2,6-di-t-butyl-1,3,5,7,8-pentam, ethyl-4bora-3a, 4a-diaza-s-indacene (FEW Chemicals GmbH, Wolfen, Germany) as described (31). Isolated ATII cells were incubated with labeled PF5080 for 20 min and analyzed by confocal microscopy. To study PFC uptake *in vivo*, male Wistar rats were anesthetized with a mixture of ketamine (10 mg/kg) and pentobarbital natrium (20 mg/kg) intraperitoneally. A tube with side port for intratracheal administration of PFC was inserted *via* tracheostomy. After intratracheal application of labeled PFC animals were ventilated for 10 min (31) and, thereafter, lungs were fixed with 3% neutral buffered formalin for 48 h. Fixed lungs were paraffin-embedded and cut into sections of 4 μm. Tissue distribution of labeled PF5080 was analyzed with CLSM. The animal study was approved by the local animal care and use committee, handling of animals was in accordance with the guidelines.

Statistics. Statistical comparisons were performed by ANOVA with subsequent Fisher's protected least significant difference (PLSD) test. The level of significance was set at $p < 0.05$.

RESULTS

PF5080 reduce C. pneumoniae–mediated TNF-α and MIP-2 release. Adherent ATII cells were preincubated with PF5080 followed by *C. pneumoniae*. TNF-α concentration in the supernatant was measured after 3 h (Fig. 1*A*) and MIP-2 concentration after 6 h incubation with *C. pneumoniae* (Fig. 1*B*).

Whereas the TNF-α concentration of control cells was only 21 ± 8 pg/mL, a significantly ($p < 0.0001$) higher TNF-α concentration was found after *C. pneumoniae* incubation (661 ± 41 pg/mL). Preincubation with PF5080 prevented the *C. pneumoniae*–induced increase in TNF-α (43 ± 10 pg/mL). Similar results were found for MIP-2. The MIP-2 concentration in supernatant of *C. pneumoniae*–incubated cells was significantly higher than in control cells (4786 ± 501 pg/mL *versus* 205 ± 82 pg/mL). PF5080 preincubation prevented the *C. pneumoniae*–induced MIP-2 production (573 ± 41 pg/mL).

PF5080 decrease C. pneumoniae–mediated TNFR1 expression. Because TNF-α mainly acts *via* the TNFR1, the effect of PF5080 on the TNFR1 expression was studied.

Confocal imaging revealed an increased expression of TNFR1 in response to *C. pneumoniae* after 3 h of incubation when compared with control cells. Almost no increase in the TNFR1 expression was found in cells that were preincubated with PF5080 and thereafter exposed to *C. pneumoniae* (Fig. 2*A*).

The amount of TNFR1 was quantified by Western blot analysis (Fig. 2, *B* and *C*). Incubation of ATII cells with

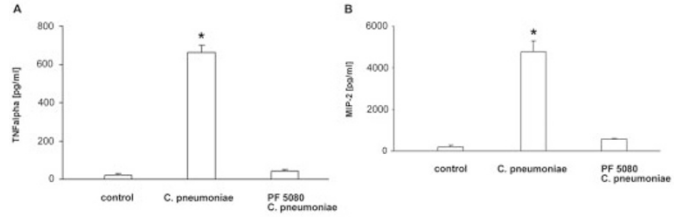

Figure 1. PF5080 reduce *C. pneumoniae*–mediated TNF-α and MIP-2 release (*A*) TNF-α and (*B*) MIP-2 concentration in the supernatant of ATII cells (control), nontreated and pretreated with PF5080 followed by *C. pneumoniae* incubation for 3 (TNF-α) or 6 (MIP-2) h. Shown are mean ± SD in pg/mL of $n = 3$ experiments, asterisk indicates $p < 0.0001$.

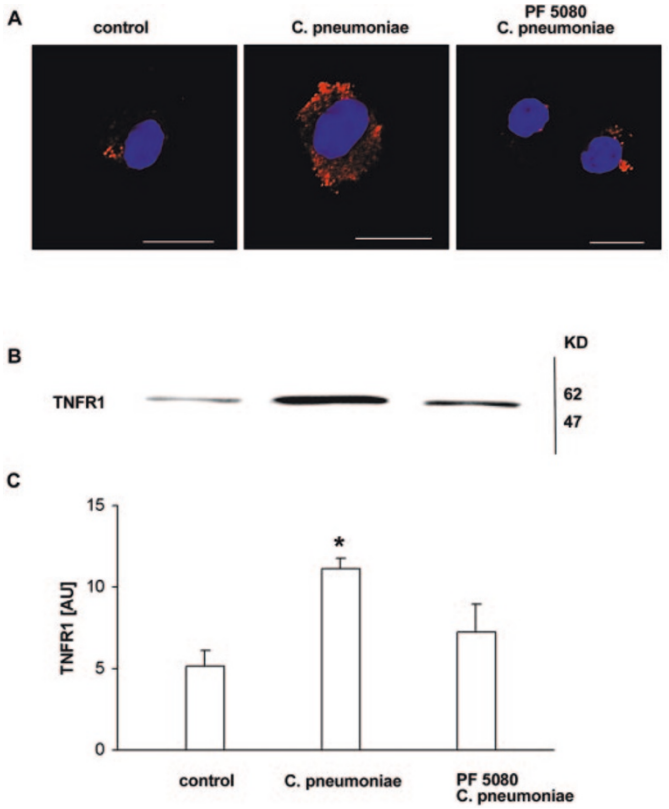

Figure 2. PF5080 decrease *C. pneumoniae*–mediated TNFR1 expression. (*A*) Representative CLSM images of untreated (control) and *C. pneumoniae* incubated (3 h) ATII cells without and with PF5080 pretreatment. TNFR1 (*red*) and nuclear DNA (*blue*), the bar equals 10 μm. (*B* and *C*) TNFR1 concentration analyzed by Western blot. Shown are mean ± SD in arbitrary units (AU) of $n = 3$ experiments, asterisk indicates $p < 0.001$.

C. pneumoniae caused a 2.2-fold increase in TNFR1 expression when compared with control cells. In contrast, PF5080 reduced *C. pneumoniae*–mediated TNFR1 expression.

PF5080 reduce C. pneumoniae–mediated NF-κB activation. NF-κB represents a key mediator in the process of *C. pneumoniae*–induced activation of TNF-α. Therefore, the effect of PF5080 on the *C. pneumoniae*–mediated NF-κB activation was studied (Fig. 3, *A* and *B*).

In noninfected control cells, IκBα was located in the cytoplasm (71.3 ± 4.6). After 30 min of *C. pneumoniae* incubation, the concentration of cytoplasmatic IκBα was significantly reduced (29.5 ± 1.2). The *C. pneumoniae*–mediated decrease of IκBα was prevented by a preincubation with PF5080 (65.9 ± 3.8).

Staining with an anti-p65 antibody (recognizes p65 only when IκBα is not bound to p65) showed an activation of p65 in the nucleus after 30 min of *C. pneumoniae* incubation (179 ± 21). Similar to noninfected control cells (6.1 ± 2) almost no activation of p65 was found in the nucleus of *C. pneumoniae* incubated cells that were pretreated with PF5080 (9.1 ± 1.6). In PF5080 pretreated cells without any subsequent *C. pneumoniae* incubation no IκBα decrease nor p65 activation was found.

The amount of cytoplasmatic IκBα and of nuclear p65 was quantified by Western blot analysis (Fig. 3, *C* and *D*). After *C. pneumoniae* incubation cytoplasmatic IκBα protein

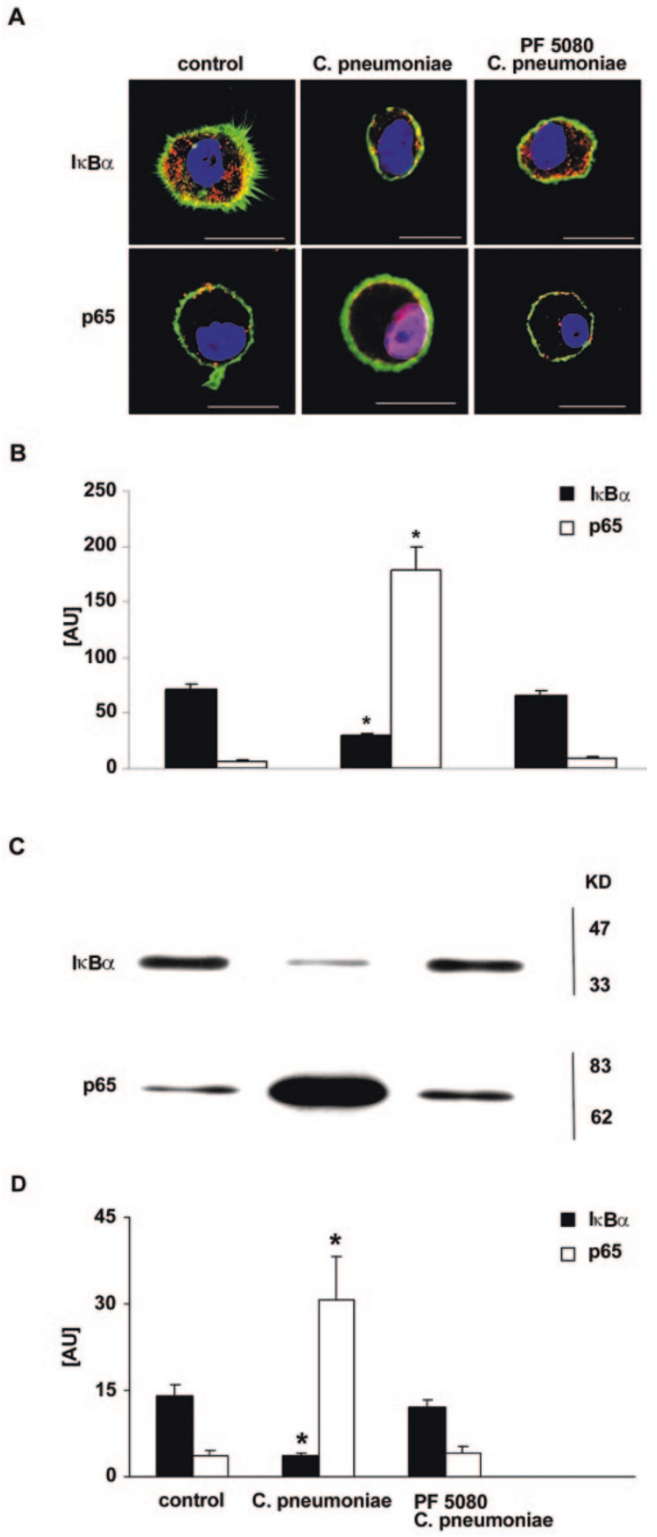

Figure 3. PF5080 reduce *C. pneumoniae*–mediated NF-κB activation. (*A* and *B*) Representative CLSM images of untreated (control) and *C. pneumoniae*–incubated (30 min) ATII cells without and with PF5080 pretreatment. IκBα and p65 (*red*), F-actin cytoskeleton (*green*), and nuclear DNA (*blue*); areas of blue-red overlap are *pink*; the bar equals 10 μm. Cytoplasmatic IκBα and nuclear NF-κB p65 were quantified on confocal images. Shown are mean ± SD in arbitrary units (AU) of $n = 3$ experiments, asterisk indicates $p < 0.0001$ for IκBα in cytoplasm and $p < 0.0001$ for p65 in nucleus. (*C* and *D*) Cytoplasmatic IκBα and nuclear p65 were quantified by Western blot. Shown are mean ± SD in arbitrary units (AU) of $n = 3$ experiments, asterisk indicates $p < 0.0001$ for IκBα in cytoplasm and $p < 0.0001$ for p65 in nucleus.

was significantly lower (3.7 ± 0.3 *versus* 14 ± 2) and nuclear p65 protein concentration was significantly higher (31 ± 7.5 *versus* 3.6 ± 1.1) than in control cells. Pretreatment of cells with PF5080 prevented the *C. pneumoniae* induced decrease in IκBα (12.2 ± 1) and increase in p65 (4 ± 1.3).

PF5080 decrease C. pneumoniae–mediated TLR4 expression. *C. pneumoniae* interact with the TLR 4 of ATII cells to activate the intracellular cascade that leads to TNF-α secretion. The effect of PF5080 on these changes in TLR4 were studied.

Whereas *C. pneumoniae* incubation is associated with a mobilization of TLR4 from the cytoplasm to the external cell surface and a subsequent increase in TLR4 expression, preincubation of cells with PF5080 prevented the increase in TLR4 expression (Fig. 4*A*).

The amount of TLR4 protein was measured by Western blot analysis (Fig. 4*B*) and quantified (Fig. 4*C*). The TLR4 protein expression was significantly (1.5-fold) higher in *C. pneumoniae*–exposed cells when compared with control cells. TLR4 expression in PF5080-pretreated cells followed by incubation with *C. pneumoniae* remained at control levels.

In ATII cells that were incubated with PF5080 but not *C. pneumoniae*, the TLR4 expression remained at the low level of noninfected (control) cells.

PF5080 reduce the C. pneumoniae contact with ATII pneumocytes. Since pretreatment of ATII-cells with PF5080 prevented *C. pneumoniae*–induced effects, we evaluated whether preincubation affects the adherence and internalization of *C. pneumoniae*. ATII cells were pretreated with PF5080 (30 min), followed by *C. pneumoniae* incubation (24 h). Bacteria were detected with an anti- *C. pneumoniae* MOMP-specific antibody. Furthermore, the F-actin cytoskeleton and the nuclear DNA were stained (Fig. 5*A*).

In control (uninfected) cells, no bacteria were identified. In cells incubated with *C. pneumoniae* in the absence of PF5080, bacteria were found in the perinuclear region of 88 ± 6% all ATII cells and in 50% of these cells bacteria were adherent to cell surface. In 12 ± 5% of cells bacteria were neither adherent nor internalized.

After pretreatment with PF5080, bacteria were adherent to the cell surface in 32 ± 4% of cells. However, only in about 5 ± 1% cells bacteria were found in the perinuclear region. In

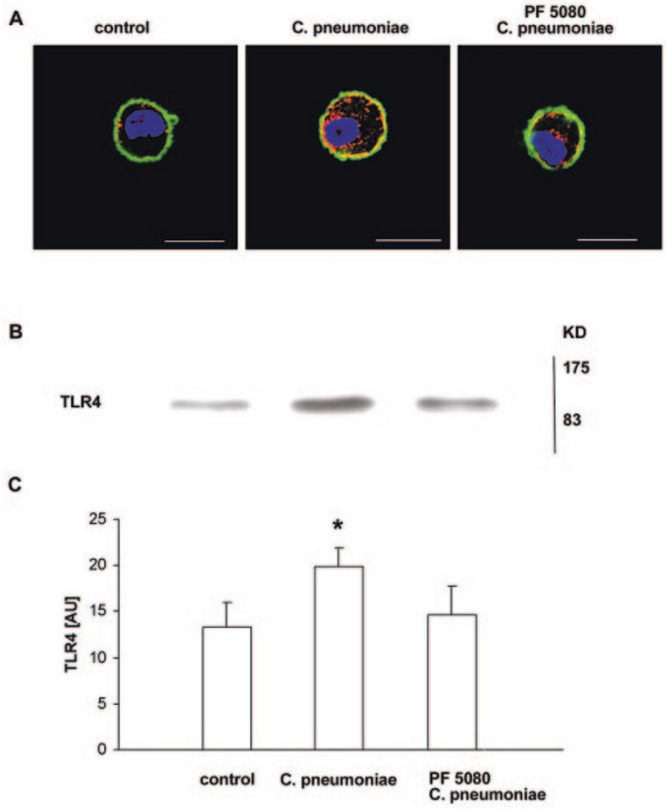

Figure 4. PF5080 decrease *C. pneumoniae*–mediated TLR4 expression. (*A*) Representative CLSM images of untreated (control) and *C. pneumoniae* incubated (30 min) ATII cells without and with PF5080 pretreatment. TLR4 (*red*), F-actin cytoskeleton (*green*), nuclear DNA (*blue*); areas of overlap between red and green are *yellow*; the bar equals 10 μm. (*B* and *C*) TLR4 concentration was analyzed by Western blot. Shown are mean ± SD in arbitrary units (AU) of $n = 4$ experiments, asterisk indicates $p < 0.0001$.

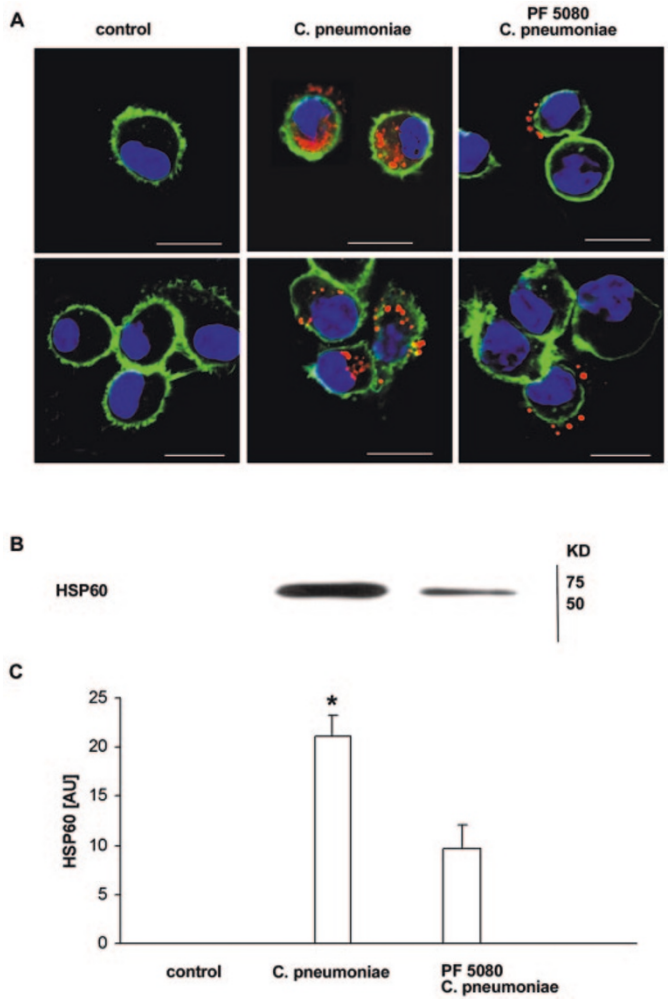

Figure 5. PF5080 reduce the *C. pneumoniae* contact with type II pneumocytes. (*A*) Representative CLSM images of untreated (control) and *C. pneumoniae*–incubated (24 h) ATII cells without and with PF5080 pretreatment. Nuclear DNA (*blue*), F-actin cytoskeleton (*green*), *C. pneumoniae* (*red*); the bar equals 10 μm. (*B* and *C*) Chlamydial HSP60 concentration was analyzed by Western blot technique. Shown are mean ± SD in arbitrary units (AU) of $n = 4$ experiments, asterisk indicates $p < 0.0001$.

the majority of cells (63 ± 4%) bacteria were neither adherent nor internalized.

The concentration of cHSP60 was analyzed by Western blot analysis and quantified after 24 h of *C. pneumoniae* incubation (Fig. 5, *B* and *C*). In uninfected ATII cells, no cHSP60 was detected. Expression of cHSP60 was significantly enhanced after 24 h of *C. pneumoniae* incubation. Preincubation of ATII cells with PF5080 partially prevented the *C. pneumoniae*–induced increase in cHSP60.

PF5080 internalize in lamellar bodies and membrane structures of ATII cells. Finally, isolated ATII cells were incubated with labeled PF5080 to study whether PF5080-mediated effects are caused by PFC incorporation into cellular membranes. The labeling was found in lamellar bodies and the cellular membrane (Fig. 6*A*).

After intratracheal instillation of labeled PF5080, an intensive staining was detected in cellular membranes of alveolar epithelial cells (Fig. 6*B*).

DISCUSSION

C. pneumoniae induce an inflammatory reaction in alveolar type II cells (8). PFC exhibit anti-inflammatory properties and have a beneficial effect in animal models of bacterial infection (14,15). Thus, intratracheal PFC application could be a promising supplementary therapy for patients with bacterial infections. However, the mechanism of PFC activity is still poorly understood.

This study shows that incubation of isolated ATII-cells prevents the *C. pneumoniae*–induced production of the pro-inflammatory cytokine TNF-α as well as its receptor TNFR1 and chemokine MIP-2. PFC have been shown before to prevent the TNF-α production of either isolated cells or in animal models of lung injury (12,16).

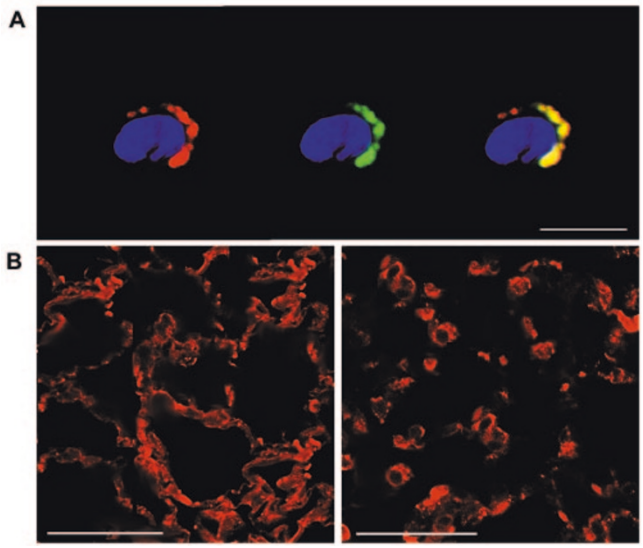

Figure 6. PF5080 internalize in lamellar bodies and membrane structures of ATII cells. (*A*) Representative CLSM images of ATII cells after incubation with labeled PF5080 (*red*) for 10 min. Lamellar bodies were stained with MAb 3C9 (*green*), nuclear DNA (*blue*); areas of overlap between red and green are *yellow*; the bar equals 10 μm. (*B*) Representative CLSM images of rat lung after intratracheal instillation of labeled PF5080. Red-labeled PF5080 is found in membranes of ATII cells; the bar equals 50 μm.

In the present study, for the first time, the underlying pathway has been studied in detail. PF5080 prevented the *C. pneumoniae*–induced activation of NF-κB. Furthermore, after PF5080 incubation, the expression of TLR4 did not increase in the presence of *C. pneumoniae*. Consequently, after PF5080 incubation in only 5% of ATII cells, *C. pneumoniae* were found internalized and in only 34% attached to the cellular surface.

The most likely explanation of the PFC effect is the integration of the lipophilic PFC into lamellar bodies and the cellular membrane as shown in the present study for ATII cells. A similar effect has been previously described for lamellar bodies (31) and for erythrocytes (27). Therefore, a PFC induced stabilization of cellular membranes (27) is the most likely explanation how PFC prevent the internalization of *C. pneumoniae*.

Extracellular *C. pneumoniae* require an intact host cell surface for attachment and subsequent activation of specific cell signaling pathways (32). We have recently shown that contact of *C. pneumoniae* with microvilli of ATII cells induces calcium-dependent changes in F-actin/β-tubulin cytoskeleton involved in NF-κB activation (33). *C. pneumoniae* appear to use several mechanism to enter host cells, including receptor-mediated endocytosis. Recently, a mannose 6-phosphate/IGF-II receptor was shown to enhance infection of endothelial cells by *C. pneumoniae* (34).

We demonstrated that constitutively expressed TLR4 in type II pneumocytes responds rapidly to *C. pneumoniae* contact and mediates the cellular responses to *C. pneumoniae* at a post-transcriptional level. The *C. pneumoniae*–induced NF-κB activation was reduced by a blockade of the TLR4 receptor (5). Either *C. pneumoniae* contact or the cHSP60 induce a TLR-mediated translocation of NF-κB and a secretion of cytokines (35–38).

In the present study, PFC did not entirely prevent the adherence of *C. pneumoniae* to ATII cells. However, PFC did prevent the internalization of bacteria. However, there was no significant *C. pneumoniae*–induced NF-κB activation or TNF-α secretion in PFC-pretreated cells.

It could be assumed that PFC directly alter the cellular surface membrane (including its receptor molecules and the NF-κB complex), thereby reducing the intracellular signalling cascade and the subsequent inflammation. The present data are confirmed by a study, showing that PFC treatment in lung of respiratory syncytial virus–infected mice reduced inflammatory activity by blocking the activation of the NF-κB activation (19). Thus, it can be concluded that PFC do not only provide a mechanical or physical barrier, but do alter signaling pathway programs of host cells and thus influence intracellular processes.

The present data seem to support the idea to use PFC (*e.g.* by inhalation) within the treatment concept of *C. pneumoniae* infection. However, the present study has two limitations that require further investigation.

First of all, we did only study the effect of an incubation of ATII-cells with PFC prior to *C. pneumoniae* exposure. At the moment, there are no data available to show the effect of PFC on ATII-cells with internalized *C. pneumoniae*.

Secondly, further studies are needed to verify the shown *in vitro* effect *in vivo* as well. We have previously shown that PF5080 does not have an impact on bacterial growth of group B streptococci *in vitro* (39). However, *in vivo* data on preterm rabbits with connatal GBS pneumonia show a reduced amount of GBS in the lungs of PF5080-treated animals (15). Therefore, the protective effect of the present study on isolated ATII cells could be even more pronounced in an animal model of *C. pneumoniae* infection.

In the past, clinical studies failed to show a benefit of partial liquid ventilation when compared with conventional therapy of severe respiratory failure. Nevertheless, liquid ventilation still represents a promising therapy, however, prior to a future clinical application, the molecular mechanism of PFC activity has to be better understood. The present study provides some further insight in the complex process of anti-inflammatory activity of PFC and will thus help to use PFC in a clinical setting more appropriate in the future.

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