Laser-assisted microdissection and real-time PCR detect anti-inflammatory effect of perfluorocarbon

Katharina von der Hardt, Michael Andreas Kandler, Ludger Fink, Ellen Schoof, Jörg Dötsch, Rainer Maria Bohle, and Wolfgang Rascher.

Von der Hardt, Katharina, Michael Andreas Kandler, Ludger Fink, Ellen Schoof, Jörg Dötsch, Rainer Maria Bohle, and Wolfgang Rascher. Laser-assisted microdissection and real-time PCR detect anti-inflammatory effect of perfluorocarbon. Am J Physiol Lung Cell Mol Physiol 285: L55–L62, 2003. First published February 21, 2003; 10.1152/ajplung.00198.2002.—The aim of this study was to identify cell types involved in the anti-inflammatory effect of ventilation with perfluorocarbon in vivo. Fifteen anesthetized, surfactant-depleted piglets received either aerosolized perfluorocarbon (Aerosol-PFC), partial liquid ventilation (rLV) at functional residual capacity (FRC) volume (FRC-PLV), or intermittent mandatory ventilation (control). After laser-assisted microdissection of different lung cell types, mRNA expression of IL-8 and ICAM-1 was determined using TaqMan real-time PCR normalized to hypoxanthine phosphoribosyltransferase (HPRT). IL-8 mRNA expression (means ± SE; control vs. Aerosol-PFC) was 356 ± 142 copies IL-8 mRNA/copy HPRT mRNA vs. 3.5 ± 1.8 in alveolar macrophages (P < 0.01); 208 ± 108 vs. 2.7 ± 0.8 in bronchialolar epithelial cells (P < 0.05); 26 ± 11 vs. 0.7 ± 0.2 in alveolar septum cells (P < 0.01); 2.8 ± 1.0 vs. 0.8 ± 0.4 in bronchiolar smooth muscle cells (P < 0.05); and 1.1 ± 0.4 vs. 0.2 ± 0.05 in vascular smooth muscle cells (P < 0.05). With FRC-PLV, IL-8/HPRT mRNA expression was significantly lower in macrophages, bronchiolar epithelial, and vascular smooth muscle cells. ICAM-1 mRNA expression in vascular endothelial cells remained unchanged. Predominantly, alveolar macrophages and bronchiolar epithelial cells were involved in the inflammatory pulmonary process. The anti-inflammatory effect of Aerosol-PFC was most pronounced.

aerosolized perfluorocarbon; interleukin-8; surfactant-depleted piglets; polymerase chain reaction


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To identify cells involved in the early pulmonary inflammatory reaction, we determined mRNA expression of IL-8 in specific pulmonary cells, such as alveolar macrophages, bronchiolar epithelial cells, bronchiolar and vascular smooth muscle cells, and cells of the alveolar septum. Additionally, ICAM-1 mRNA expression was measured in vascular endothelial cells. The aim was to define the relative contribution of specific pulmonary cells to the inflammatory response and the possible beneficial influence of ventilation with perfluorocarbon.

MATERIALS AND METHODS

The model of surfactant-depleted piglets was identical with the model described earlier (14, 29).

Subjects

Fifteen piglets with a body wt of 4.01 ± 0.35 kg were included in the study. The animal experiment was approved by the Animal Care Committee of the University of Erlangen-Nürnberg and the Government of Mittelfranken, Germany, and performed according to the guidelines of National Institutes of Health. Data of 14 piglets were available for mRNA evaluation. After mRNA extraction and real-time PCR for the housekeeping gene, one piglet of the control group had to be excluded, since mRNA had degraded to a quality too low for further analysis.

Animal Preparation, Lung Injury, and Therapy Groups

The piglets were transported from the breeder to the laboratory immediately before the experiment started. General anesthesia was performed using midazolam, ketamine, fentanyl, and vecuronium. After tracheotomy, a sheath (4.5 French, check-flo performer introducer set; Cook, Mönchengladbach, Germany) was placed into the right jugular vein, a thermodilution catheter (4 F, Arrow, Erding, Germany) into the pulmonary artery, and an arterial catheter (20 gauge, Arrow) and a sensor for online blood gas monitoring (Paratrend 7; Agilent, Böblingen, Germany) into the left femoral artery. Intermittent mandatory ventilation (IMV) was performed with a time-cycled, pressure-controlled neonatal respirator (Infant Star 950; Mallinckrodt, Hennef, Germany) starting with a peak inspiratory pressure (PIP) of 20 cmH₂O, a positive end-expiratory pressure (PEEP) of 4 cmH₂O, an inspiratory fractional oxygen concentration (FIO₂) of 1, and a frequency of 40 breaths/min, augmented to 50 breaths/min before lavage. Respiratory gas was humidified, and temperature was kept at 39°C (MR 700 AGM; Fisher & Paykel, Welzheim, Germany). Lung injury was induced by repeated saline lung lavage (17) (0.9% NaCl at 39°C) using 30 ml/kg per side. Lung injury was considered successful when the arterial Pao₂ remained <80 mmHg for a period of 60 min. During lavage, PIP and PEEP were increased to 32 and 8 cmH₂O, respectively. Arterial oxygen tension was measured every 15 min during therapy and every 30 min during the observation period (ABL 330; Radiometer, Copenhagen, Denmark).

The animals were randomized to three different therapy groups (Aerosol-PFC, FRC-PLV, and control). In all animals, respiratory support was held constant at identical respiratory settings (PEEP 8 cmH₂O, PIP 32 cmH₂O, FIO₂ 1.0, 50 breaths/min). Piglets in the Aerosol-PFC group received 10 ml·kg⁻¹·h⁻¹ perfluorocarbon (FC77, 3 M; Neuss, Germany) aerosolized by an aerosolization catheter (Trudell Medical, London, Canada) (17) at the distal end of the endotracheal tube for 2 h. In the FRC-PLV group, 30 ml/kg of FC77 were filled into the lung via a side port of the tube connector over a period of 30 min. A continuous replacement of FC77 (20 ml·kg⁻¹·h⁻¹) was performed in the FRC-PLV group to account for evaporative loss. Therapy with FC77 was stopped after 2 h. After an additional observation period of 6 h (8 h after establishment of lung injury) with constant respiratory parameters, animals were killed. The trachea was clamped at a PEEP of 8 cmH₂O, and lungs and heart were removed en bloc. Tissue-freezing medium (Leica, Nussloch, Germany) was instilled to prevent alveolar collapse. Two samples of corresponding parts of the inferior lobe of the lung were chosen for analysis to ensure comparability between the groups. Samples were frozen in liquid nitrogen and kept at −80°C until analysis.

Cryostat Sectioning and Staining

Ten-micrometer sections of tissue-freezing medium-embedded lung tissue samples were prepared in a cryotome (Leica, Nussloch, Germany) using 30 ml/kg of tissue-freezing medium (Leica, Nussloch, Germany) and immersed in 70%, 90%, and 100% ethanol. Staining was performed for 60 s in hemalaun.

Laser-Assisted Microdissection

Cell picking was performed as described in detail (10, 11, 12). The ultraviolet (UV) laser microbeam (P.A.L.M., Bernried, Germany) used for microdissection consisted of a nitrogen laser of high-beam precision (wavelength 337 nm), which was coupled to an inverted microscope (Axiovert 135; Zeiss, Jena, Germany) via the epifluorescence illumination path. Microscope stage and micromanipulator were digitally controlled and moved by computer mouse. Alveolar macrophages, bronchiolar epithelial cells, endothelial cells, smooth muscle cells of bronchioli and blood vessels, and alveolar septum cells were identified by light microscopy. Adjacent tissue was removed by UV laser photolysis under visual control. A sterile needle linked to the micromanipulator served for picking the cell(s) via adhesive forces with direct transfer into a reaction tube. Needles with the adherent cell(s) were transferred into a reaction tube containing 10 μl of first-strand buffer (52 mM Tris-HCl, pH 8.3, 78 mM KCl, and 3.1 mM MgCl₂). Per tube, 16 alveolar macrophages (Fig. 1, A–C), ~20 bronchiolar epithelial cells (Fig. 1, D–F), or 3 circles of endothelium (Fig. 1, G–I) or bronchiolar or vascular smooth muscle cells were collected. Intimal endothelial cells were separated from the media smooth muscle cells by laser microdissection at the level of the lamina elastica interna. Specimens were immediately frozen in liquid nitrogen.

Reverse Transcription

cDNA synthesis was performed within 12 h after cell picking using products purchased from Perkin-Elmer (10× PCR buffer II, MgCl₂, random hexamers, RNase inhibitor, murine Moloney leukemia virus (MULV) reverse transcriptase) and dNTPs (Eurobio, Raunheim, Germany). Tubes with picked cells were heated to 70°C for 10 min and then cooled on ice for 5 min. For cDNA synthesis, 2 μl PCR buffer II, 2 μl MgCl₂ (1 μl dNTP (10 μM each), 1 μl random hexamers (50 μM), 1 μl MULV reverse transcriptase, 1 μl H₂O, and 0.5 μl (5 units) RNase inhibitor were added to a total volume of 18.5 μl. Samples were incubated at 20°C for 10 min and at 43°C for 70 min. Reactions were stopped by heating to 95°C for 5 min.

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Relative mRNA Quantitation

mRNA expression of IL-8 and ICAM-1 was determined by TaqMan real-time PCR. To normalize gene expression, mRNA expression of the housekeeping gene hypoxanthine phosphoribosyltransferase (HPRT) was measured. Only samples with detection of both target gene and housekeeping gene were included in the analysis. Microdissection procedure was repeated until housekeeping gene and target gene mRNA were both detectable in the identical sample.

TaqMan Real-Time PCR

Efficiency and reliability of this method (8, 13) after laser-assisted cell picking (10, 12) have been shown previously. The approach for measurement of gene expression by TaqMan real-time PCR is based on the 5'-exonuclease activity of the Taq polymerase. Within the amplicon defined by a gene-specific oligonucleotide primer pair, an oligonucleotide probe labeled with two fluorescent dyes is designed. As long as the probe is intact, the emission of a reporter dye (i.e., 6-carboxy-fluorescein) at the 5' end is quenched by the second fluorescent dye (6-carboxy-tetramethyl-rhodamine) at the 3' end. During the extension phase of the PCR, the Taq polymerase cleaves the probe, releasing the reporter dye. An automated photometric detector combined with special software (ABI Prism 7700 Sequence Detection System; Perkin-Elmer, Foster City, CA) monitors the increasing reporter dye emission. The algorithm normalizes the signal to an internal reference (ΔRn) and calculates the threshold cycle number (C\textsubscript{T}) when the ΔRn reaches 10 times the standard deviation of the baseline. For relative quantitation as used here, comparative C\textsubscript{T} method normalizes the number of target gene copies to an endogenous reference; for example, a suitable housekeeping gene, in our study, HPRT. After cDNA synthesis, each sample was divided for target gene and housekeeping gene analysis into two aliquots of 8 μl. The TaqMan Universal Master Mix (Perkin-Elmer) was used according to the manufacturer's protocol. Eight microliters of cDNA (reverse transcription mixture) and oligonucleotides with a final concentration of 900 nM of primers and 200 nM of hybridization probe were added to 50 μl of reaction mix. The oligonucleotides of each target of interest were designed by Primer Express software (Perkin-Elmer). Primers and probes were purchased from Eurogentec, Seraing, Belgium. The thermocycler parameters were 50°C for 2 min and 95°C for 6 min, followed by 55 cycles of 95°C for 20 s and 60°C and 73°C for 30 s each. Primers and TaqMan probes used are listed in Table 1.

Data Analysis

Relative quantitation is given by the following equation (for deduction see Ref. 11)

\[
\frac{T_0}{R_0} = K \times (1 + E)^{(C_T,R - C_T,T)}
\]  

(1)
where $T_0$ is the initial number of target gene copies, $R_0$ is the initial number of housekeeping gene copies, $C_r T$ is the threshold cycle of target gene, $C_r R$ is the threshold cycle of housekeeping gene, $E$ is efficiency of amplification, and $K$ is constant.

In routinely applied dilution series, the efficiency of the primer/probe sets was shown to be 100% (=1). For evaluation of target gene mRNA expression in relation to housekeeping gene mRNA expression, the difference of CT values between the different housekeeping gene and target gene was calculated, post hoc tests were applied, respectively.

To reduce run-to-run variations in the immunoreactivity, all stainings for semiquantitative evaluation were performed in the same run. For scoring of immunoreactivity, the following grading was used: 0 = no, 1 = mild, 2 = moderate, and 3 = strong staining for IL-8. Immunoreactivity in alveolar macrophages, bronchiolar epithelial cells, alveolar septum, bronchiolar smooth muscle cells, and vascular smooth muscle cells was determined. IL-8 immunoreactivity was summarized to form an immunohistochemical summary score in cell types not showing constitutive IL-8 expression (in alveolar macrophages, bronchiolar epithelial cells, and alveolar septum cells).

**RESULTS**

**HPRT mRNA expression.** HPRT mRNA was detectable in 73% of alveolar macrophage specimens, in 85% of bronchiolar epithelial cell specimens, in 92% of alveolar septum and endothelial cell specimens, and in 100% of bronchiolar and vascular smooth muscle cell specimens.

Analysis of the HPRT mRNA expression in alveolar macrophages with the exact, defined number of 16 cells per tube and in bronchiolar epithelial cells with an approximate number of 20 cells per tube showed no regulation of HPRT mRNA expression under the experimental setting. For alveolar macrophages, $C_T$ values for HPRT mRNA were (means ± SE) 38.48 ± 0.44 in Aerosol-PFC, 40.31 ± 0.91 in FRC-PLV, and 39.5 ± 0.63 in the control group. For bronchiolar epithelial cells, $C_T$ values for HPRT mRNA were (means ± SE) 37.13 ± 0.53 in Aerosol-PFC, 38.14 ± 0.64 in FRC-PLV, and 39.16 ± 0.41 in the control group.

**Target Genes**

**IL-8 mRNA expression.** IL-8 mRNA was detectable in 69% of alveolar macrophage specimens, in 83% of bronchiolar epithelial cell specimens, 85% of alveolar septum, 100% of bronchiolar smooth muscle cell specimens, and 83% of vascular smooth muscle cell specimens.

IL-8 mRNA expression was significantly lower in the Aerosol-PFC group compared with the control group in all cell types analyzed. IL-8 mRNA expression (means ± SE; control vs. Aerosol-PFC) was: 356 ± 142 copies of IL-8 mRNA per one copy of HPRT mRNA vs. 3.5 ± 1.8 in alveolar macrophages ($P < 0.01$); 208 ± 108 vs. 2.7 ± 0.8 in bronchiolar epithelial cells ($P < 0.05$); 26 ± 11 vs. 0.7 ± 0.2 in alveolar septum cells ($P < 0.01$); 2.8 ± 1.0 vs. 0.8 ± 0.4 in bronchiolar smooth muscle cells ($P < 0.05$); and 1.1 ± 0.4 vs. 0.2 ± 0.05 in vascular smooth muscle cells ($P < 0.05$). In the FRC-PLV group, the ratio of IL-8/HPRT mRNA expression was significantly lower in alveolar macrophages (14.8 ± 4.4), bronchiolar epithelial cells (8.2 ± 2.8), and vascular smooth muscle cells (0.27 ± 0.09) compared with control (Fig. 2). Within each group, maximum IL-8 mRNA expression was found in alveolar macrophages, followed by bronchiolar epithelial cells, alveolar septum, and bronchiolar smooth muscle cells, and was lowest in vascular smooth muscle cells (Fig. 3).

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**Table 1. Primers and TaqMan probes used for TaqMan real-time PCR**

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<tr>
<th>HPRT</th>
<th>Primer/probe</th>
<th>Amplicon length</th>
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<tr>
<td><strong>Forward</strong></td>
<td>5'-TGGAAAGAATGCTGTGTTGGAAG-3'</td>
<td><strong>Reverse</strong></td>
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<tr>
<td><strong>TaqMan probe</strong></td>
<td>5'(FAM) - ACATCGATCGAAGAACACTGGT - TAMRA - (TAMRA)3'</td>
<td><strong>TaqMan probe</strong></td>
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**IL-8**

| **Forward** | 5'-TTCTCGAGCTCTGTCGAGG-3' | **Reverse** | 5'-GGTGGAAAGCTGGAAGTGTC-3' |
| **TaqMan probe** | 5'(FAM) - TTCTCGGAAAGCTAGTGCAACT - TAMRA - (TAMRA)3' | **TaqMan probe** | 5'(FAM) - TGGCGATCGAAGAACACTGGT - TAMRA - (TAMRA)3' |

**ICAM-1**

| **Forward** | 5'-CTGGCAGACGAAAGTGGT-3' | **Reverse** | 5'-GTCTGCTGCAAGGCTGCAAGT-3' |
| **TaqMan probe** | 5'(FAM) - TGGAGCTCTAGTGCTGCTGACT - TAMRA - (TAMRA)3' | **TaqMan probe** | 5'(FAM) - CTGGCAGACGAAAGTGGT - TAMRA - (TAMRA)3' |

**FAM, 6-carboxy-fluorescein; TAMRA, 6-carboxytetramethyl-rhodamine.**

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**Immunohistochemistry**

IL-8 immunohistochemistry was established in lung tissue from a healthy, untreated porcine lung and performed in each animal of the Aerosol-PFC, FRC-PLV, and control groups (2 lung tissue samples taken from the inferior lung lobe) using serial 5-μm cryostat sections and five different primary monoclonal mouse anti-pig IL-8 antibody (clone NB8; Endogen; Woburn, MA)-working dilutions between 0 μg/ml and 10 μg/ml. A dilution of 5 μg/ml turned out to be optimal, thus lung tissue (2 samples each of the lower lobe) from all animals of each group was immunostained.

A rabbit anti-mouse immunoglobulin “link” (Dako, Hamburg, Germany) in a dilution of 1:40 was used as secondary antibody. The final incubation was with the alkaline phosphatase-anti-alkaline phosphatase complex (1:50, Dako) (5). The samples were thoroughly washed in Tris-buffered saline, pH 7.4, between the steps. Alkaline phosphatase was allowed to react with new fuchsin (100 μg/ml) and levamisole (400 μg/ml) at room temperature. All incubation steps lasted 25 min. Sections were counterstained with hematoxylin and mounted in gelatin. Negative controls were performed using mouse anti-rabbit immunoglobulin (clone MR12/52, 1 μg/ml, Dako) as primary antibody.
ICAM-1 mRNA expression. ICAM-1 mRNA was detectable in 82% of all endothelial cell specimens. The difference of CT values between target gene and housekeeping gene of each lung specimen could finally be included for analysis. The values for ICAM-1 mRNA expression normalized to HPRT mRNA expression were not significantly different between the three treatment groups: 6.98 ± 2.15 in the Aerosol-PFC group, 9.23 ± 4.53 in the FRC-PLV group, and 9.05 ± 4.55 in the control group (2ΔCT-HPRT - 2ΔCT-ICAM-1; means ± SE).

Immunohistochemical Staining

Serial sections and differential immunostaining using primary antibody concentrations between 1 μg/ml and 10 μg/ml revealed a constitutive IL-8 expression (protein level) in bronchiolar smooth muscle cells and in vascular smooth muscle cells in healthy, untreated porcine lungs as well as in all groups investigated. All primary antibody negative controls showed no immunoreactivity (Fig. 4A).

In alveolar septum cells and alveolar macrophages of healthy, untreated porcine lung tissue, IL-8 immunostaining (antibody dilutions from 2 μg/ml to 5 μg/ml) was completely negative. At a dilution of 10 μg/ml, a very weak cytoplasmic IL-8 immunoreactivity could be observed.

Data for mean IL-8 immunohistochemical scores in alveolar macrophages, bronchiolar epithelial cells, and alveolar septum cells are presented in Table 2. The immunohistochemical summary score of IL-8 protein expression in alveolar macrophages, bronchiolar epi-

Fig. 2. Pulmonary IL-8 mRNA expression normalized to hypoxanthine phosphoribosyltransferase (HPRT) mRNA expression (2ΔCT-HPRT - 2ΔCT-IL-8; means ± SE) in surfactant-depleted neonatal piglets treated with aerosolized perfluorocarbon (Aerosol-PFC, n = 5), partial liquid ventilation (FRC-PLV, n = 5), or intermittent mandatory ventilation (IMV; control, n = 4). A: in alveolar macrophages, n (samples) = 27. B: in bronchiolar epithelial cells, n (samples) = 28. C: in alveolar septum, n (samples) = 28. D: in bronchiolar smooth muscle cells, n (samples) = 28. E: in vascular smooth muscle cells, n (samples) = 28. *P < 0.05, **P < 0.01, compared with control. The y-axis scales are markedly different among the graphs.

Fig. 3. Pulmonary IL-8 mRNA expression normalized to HPRT mRNA expression (2ΔCT-HPRT - 2ΔCT-IL-8; means ± SE) in alveolar macrophages (A), bronchiolar epithelial cells (B), alveolar septum (S), bronchiolar smooth muscle cells (M), and vascular smooth muscle cells (V) in surfactant-depleted neonatal piglets treated with Aerosol-PFC (A), FRC-PLV (B), or IMV (control; C). The y-axis scales are markedly different among the graphs. *P < 0.05, **P < 0.01, ***P < 0.001 compared with vascular smooth muscle cells. *P < 0.05, compared with bronchiolar smooth muscle cells; n samples and n piglets are identical with data shown in Fig. 2.
thelial cells, and alveolar septum cells was significantly reduced by Aerosol-PFC compared with FRC-PLV and control animals. Immunostaining for IL-8 in lung tissue of a piglet treated with Aerosol-PFC or IMV is presented in Fig. 4, B and C, respectively.

**Arterial Oxygen Tension**

As published previously (14), arterial oxygen tension significantly increased during treatment in the Aerosol-PFC and FRC-PLV groups compared with control. In the FRC-PLV group, this effect subsided shortly after the end of PFC substitution, and PaO₂ decreased from a maximum of 384 ± 28 mmHg to 95 ± 21 mmHg, whereas the improvement in the Aerosol-PFC group persisted (endpoint PaO₂: Aerosol-PFC 406 ± 27 mmHg, FRC-PLV 217 ± 51 mmHg, control 68 ± 8.4 mmHg; P < 0.001 Aerosol-PFC vs. control).

**DISCUSSION**

Analysis of commonly used tissue homogenates bears the risk of masking genetic deviations or expression changes of an individual cell type by the bulk of surrounding cells. To overcome the problem of tissue heterogeneity, cells have to be harvested selectively for further analysis. Therefore, microdissection techniques were developed, and, in particular, the laser-based microdissection systems make retrieval of target cells simple, rapid, and precise. Laserphotolysis of adjacent tissue is performed under visual control. Cells and groups of cells are only transferred into the reaction tube when no contaminating tissue still adherent to the cells of interest can be seen by light microscopy. Furthermore, mRNA of adjacent cells will be destroyed by the laser beam. Therefore, the contamination rate is expected to be up to zero in alveolar macrophages and bronchiolar epithelial cells. Vascular endothelial cells have been separated at the level of the lamina elastica interna. Due to the very thin endothelial layer, contamination of adjacent cells of the vascular wall is inevitable but little. For microdissection of vascular smooth muscle cells, photolysis of the thin layer of endothelial cells cannot be performed without the risk of destroying smooth muscle cell mRNA. However, compared with the amount of smooth muscle cells obtained by microdissection, the relative amount of contaminating endothelial cells or connective tissue will be considerably small.

Betsuyaku and coworkers (2) have already demonstrated remarkable differences between lung homogenate gene expression and a cell type-specific measurement after microdissection.

In our study, IL-8 mRNA expression varied up to 300-fold in different cell types microdissected from identical cryosections. This emphasizes the capability of this new method to distinguish between the mRNA

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<th>Table 2. IL-8 immunohistochemistry score</th>
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<td><strong>Aerosol-PFC</strong></td>
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<tr>
<td>Alveolar macrophages</td>
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<td>Bronchiolar epithelial cells</td>
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<td>Alveolar septum</td>
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<td>Summary score</td>
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Values are means ± SE. IL-8 immunohistochemistry score of alveolar macrophages, bronchiolar epithelial cells, and alveolar septum cells. A summary score of all cell types in surfactant-depleted piglets treated either with aerosolized perfluorocarbon (Aerosol-PFC), partial liquid ventilation (FRC-PLV), or intermittent mandatory ventilation (Control). †P < 0.05 vs. Control, ‡P < 0.01 vs. Control, *P < 0.05, §P < 0.01 vs. FRC-PLV.
expression of different cells and confirms that cryosectioning does not level cell type-specific differences in mRNA expression by mRNA smear (3). With the use of identical cell numbers picked per tube, HPRT mRNA expression in alveolar macrophages and bronchiolar epithelial cell specimens did not differ between the treatment groups. Thus HPRT was shown to be stable and not regulated in this setting and proven to be a suitable housekeeping gene.

Analysis of IL-8 mRNA expression on a cellular level by laser-assisted microdissection identified alveolar macrophages as the predominant source in IL-8 mRNA synthesis in this lung injury model. Consequently, the anti-inflammatory effect of Aerosol-PFC therapy was most pronounced in alveolar macrophages, identifying their crucial role in the regulation of the pulmonary inflammatory reaction. IL-8 mRNA expression was shown not only to be present but also to be regulated by different treatment regimens in all other cell types studied (bronchiolar epithelial cells, bronchiolar and vascular smooth muscle cells, and cells infiltrating the alveolar septum). The reduced levels of IL-8 mRNA expression seen on the cellular level following treatment with FRC-PLV and Aerosol-PFC are in line with reduced IL-8 mRNA expression previously demonstrated in samples of whole tissue mRNA extraction (29). This underlines the value of representative results obtained by whole tissue mRNA extraction. As the differences in IL-8 mRNA expression between the therapy groups were confirmed by immunohistochemistry, the mRNA findings were reflected qualitatively on the protein level.

ICAM-1 mRNA expression was similar in endothelial cells of the Aerosol-PFC, FRC-PLV, and control groups. Variation in mRNA expression of ICAM-1 in endothelial cells, therefore, could not be identified to contribute significantly to the early inflammatory process observed in this porcine model. However, since ICAM-1 is known to contribute to pulmonary inflammatory reactions, mRNA expression of ICAM-1 might be significantly different in other cell types not investigated in this study. Moreover, regulation of this gene may manifest itself by mRNA stabilization and prolongation of its half-life or by modification of translational and posttranslational processes rather than by induction of mRNA (19).

Treatment of experimental neonatal respiratory distress syndrome with Aerosol-PFC has been shown not only to improve gas exchange and lung mechanics persistently (14) but to also reduce the early pulmonary inflammatory reaction, reflected by reduced interleukin mRNA expression in lung tissue (29). Consecutive reduction of lung injury may diminish the risk for preterm infants to develop BPD. The findings of the present study demonstrated also on the cellular level the anti-inflammatory effect of Aerosol-PFC, which was at least as potent as the effect of PLV at FRC volume. The exact mechanism of the anti-inflammatory effect of FC77 cannot be elucidated by our study. Improvement of pulmonary compliance (14) with consecutive reduction of shear stress forces will account for an anti-inflammatory response. Direct anti-inflammatory effects of perfluorocarbons on human neutrophils or alveolar macrophages have been demonstrated by others (9, 28). The effect of perfluorochemicals in the presence of hyperoxia has been discussed controversially in literature. Pretreatment with perfluorochemicals aggravated lung injury induced by hyperbaric oxygen treatment (22). Perfluorochemical emulsion blood replacement did not alter hyperoxia-induced lung injury (1). On the other side, intraperitoneal or intravenous administration of FC77 decreased acid-induced lung permeability changes independently from hyperoxia exposure (18). In our study, all animals were exposed to 100% inspired oxygen. Although animals in the Aerosol-PFC group were hyperoxic and animals in the control group were not, inflammatory response was lower in the PFC-treated group. Our study was not designed to detect a possible protective effect of PFCs against hyperoxia. Hyperoxia was not the mechanism of lung injury but resulted from PFC treatment in surfactant-depleted piglets with severe acute respiratory distress syndrome.

Although many cell types were shown to be involved in the regulation of the inflammatory process, alveolar macrophages could be identified to contribute predominantly to the increase of IL-8 mRNA, seen in whole lung tissue specimens. This finding points to alveolar macrophages as suitable cellular targets for the development of new anti-inflammatory therapeutic approaches. In summary, predominantly alveolar macrophages but also bronchiolar epithelial cells and to a lesser extent cells of the alveolar septum and smooth muscle cells are involved in the pulmonary inflammatory process in surfactant-depleted piglets with acute respiratory distress syndrome. On a cellular level, the anti-inflammatory effect of PFC therapy was most pronounced with Aerosol-PFC.

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