Expression of Xenobiotic Metabolizing Enzymes in Different Lung Compartments of Smokers and Nonsmokers

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BACKGROUND: Cytochrome P450 monooxygenases (CYP) play an important role in the defense against inhaled toxicants, and expression of CYP enzymes may differ among various lung cells and tissue compartments.

METHODS: We studied the effects of tobacco smoke in volunteers and investigated gene expression of 19 CYPs and 3 flavin-containing monoxygenases, as well as isoforms of glutathione S-transferases (GST) and uridine diphosphate glucuronosyltransferases (UGT) and the microsomal epoxide hydrodase (EPHX1) in bronchoalveolar lavage cells and bronchial biopsies derived from smokers (n = 8) and nonsmokers (n = 10). We also investigated gene expression of nuclear transcription factors known to be involved in the regulation of xenobiotic metabolism enzymes.

RESULTS: Gene expression of CYP1A1, CYP1B1, CYP2A6, GSTPI, and EPHXI was induced in bronchoalveolar lavage cells of smokers, whereas expression of CYP2B6/7, CYP3A5, and UGT2A1 was repressed. In bronchial biopsies of smokers, expression of CYP1A1 and CYP1B1 was repressed. Induction of CYP1A1 and CYP1B1 transcript abundance resulted in increased activity of the coded enzyme. Finally, expression of the liver X receptor and the glucocorticoid receptor was significantly up-regulated in bronchoalveolar lavage cells of smokers.

CONCLUSIONS: We found gene expression of pulmonary xenobiotic metabolizing enzymes and certain key transcription factors to be regulated in bronchoalveolar lavage cells and bronchial biopsies of smokers. The observed changes demonstrate tissue specificity in xenobiotic metabolism, with likely implications for the metabolic activation of procarcinogens to ultimate carcinogens of tobacco smoke.

KEY WORDS: cytochrome P450 monooxygenases, metabolism, smoking, transcription factors, xenobiotic metabolizing enzymes. Environ Health Perspect 114:1655–1661 (2006). doi:10.1289/ehp.8861 available via http://dx.doi.org/ [Online 19 July 2006]

The lung serves as a primary site for xenobiotic metabolism, and many xenobiotics are substrates for cytochrome P450 catalyzed oxidations. Notably, the lung is composed of > 40 different cell types with different levels of metabolic competence (Ding and Kaminsky 2003). Alveolar macrophages and bronchial epithelial cells are part of > 40 different cell types in lung tissue and differ in their biological functions (Hocking and Golde 1979; Hukkanen et al. 2002). Indeed alveolar macrophages clear the airways of tobacco smoke particles and therefore constitute an important defense mechanism (Drath et al. 1979). Furthermore, the lung epithelium takes part in the detoxification of tobacco smoke through metabolic activation by phase I and phase II enzymes (Crawford et al. 1998; Han et al. 2005). Although a number of CYP isoforms have been identified in human lung tissue, a comprehensive survey of most human pulmonary xenobiotic metabolizing enzymes in different human lung tissue compartments has not been performed.

Tobacco smoke constitutes a complex mixture of thousands of toxicants, some of which require tissue-specific activation to become genotoxic carcinogens and others become metabolically activated poisons (Smith et al. 2003; Yamazaki et al. 1992). For smokers, the risk of developing lung cancer has been shown to be, at least in part, dependent on pulmonary metabolism of smoke constituents (Rubin 2001). Particular evidence stems from studies with smokers in which genetic polymorphisms of certain xenobiotic metabolizing enzymes were linked to the development of lung cancer (Bartsch et al. 2000; London et al. 1999). Currently available data suggest that genetic variability in coding sequences of P450 enzymes, antioxidants, or DNA repair genes contribute to the risk of developing bronchogenic carcinomas (Caporaso 2002).

Cigarette smoke may affect the capacity of lung tissue to dispose of foreign chemicals by changing expression and coded activity of xenobiotic metabolizing enzymes. Additionally, cigarette smoke may affect the pharmacokinetics of inhaled drugs by modulating activity of specific drug metabolizing enzymes (Durak et al. 1996).

Therefore, knowledge of expression and activity of xenobiotic and drug metabolizing enzymes in lung tissue of smokers gives us a better understanding of metabolically induced toxicity of tobacco smoke constituents and allows us to assess the capacity for tissue specific detoxification.

We investigated the gene expression of 19 cytochrome P450 monooxygenases (CYPs) and 3 flavin-containing monoxygenases (FMOs), as well as uridine diphosphate glucuronosyltransferase (UGT) 2A1 (UGT2A1), epoxide hydrolase (EPHX1), glutathione-S-transferase (GST) A2 (GSTA2), GSTPI, and GSTM1 in lung cells obtained from bronchoalveolar lavage (BAL) fluid and in bronchial biopsies (BBs) of smokers (n = 8) and nonsmokers (n = 10).

Additionally, transcriptional regulation of CYPs depends, at least in part, on promoter activation through binding of transcription factors and nuclear receptors to cognate DNA recognition sites (Borlak and Thum 2001; Pascussi et al. 2003). We therefore studied expression of the aryl hydrocarbon receptor (AHR), constitutive androstane receptor (CAR), pregnane X receptor (PXR), liver X receptor (LXR), and glucocorticoid receptor (GR) (Gibson et al. 2002; Tirona et al. 2003) for their established role in CYP gene regulation in BAL cells and BBs derived from smokers and nonsmokers.

Methods

Study subjects. All subjects were volunteers and gave written consent after being fully informed about the purpose and nature of the study. The study was approved by the Ethical Committee of Hannover Medical School.

Ten nonsmokers and eight smokers were enrolled. All subjects had no history of allergic or other diseases. Only subjects with forced expiratory volume in 1 sec (FEV1) > 75% (predicted); normal electrocardiogram, differential blood cell count, blood coagulation, serum parameters (gamma-glutamyl-transferase, aspartate aminotransferase, alanine aminotransferase, urea, creatinine, sodium, potassium, IgE); and negative skin-prick test (ALK-SCHERAX Arzneimittel GmbH, Hamburg, Germany) were enrolled.

We thank C.R. Lai (Drug Research and Medical Biotechnology, Fraunhofer ITEM) and B. Volkmann (Immunology/Allergology and Clinical Inhalation, Fraunhofer ITEM) for technical assistance.

This work was supported by a grant from the Lower Saxony Ministry of Culture and Science to J.B.

The authors declare they have no competing financial interests.

Received 17 November 2005; accepted 19 July 2006.
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(0.05–0.1 mg/kg). Lidocaine (maximum: 6 mg/kg) was given as a local anesthetic of the upper and lower airways. Drug administration and timing was strictly controlled. The bronchoscope (BF 160 P, Olympus Optical Co. Europe GmbH, Hamburg, Germany) was wedged into the medial segment of the middle lobe, and BAL was performed with 6 × 20 mL sterile saline solution. Lavage fluid from the first 20 mL was discarded. After lavage, the bronchoscope was passed to the anterior segment of the left upper lobe and three bronchial biopsies were taken distal from the carina. The biopsy samples were immediately placed in RNA isolation buffer (Macherey-Nagel GmbH & Co. KG, Düren, Germany), snap-frozen in liquid nitrogen, and stored at −80°C until RNA isolation. Differential cell counts were obtained from cytospin slides, with 300 cells/slide being counted (Table 2).

**RNA isolation and reverse transcription.**

RNA was isolated from BAL pellets (1 × 10⁶ cells/pellet) and biopsy materials using the Total RNA Isolation System (Macherey-Nagel GmbH & Co. KG) according to the manufacturer’s recommendation. Quality and quantity of isolated RNA was assayed by capillary electrophoresis (Bioanalyzer 2100; Agilent Technologies Deutschland GmbH, Böblingen, Germany) following the manufacturers instructions. We used 1 µg total RNA from each sample for reverse transcription, as described previously (Thum and Borlak 2002). The resulting cDNA was frozen at −20°C until further experimentation.

**Thermocycler reverse transcriptase-polymerase chain reaction (RT-PCR).**

PCR was carried out using a thermal cycler (T3; Biometra GmbH, Goettingen, Germany) as described previously (Thum and Borlak 2001). In brief, the following PCR conditions were used: denaturation, 94°C (45 sec); primer annealing, 54–58°C (60 sec); and extension, 72°C (60 sec). Detailed oligonucleotide sequence information is given in Table 3. We checked for DNA contamination by direct amplification of RNA extracts before conversion to cDNA. Any contamination of RNA extracts with genomic DNA could therefore be excluded. The optimal PCR cycles were derived by studying PCR products at different numbers of PCR cycles. As a consequence, PCR-reactions were performed within the linear range of amplification, and transcript expression levels were calculated as the ratio of the gene of interest (numerator) versus an established housekeeping gene (cyclophilin A, denominator). We observed no significant differences in the gene expression results when experiments were repeated with the same cDNA, indicating good reproducibility of the method. Amplification products were separated on a 1.5% agarose gel, stained with ethidium bromide, photographed on a transilluminator (Kodak Image Station 440; Kodak GmbH, Stuttgart, Germany), and quantified using the Kodak ID 3.5 network software.

**Ethisoxyresorufin-O-demethylase (EROD) assay.** Immediately after bronchoscopy, approximately 0.1 × 10⁶ cells were separated from BAL fluid and put into 500 µL Dulbecco’s

**Table 1. Subject characteristics.**

| Subjects | Age (median range) | Sex | FEV₁ (% predicted) | FEV₁/FVC (%) | Cigarettes/day | Pack-years | Cotinine (ng/mL) | Interval (min) |
|----------|-------------------|-----|-------------------|--------------|---------------|------------|-----------------|---------------|
| Nonsmokers (n = 10) | 23 | M | 105.8 | 73.7 | 0 | 0 | 8.5 | — |
| 36 | F | 113.7 | 85.8 | 0 | 0 8.5 | 13.5 | — |
| 21 | F | 92.2 | 77.1 | 0 | 0 | 0 | 0 | — |
| 24 | F | 101.2 | 78.8 | 0 | 0 | 0 | 0 | — |
| 28 | M | 106.0 | 76.3 | 0 | 0 | 3.75 | 20.4 | — |
| 24 | F | 123.5 | 88.7 | 0 | 0 | 2.3 | — | — |
| 25 | F | 105.5 | 83.8 | 0 | 0 | 0 | 0 | — |
| 26 | M | 98.6 | 81.7 | 0 | 0 | 0 | 0 | — |
| 26 | F | 85.5 | 79.7 | 0 | 0 | 0 | 0 | — |
| 23 | F | 106.0 | 83.7 | 0 | 0 | 0 | 0 | — |
| Median (range) | 24.5 (21–36) | — | 105.8 (85.5–123.5) | 80.7 (73.7–86.7) | 0 (0) | 0 (0–3.75) | 0 (0–20.4) | — |
| Smokers (n = 8) | 28 | M | 107.6 | 85.2 | 20 | 13.0 | 194.8 | 97 |
| 30 | M | 98.1 | 75.8 | 20 | 3.0 | 175.8 | 137 | — |
| 22 | F | 113.2 | 83.2 | 20 | 7.0 | 400.0 | 157 | — |
| 30 | M | 95.4 | 73.5 | 20 | 13.0 | 241.4 | 88 | — |
| 29 | M | 89.2 | 78.6 | 20 | 2.5 | 156.8 | 93 | — |
| 26 | M | 108.2 | 74.6 | 25 | 15.0 | 310.0 | 136 | — |
| 26 | F | 99.0 | 83.7 | 20 | 12.0 | 369.0 | 88 | — |
| 22 | M | 77.2 | 75.3 | 20 | 31.0 | 102.8 | 70 | — |
| Median (range) | 28.5 (22–45) | — | 98.6 (77.2–113.2) | 77.2 (73.5–85.2) | 20 (20–25) | 12.5 (2.5–31.0) | 218.1 (102–400) | 90.5 (70–157) |

*Abbreviations: F, female; M, male.

*p < 0.01 compared with nonsmokers.

**Table 2. Basic BAL fluid data given as median (range).**

| Subjects (n) | Recovery (mL) | Total cells (10⁶) | Macrophages | Neutrophils | Lymphocytes | Eosinophils |
|--------------|---------------|------------------|-------------|-------------|-------------|-------------|
| Nonsmokers (10) | 77.5 (60–88) | 90 (84–95) | 90.8 (22–6.7) | 2 (1–3) | 0.1 (0.1–0.2) | 0 (0–1) |
| Smokers (8) | 76.5 (62–86) | 90 (85–92) | 1.4 (4.9–21.2) | 4 (1–8) | 0.5 (0.1–) | 0 (0–4) |

Histology

The bronchoalveolar lavage fluid was submitted for quantification of T helper 1 (Th1) and T helper 2 (Th2) cytokines by enzyme-linked immunosorbent assay (ELISA). The bronchoalveolar lavage fluid was submitted for quantification of T helper 1 (Th1) and T helper 2 (Th2) cytokines by ELISA. The results were expressed as the median (range) of the cytokine levels in the bronchoalveolar lavage fluid. The cytokines were measured in duplicate for each sample, and the levels were expressed as the median of the duplicate measurements. The levels of cytokines were compared using the Mann-Whitney U-test, and the results were expressed as the median (range) of the cytokine levels in the bronchoalveolar lavage fluid.
modified Eagle’s medium supplemented with 5% fetal calf serum and 2 mM glutamine. Then, 7-ethoxycoumarin (2 µM, Sigma-Aldrich Chemie GmbH, Deisenhofen, Germany) was added to cells, and the plates were incubated at 37°C for 4 hr and centrifuged for 5 min (1,200 × g, 4°C). The resulting supernatant was snap-frozen in liquid nitrogen and stored at −80°C to await fluorescence detection as described by Grant et al. (1987). Samples (250 µL) were treated with 250 µL ammonium acetate (pH 4.5), and aliquots were treated with 100 µL β-glucuronidase (Sigma-Aldrich Chemie GmbH) overnight at 37°C to assay product release of β-glucuronide conjugates. After addition of 500 µL glycine buffer (pH 10.3), fluorometric analysis was carried out on a spectrophotofluorometer (Bio-Rad Laboratories GmbH, München, Germany). The fluorometric analysis of the resultant product resorufin was done with an excitation at 530 nm and an emission wavelength of 585 nm. Calibration of the system was performed with resorufin and an appropriate standard curve with a concentration range of up to 100 nM.

**Statistical analysis.** We used the Mann-Whitney U test for intergroup comparisons. A p-value < 0.05 was considered statistically significant. Unless otherwise stated, the data are expressed as median and range or as individual results.

**Results**

**Subject characteristics and BAL data.** The clinical characteristics of the study subjects are given in Table 1. There was no significant difference in FEV₁ (%) predicted and FEV₁/forced vital capacity (FVC) between smokers and nonsmokers.

Recovery of BAL fluid did not differ between smokers and nonsmokers. The total cell count in BAL fluid from smokers was nearly double the cell count in nonsmokers (p < 0.01). This was mainly due to higher numbers of macrophages and neutrophils. We found no significant differences in the absolute counts of lymphocytes and eosinophils in BAL samples between smokers and nonsmokers (Table 2).

**Gene expression of CYPs and FMOs.** Expression of CYP1A2, CYP2C8, CYP2C18, CYP2C19, CYP2D6, CYP3A4, and CYP4A11 in smokers and nonsmokers (Figure 1); however, the expression of CYP1B1 was significantly (p < 0.05) repressed.

**Transcript profiling in BAL cells.** Expression of CYP1A1 (p < 0.01), CYP1B1 (p < 0.001), and CYP2B1 (p < 0.001) was higher in BAL cells from smokers compared with those of nonsmokers (Figure 1), whereas expression of CYP2B6/7 (p < 0.01) and CYP3A45 (p < 0.001) was lower; expression of CYP2A67, CYP2E1, CYP2C9, CYP2J2, and FMO5 was not altered. CYP2F1 transcripts were detected in 3 of 10 nonsmokers and in 3 of 8 smokers, but the amount was too small for semiquantitative analysis (data not shown). Similarly, CYP4B1 was detected in 5 smokers and 1 nonsmoker, and CYP3A45 was detected in 2 nonsmokers but was below the limit of detection (LOD) in smokers (data not shown).

**Transcript profiling in BBs.** In smokers, expression of CYP1A1 (p < 0.05), CYP1B1 (p < 0.001), and CYP2C9 (p < 0.05) was increased (p < 0.05) compared with nonsmokers, and there was a trend toward increased expression of CYP3A45 (p = 0.0545; Figure 1); however, the expression of CYP2J2 was significantly (p < 0.05) repressed.

### Table 3. Oligonucleotide primers and gene regulation in BAL cells and BB samples.

| Accession no. | Gene     | Forward primer (5´–3´) | Reverse primer (5´–3´) | Product length (bp) | BAL   | BB  |
|---------------|----------|------------------------|------------------------|---------------------|-------|-----|
| D01150        | CYP1A1   | TCACAGACACAGCTGTATGAG  | GATGGGTTACATAGCTTT    | 432                 | ↑     | ↑   |
| NM_000104.2   | CYP1B1   | GCAGATTTGATCATGCTGCTT  | TGTCAGTCCTTGTGGATG    | 301                 | ↑     | ↑   |
| M38594        | CYP1A2   | TGCTCCTCACATCCTACAAAT  | TTACATGTCGCGCCATTAG   | 308                 | ↓     |     |
| U22027        | CYP2A6/7 | GTTGAGGACAGTCAGGCCT    | AGGCAGTTAGGGGAAAGT    | 1,151               |       |     |
| X13494        | CYP2B6/7 | CCATACACAGGAGGCGAGT    | GGTGTCAGATCGATCTCT    | 357                 | ↓     |     |
| XM_005922     | CYP2C8   | GATCATGTATGGCAGACCA    | CCTCGTGAAGAGGCTGAG    | 311                 |       |     |
| XM_005918     | CYP2C9   | AGCTGATGATACATGTCAGT   | ACCTGCTGAAAGGCTGAG    | 437                 | ↑     |     |
| NM_00772      | CYP2C18  | CTGTACAGTATGTCCTGCT    | CCTCGTGAAGAGGCTGAG    | 437                 | ↑     |     |
| NM_005919     | CYP2C19  | GTGACCTTGACTGACCTCT    | ACCTGCTGAAAGGCTGAG    | 311                 |       |     |
| M33189        | CYP2D6   | TCTGAGTACCTGTGCCCAT    | AAGCTGCTGAAAGGCTGAG   | 437                 |       |     |
| AF084225      | CYP2E1   | AGCAAGTCTGATAGATGG     | ATAGATCAGTCGACTGAG    | 365                 |       |     |
| J029306       | CYP2F1   | ATGAACTGCGGACCACGCG    | ACAGGCTCCTACACG6G     | 283                 |       |     |
| AF271424      | CYP2J2   | CCCAGCAAATCTCTCCAGC    | CATTTCCTGACCTGAGA     | 389                 |       |     |
| AF335278      | CYP3A1   | ACCAAACATCCCTCTACAC    | TCACATGTCCTGCTGATG    | 322                 |       |     |
| X12387        | CYP3A3   | CGAAGTCCTTGACTGACCT    | TGACAGCCGAAACACTGAAA  | 373                 |       |     |
| L26985        | CYP3A5   | TGTCAGCGAAACACTGAAA    | TGGAAGATGTCCTGCTGATG  | 472                 |       |     |
| NM_007851.1   | CYP3A7   | CTATGATACCTGTCCTGATG   | TGCGATCTTCCCACCTAC    | 474                 |       |     |
| AF208532      | CYP4A11  | CAAGTGATCCTTGGCTCTAT   | CGTATGCTCCTACCTGAG    | 280                 |       |     |
| NM_007799.1   | CYP4B1   | TGACATGTCCTCGATAGAG    | AAAGCTGATCCTGAGGCGCA  | 397                 |       |     |
| NM_000210     | EPX1     | TGAGAAGGAGGAGGCGAGT    | TCACAGGCTGCTCAGGAG    | 227                 |       |     |
| NM_002021     | FM01     | GTGTGCTGATGGTGAGGA     | GCCAAAGAGAAGCTGAGA    | 234                 |       |     |
| NM_006894     | FM03     | GGCGACGCTGACTTACCA     | GAGTCTGGCAACAAACAT    | 301                 |       |     |
| NM_001461     | FM05     | CCTCAGTCTTCTATTGACCA   | ACATATTTCTCTATCTGCA   | 400                 |       |     |
| NM_000846     | GSTA2    | GCCACGGCTCCTACTCTCA    | GCAAGGCTGATGCTTCTTTTC| 354                 |       |     |
| NM_000561     | GSTM1    | TCCCAGCTCTGCCCTACTGG   | GGCTCCTAAATATGGCTGAG | 347                 |       |     |
| XM_004016     | GSTP1    | AGCTCCTGCTTGGCTTACATC  | GGCGAGGCTGACCTGAGG    | 313                 |       |     |
| XM_003547     | UGT2A1   | TGACAGGATACAGGGCTGATC  | TCGACAGGACAACGATGAGG  | 329                 |       |     |
| Nuclear transcription factors |
| NM_001621     | AHR      | CTGCTGTTCCACAAAGATG    | GAAATCAGTCGCTGCTG     | 352                 |       |     |
| NM_005122     | NR113    | GTCAGGGCAGTCAGAGAGA    | GCAGGCTGACCTGCTG     | 350                 |       |     |
| M10901        | NR131    | GCTCGGTGATGAGTATG      | TCGACAGGACCTGCTG     | 300                 |       |     |
| U22862        | NR153    | TACACACCTGCTTCGAGTCA   | GCCAGACAGAAGCTGAGC    | 351                 |       |     |
| XM_004106     | NR212    | TTGCTTGCGATGACCTGAGT   | GGCGATGACCGAGGATTCTC  | 321                 |       |     |

**Accession numbers from GenBank (2005). Arrows indicate up- or down-regulation of significantly affected genes.**
Expression of CYP2B6/7, CYP2E1, CYP2F1, CYP2S1, CYP4A1, and FMO3 was similar to that of nonsmokers. CYP2A6/7 transcripts were detected in 2 of 10 nonsmokers and 3 of 8 smokers (data not shown). CYP3A4 was < LOD in all BBs investigated.

Gene expression of EPHX1, GSTs, and UGTs. In BAL cells of smokers, gene expression of EPHX1 and GSTP1 was significantly higher than that of nonsmokers, whereas expression of the UGT2A1 gene was significantly lower (p < 0.001) (Figure 2). Expression of GSTA2 and GSTM1 was not different between the study groups (Figure 2).

Further, in BBs taken from smokers, expression of EPHX1 was significantly lower (p < 0.001), whereas the expression of GSTA2 and GSTP1 were up to triple that in nonsmokers. Expression of GSTM1 and UGT2A1 was not significantly different in BBs of the two groups (Figure 2).

Gene expression of nuclear receptors. Gene expression of LXR (p < 0.001) and GR (p < 0.01) in BAL cells of smokers (Figure 2) was up to three times that of nonsmokers, whereas expression of the cytosolic AHR and PXR was similar. The expression of AHR, LXR, PXR, and GR was not different in BBs of both study groups. Expression of CAR was < LOD in all samples studied.

Enzyme activity of CYP1A1 and CYP1B1. 7-Ethoxyresorufin served as substrate for CYP1A1 and CYP1B1. In BAL cells derived from smokers, EROD activity was up to 3-fold that of nonsmokers (p < 0.05; Figure 3), but glucuronides were < LOD, as determined by β-glucuronidase treatment.

Discussion

In this study we aimed to investigate the regulation of gene expression of major human pulmonary xenobiotic metabolizing enzymes as well as regulatory nuclear transcription factors in smoking and nonsmoking healthy volunteers. Differences were observed when we compared BAL cells and BBs of smokers and nonsmokers. Our findings provide new insight into lung tissue–specific responses to tobacco smoke as detailed below.

Effects of tobacco smoke on pulmonary xenobiotic metabolizing enzymes. Lung tissue and cell types within the lung differ in their capacity to oxidize xenobiotics (Hecht 1999) because CYPs are not uniformly expressed (Ding and Karminsky 2003). As the majority of airborne toxicants enter the body through the respiratory tract, the pulmonary epithelium is exposed to high concentrations before systemic circulation, which, in turn, requires an effective local defense system. Our findings of a simultaneous induction of CYP1A1 and CYP1B1 in different lung compartments after exposure to tobacco smoke demonstrate up-regulation of the pulmonary enzyme system and agree well with other reports (McLemore et al. 1990). Regulation of CYP1A1 and CYP1B1 is not fully understood, but transcriptional activation by the AHR constitutes a major mechanism. Upon translocation into the nucleus, the AHR forms a heterodimer with its nuclear counterpart ARNT (AHR nuclear translocator) and drives gene expression of the AHR–responsible gene family, including CYP1A1 and CYP1B1 (Borlak and Thum 2001). Thus, the strong induction of CYP1A1 and CYP1B1 can be explained, at least in part, in terms of AHR-mediated transcriptional activation. Although we did not observe changes of the AHR at the gene expression level, Martey et al. (2005) recently reported that cigarette smoke extract led to an activation of the AHR in cultured human lung fibroblasts based on induced nuclear translocation of the AHR (Martey et al. 2005). The observed up-regulation of AHR-responsive genes in lung tissue of smokers is likely mediated by the activation of AHR. The present study does, however, contradict the findings.

| Gene | BAL | BB |
|------|-----|----|
| CYP2E1 | NS | NS |
| CYP2F1 | NS | NS |
| CYP2S1 | NS | NS |
| CYP2S2 | NS | NS |
| CYP2S3 | NS | NS |
| CYP2S4 | NS | NS |
| CYP2S5 | NS | NS |
| CYP4A1 | NS | NS |
| CYP4B1 | NS | NS |
| CYP4F1 | NS | NS |
| CYP4V1 | NS | NS |
| CYP4V2 | NS | NS |
| CYP4V3 | NS | NS |
| CYP4V4 | NS | NS |
| CYP4V5 | NS | NS |
| CYP4V6 | NS | NS |
| CYP4V7 | NS | NS |
| CYP4V8 | NS | NS |
| CYP4V9 | NS | NS |
| CYP4V10 | NS | NS |
| CYP4V11 | NS | NS |
| CYP4V12 | NS | NS |
| CYP4V13 | NS | NS |
| CYP4V14 | NS | NS |
| CYP4V15 | NS | NS |
| CYP4V16 | NS | NS |
| CYP4V17 | NS | NS |
| CYP4V18 | NS | NS |
| CYP4V19 | NS | NS |
| CYP4V20 | NS | NS |
| CYP4V21 | NS | NS |

Figure 1. Gene expression of various CYPs in BAL cells and BB material obtained from nonsmokers (n = 10) and smokers (n = 8). NS, not significant. Data are represented as plots from individual volunteers, with solid lines indicating medians. Results are presented as the ratio of the gene of interest/cyclophilin; only significantly altered genes are shown.

*p < 0.05, **p < 0.01, ***p < 0.001.
of Anttila et al. (1991), who did not find CYP1A1 expression in alveolar macrophages, regardless of smoking status. This may be due to different experimental approaches because Anttila et al. employed immunohistochemistry, whereas we used a sensitive RT-PCR method to determine CYP1A1 and CYP1B1 expression and a fluorometric assay to demonstrate increased activity of the coded enzymes. Indeed, Willey et al. (1996) were initially unable to detect CYP1A1 expression in alveolar macrophages, but upon application of more sensitive techniques these investigator were able to detect CYP1A1 gene expression (Willey et al. 1997).

CYP2A enzymes metabolize a variety of carcinogens including 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), which can lead to the development of lung cancer (Koskela et al. 1999). In the present study, we detected expression of CYP2A6/7 in both alveolar macrophages and bronchial biopsy material, but we did not find any difference in regard to smoking status. This contrasts the finding of Crawford et al. (1998), who demonstrated repression of CYP2A6/7 in human bronchial epithelial cells of smokers. The somewhat high interindividual variation of CYP2A6/7/gene expression observed in the present study and in the study of Crawford et al. (1998) make comparison of the data difficult.

We also observed significant induction of CYP2S1 in BAL cells of smokers. Expression of CYP2S1 in lung tissue has been previously reported (Rylander et al. 2001), but regulation by cigarette smoke has not been determined so far. One study (Rivera et al. 2002) demonstrated inducibility of CYP2S1 in mouse lungs after systemic administration of dioxins. The observed induction of CYP1A1 and CYP2S1 in bronchoalveolar macrophages and bronchial biopsies of smokers is of importance because of their contribution to the metabolic activation of tobacco smoke components, namely polycyclic aromatic hydrocarbons, which can lead to the development of lung cancer (Georgiadis et al. 2005).

CYP2B6/7 and CYP3A5 play an important role in the metabolic activation of NNK and of benzo[a]pyrene (Code et al. 1997; Hecht 1999), but transcript levels and enzyme activity were repressed in BAL cells derived from smokers. Previously, Hukkanen et al. (2003) demonstrated decreased CYP3A5 expression in alveolar macrophages of smokers. We now extend the findings of Hukkanen et al. (2003) to BBs and report a trend toward increased CYP3A5 expression in smokers (p < 0.0545), which could result in local metabolic activation of carcinogens. Notably, the CYP3A family of monoxygenases plays a key role in pulmonary drug metabolism. Inhaled drugs, such as salmeterol, tiotropium, theophylline, or glucocorticoids (e.g., budesonide, prednisone), with an established role in chronic obstructive pulmonary disease or asthma treatment (Global Initiative for Asthma 2005; Global Initiative for Chronic Obstructive Lung Disease 2005) are substrates for CYP3A isoforms (Jonsson et al. 2000).

Figure 2. Gene expression of FM05, various phase II enzymes, and key regulatory transcription factors in BAL cells and BB material obtained from nonsmokers (n = 10) and smokers (n = 8). NS, not significant. Data are represented as plots from individual volunteers, with solid lines indicating medians. Results are presented as the ratio of the gene of interest/cyclophilin; only significantly altered genes are shown.

* p < 0.05. ** p < 0.01. *** p < 0.001.

Figure 3. Metabolism of 7-ethoxyresorufin in BAL cells obtained from nonsmokers (n = 10) and smokers (n = 8) with and without glucuronidase treatment. Data represent mean ± SE of the individual incubation experiments, with approximately 100,000 cells per experiment.

* p < 0.05.
We also observed induction of the carci-
gen-metabolizing enzymes GSTP1 in BAL cells and BBs and GSTA2 in BBs from smokers. This is likely an adaptive response to chronic exposure to tobacco smoke components. GSTP1 overexpression inhibited cytokotoxic effects of cigarette smoke extract on human fibroblast-derived cells and depletion of GSTP1-induced apoptosis in lung fibroblasts (Ishii et al. 2001, 2003). Moreover, Crawford et al. (2000) showed that mRNA levels of GSTs expressed by bronchial epithelial cells from patients with bronchogenic carcinoma are significantly lower compared with subjects without carcinoma. Thus, increased expression of GSTP1 in healthy smokers might protect against accumulation of carcinogens.

Effects on nuclear receptors. GR and LXR were significantly up-regulated in BAL cells, but no clear correlation between CYP mono-
oxygenases and nuclear receptor gene expres-
sion was observed. However, the up-regulation of GR fits well to the increased expression of CYP2C9 because GR plays an important role in transcriptional regulation of the CYP2C9 gene (Gerbal-Chaloin et al. 2002). CYP regula-
tion is, in most cases, not dependent only on a specific nuclear factor, but requires a com-
plex network of interacting factors (Waxman 1999).

Study limitations. We did not recruit for a sex-balanced study group; for example, 8 of 10 were females in the nonsmoking group, whereas 7 of 8 were males in the smoking group. Nonetheless, when gene expression was compared between men and women, we found no significant differences. Furthermore, within different lung tissue compartments, we observed opposite effects in expression of cer-
tain genes (i.e., CYP3A5, EPHX1) for smokers, but these were independent of the sex. Although drug administration before the bronchoscopic procedures was strictly con-
trolled, we cannot rule out minor effects of the drugs used (e.g., midazolam, lidocaine, atropine) on gene expression within lung tis-
sue. Notably, the same drugs were given to both smokers and nonsmokers, usually ≤15 min before the bronchoscopic proce-
dures. This short interval makes major effects on gene expression unlikely.

Conclusions We observed profound effects of tobacco smoke exposure in BBs and BAL cells of vol-
teers and found CYP1A1, CYP1B1, and
GSTP1 to be up-regulated in BBs and alveolar macrophages of smokers, whereas others tran-
scripts were differentially expressed and, in some cases, even oppositely regulated (i.e., CYP3A5, EPHX1). Differences in the expres-
sion of xenobiotic metabolizing enzymes may suggest local and tissue-specific susceptibility in metabolically activated toxicity.

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Grant MH, Burke MD, Hawkesworth GM, Guthie SJ, Engeset J, Petrie JC. 1987. Human adult hepatocytes in primary mono-
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1995; Zevin and Benowitz 1999). Therefore, metabolism of CYP3A enzyme activity in smokers may require dose adjustment for some inhaled drugs.

Furthermore, the CYP epoxygenases CYP2C and CYP2J2 are key players in the metabolism of arachidonic acid, resulting in the production of epoxyeicosatrienoic acids, some of which affect vascular and bronchial smooth muscle tone (Fisslthaler et al. 1999; Thum and Borlak 2004; Zeldin et al. 1995).

In the present study, we observed reduced expression of CYP2J2 in BBs of smokers, whereas CYP2C9 was significantly induced in this lung compartment. This shift in the expression of the CYP epoxygenase gene might alter production of epoxy fatty acids, some of which are considered to be signalling molecules affecting vascular- and smooth muscle cell tone. Undoubtedly, future studies are needed to determine the regulation and the role of epoxyeicosatrienoic acids in smokers.

In addition, genetic variability in the coding of various CYP genes may affect enzyme activity (reviewed by Daly et al. 1994; Ingelman-Sundberg 2001). Likewise, changes in the expression of CYP transcripts may influence an individual’s capacity to convert different precarcinogenic compounds into their ultimate carcinogens; therefore, these CYP tran-
scripts are of major importance for an individual’s susceptibility of developing chemically induced cancer. Certain CYP mono-
oxygenases have an established role in an activation of precarcinogens to ultimate car-
icogens of inhaled tobacco smoke toxiancients (e.g., CYP1A1, CYP1A2, CYP1B1, CYP2E1, CYP3A4). The simultaneous induction of CYP1A1 and CYP1B1 in both types of lung cells obtained from BAL and pulmonary epithelial cells after smoking cigarettes, as found in the present study, may therefore enhance an individual susceptibility for chemi-

cally induced lung cancer.

In addition, expression of EPHX1 was increased in BAL cells of smokers, but decreased in BBs. This is important because in the absence of EPHX1 the tobacco smoke carcinogen benzo[a]pyrene is primarily metabolized to the noncarcinogenic 7,8-benzo-

phenolic product (Shou et al. 1994). Bartsch et al. (1992) observed a significant increase in the activity of pulmonary EPHX1 within smokers, but EPHX1 activity was determined in preparations of parenchymal lung tissue, which consists of many different cell types. Besides, the lung tissue specimens were taken from patients with either lung cancer or non-
neoplastic lung diseases. Thus, a direct com-
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