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Comparison of metabolites in exhaled breath and bronchoalveolar lavage fluid samples in a mouse model of asthma

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Neuhaus S, Seifert L, Vautz W, Nolte J, Bufe A, Peters M. Comparison of metabolites in exhaled breath and bronchoalveolar lavage fluid samples in a mouse model of asthma. *J Appl Physiol* 111: 1088–1095, 2011. First published July 21, 2011; doi:10.1152/jappphysiol.00476.2011.—**Background:** A multi-capillary column ion mobility spectrometer (MCC/IMS) was developed to provide a method for the noninvasive diagnosis of lung diseases. The possibility of measuring the exhaled breath of mice was evaluated previously. The aim of the present study was to reveal whether mice affected by airway inflammation can be identified via MCC/IMS. **Methods:** Ten mice were sensitized and challenged with ovalbumin to induce allergic airway inflammation. The breath and volatile compounds of bronchoalveolar lavage fluid (BALF) were measured by MCC/IMS. Furthermore, histamine, nitric oxide, and arachidonic acid were determined as inflammatory markers in vitro. **Results:** Six volatile molecules were found in the BALF headspace at a significantly higher concentration in mice with airway inflammation compared with healthy animals. The concentration of substances correlated with the numbers of infiltrating eosinophilic granulocytes. However, substances showing a significantly different concentration in the BALF headspace were not found to be different in exhaled breath. Histamine and nitric oxide were identified by MCC/IMS in vitro but not in the BALF headspace or exhaled breath. **Conclusion:** Airway inflammation in mice is detectable by the analysis of the BALF headspace via MCC/IMS. Molecules detected in the BALF headspace of asthmatic mice at a higher concentration than in healthy animals may originate from oxidative stress induced by airway inflammation. As already described for humans, we found no correlation between the biomarker concentration in the BALF and the breath of mice. We suggest using the model described here to gain deeper insights into this discrepancy.

allergic airway inflammation; ion mobility spectrometry

ION MOBILITY SPECTROMETRY (IMS) is a sensitive, fast, and cost-effective method for the detection of gas-phase analytes that is increasingly used for biological applications (4, 8, 13, 18, 21). It is a powerful tool to reveal comprehensive information of a mixture's compounds, e.g., in exhaled breath (21), particularly when a multi-capillary column (MCC) is used to preseparate the sample. However, in contrast to gas-chromatographic mass spectrometry (GC/MS) analysis, direct identification of molecules is not possible. Meanwhile, databases have been built and validated by GC/MS reference measurements to identify molecules detected by MCC/IMS due to their specific location in the chromatogram. First studies have already been performed to measure the breath of patients by this method: for instance, Westhoff et al. (23) showed the possibility of identifying patients suffering from lung carcinoma by using the

MCC/IMS approach. However, substantial information about the nature of the detected substances is missing due to a lack of possibilities of carrying out further analysis of molecules in bronchoalveolar lavage fluid (BALF) and lung tissue. We have, therefore, adapted this method for the measurement of exhaled breath of mice, giving the opportunity of performing cellular and molecular analysis to get further insights into the origin of a disease-specific molecular pattern in breath (20). In the present study, we used a mouse model of asthma to identify substances characteristic for inflammatory responses in the exhaled breath and BALF of mice. In the literature, studies can be found showing characteristic markers in exhaled breath of patients with airway inflammation. Those data were predominantly derived from experiments analyzing exhaled breath condensate (EBC). Corradi et al. (7), for instance, showed that increased concentrations of aldehydes as a marker of lipid peroxidation in human chronic obstructive pulmonary disease patients are detectable in EBC and sputum. Other authors have shown that products from the metabolism of lipid mediators, such as prostaglandins, may also be detectable in exhaled breath condensate (3, 17). Brussino et al. (3) revealed that oxidative stress during allergen challenge is accompanied by lipid degradation and could be detected in EBC.

However, data on lipid degradation products derived from asthma patients are heterogeneous; for instance, malondialdehyde is increased in the EBC of children with asthma exacerbation while the concentration of other aldehydes, such as nonanal or heptanal, is decreased or unchanged, respectively (6). Possibilities to further analyze the mechanisms accounting for this discrepancy are limited in humans, further underlining the importance of an animal model.

We have already demonstrated that the breath of mice can be collected and analyzed by MCC/IMS, enabling us to investigate the molecular pattern of breath in models of lung disease (20). Therefore, the aim of this study was to evaluate both the exhaled breath of mice and volatile compounds in the BALF headspace to show whether it is possible to differentiate mice with and without airway inflammation using MCC/IMS.

METHODS

Ion mobility spectrometry. MCC/IMS measurement and analysis was performed as described previously (18–20, 22). Briefly, a multicapillary column (OV-5, 20 cm, 40°C) preseparates the gas phase analytes by gas chromatography due to specific polarity (Fig. 1). After preseparation through MCC, the analytes enter the IMS (Fig. 1, no. 1) and are ionized indirectly by proton transfer from reactant ions formed by a ⁶³Ni (550 MBq) radiation source (Fig. 1, no. 2). The ions are conducted into the drift tube (Fig. 1, no. 3) through a periodically opening ion shutter (300 μs) where they are accelerated in an electric field (300 V/cm) (Fig. 1, no. 4) drifting against a 100 ml/min stream of synthetic air at a distance of 12 cm. The drift gas decelerates the

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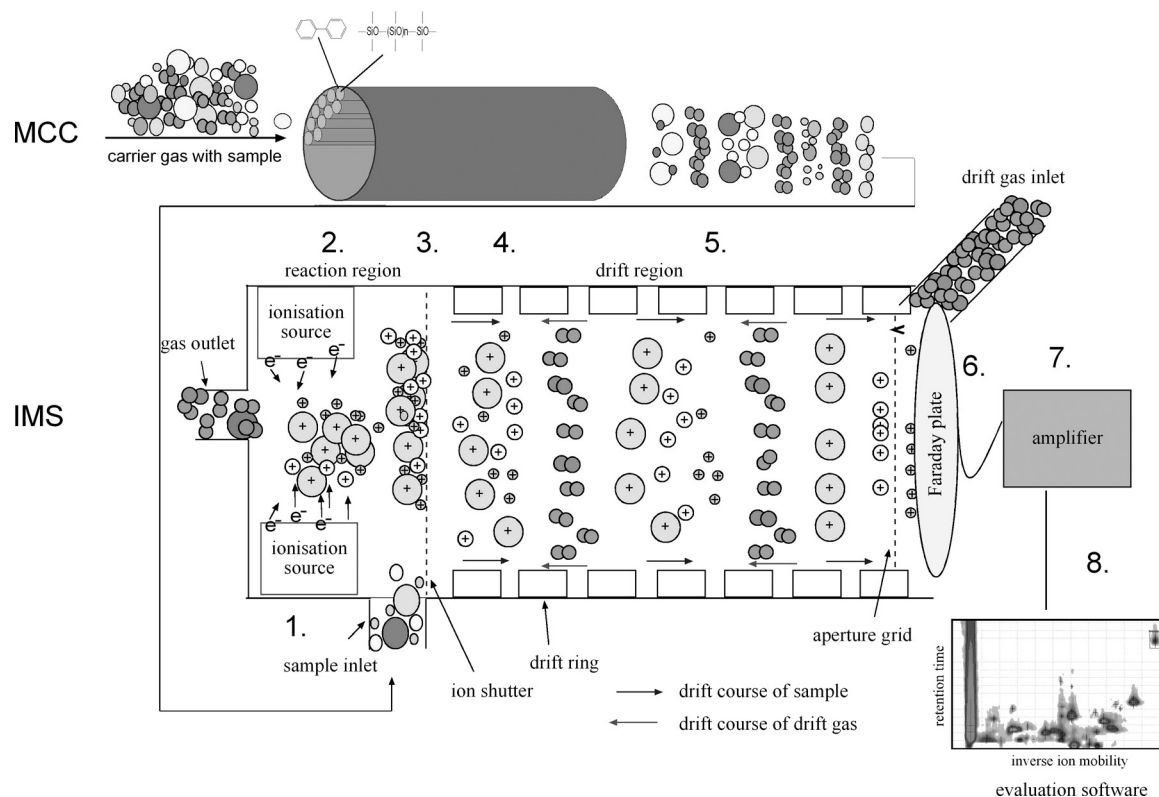


Fig. 1. Illustration of a multi-capillary column (MCC) ion mobility spectrometer (IMS). An MCC preseparates the gas phase analytes. Subsequently, the analytes enter the IMS (1) and are ionized indirectly by proton transfer from reactant ions formed by a ^{63}Ni radiation source (2). The ions are conducted into the drift tube (3), drifting against a stream of synthetic air (4). The drift gas decelerates the ions due to multiple collisions, depending on the molecular weight, size, and shape of the ions (5), resulting in a molecule-specific drift time. The ions are detected on a Faraday plate (6), and signals are amplified (7). The intensity measured at the Faraday plate is a measure for the concentration and enables quantification using a calibration carried out earlier (8).

ions due to multiple collisions, depending on the molecular weight, size, and shape of the ions (Fig. 1, no. 5), resulting in a molecule-specific drift time. The ions are detected on a Faraday plate (Fig. 1, no. 6), and signals are amplified (Fig. 1, no. 7). The retention time (RT) in the MCC and the inverse ion mobility ($1/K_0$), which depends on drift time in the IMS, enable the identification of the ions by comparison with a database. The intensity measured at the Faraday plate is a measure for the concentration and enables quantification using a calibration carried out earlier (Fig. 1, no. 8). The reaction ion peak (RIP) was normalized to 1 arbitrary unit. All signal intensities detected were related to the RIP. Samples were measured over a time period of 1,500 s. To guarantee that the system was gas-tight and, therefore, free from contamination with ambient air, frequent control measurements with humid synthetic air were performed.

Animals. Female, 5- to 6-wk-old BALB/c mice (Charles River, Sulzfeld, Germany) were used and acclimated to the animal facility for 2 wk prior to the experiments. Food and water were provided ad libitum. Animal experiments were approved by the appropriate governmental authority (Landesamt für Natur, Umwelt und Verbraucherschutz Nordrhein-Westfalen, Recklinghausen, Germany).

Sensitization and airway challenge. Twenty mice were sensitized intraperitoneally by injecting 20 μg ovalbumin (OVA; GradeV; Sigma-Aldrich, St. Louis, MO) emulsified in 2.2 mg aluminum hydroxide (ImjectAlum, Pierce, Rockford, IL) in a total volume of 200 μl on days 1 and 14. On days 28 and 38, one group of mice (OVA/OVA, $n = 10$) was challenged via the airways by exposure to OVA aerosol for 30 min. OVA aerosol was generated with a PARI-Boy aerosol generator from a 1% OVA solution. Control mice (OVA/PBS, $n = 10$) were treated with PBS aerosol. Thus the same level of stress was induced in both groups of mice without the induction of airway inflammation in the control mice. In experiments performed to ana-

lyze BALF headspace OVA/OVA mice were compared with nonsensitized (untreated) mice. This gave us the opportunity to correlate systemic T-cell response (IL-5) to metabolites detected in the BALF headspace.

Sampling of breath for MCC/IMS analysis. The breath of the mice was measured on days 39, 40, and 41 after the onset of the experiment. For this purpose, mice were anesthetized by intraperitoneal injection of 65 $\mu\text{g/g}$ ketamine (Ratiopharm, Ulm, Germany) and 13 $\mu\text{g/g}$ xylazine (Bayer, Leverkusen, Germany). After the relaxation of muscles, mice were orotracheally intubated with a 1-mm-diameter endotracheal tube as described elsewhere (2).

Subsequently, the tube was connected to a three-way bypass. This enabled airflow through a pneumotachometer of a plethysmographic box (type 871, Hugo Sachs Electronics, March-Hugstetten, Germany) for control of the sample flow to the MCC/IMS sample loop. Thus only exhaled breath was introduced into the MCC/IMS for analysis. The mice breathed synthetic air during the sample collection. Pressure change during exhalation was identified by the pneumotachometer, thereby activating a valve enabling the collection of exhaled breath by a peristaltic pump. Eleven milliliters of exhaled breath were collected on the sample loop of the MCC/IMS before applying the sample to the MCC column. To ensure the gas-tightness of the system, we performed control measurements in which we contaminated ambient air with isoflurane, a molecule that can be sensitively detected by IMS (20). These control measurements showed that during measuring the mice breathed air not contaminated by ambient air (data not shown).

Measurement of volatile metabolites in the BALF headspace, histamine, and nitric oxide samples through MCC/IMS. To reveal whether there are differences between mice with and without airway inflammation detectable by MCC/IMS, the BALF of five healthy

(untreated) mice and six mice with airway inflammation (OVA/OVA) was prepared. BALF (500 µl) was put into a new glass bottle with 100 ml volume, heated to 37°C, and incubated for 30 min. Histamine, arachidonic acid, and nitric oxide were measured without previous incubation in a similar bottle. NO was analyzed by IMS without pre-separation through MCC.

An airflow of 30 ml/min synthetic air was conducted through the glass bottle for 60 s to apply the different samples to the sample loop of the IMS.

Measurement of volatile metabolites in the BALF headspace by solid phase microextraction-GC/MS. A 20 ml glass bottle containing 500 µl BALF was closed by a seal with a septum. A solid phase microextraction (SPME) fiber (PDMS 100 µm) was exposed for over 30 min to the BALF headspace. The adsorbed analytes were desorbed thermally in the injector of an MSD GC/MS System at 250°C splitless for 1 min (MSD, Agilent Technologies 6890N GC/ 5973 mass-selective detector; Gerste, Mühlheim, Germany).

The initial oven temperature of 35°C was maintained for 2 min, increased by 7°C/min to 250°C, and held for 27 min. A HP-5MS capillary column (60 m, 0.25 mm inner diameter, 0.25 µm film thickness; WiCom, Heppenheim, Germany) was used for the compound separation with helium as the carrier gas at a constant flow-rate of 1.0 ml/min. Electron ionization mode was used with 70 eV and the ions were registered in a mass range of m/z 33–450 u.

Analysis of allergic phenotype of sensitized and challenged mice. On day 43 after experimental onset, the mice were killed and BALF was prepared. Furthermore, spleen cells were collected for cell culture. Restimulation of splenic cell culture with ovalbumin was performed and the BALF cells were analyzed as described earlier (14, 15). IL-5 concentration in cultures of spleen cells was measured with a BD OptEIA mouse IL-5 kit, and immunoglobulin E concentration in the BALF was detected by using a BD OptEIA mouse IgE kit according to the manufacturer's instructions. The ELISA plate reader Dynatech MR5000 (Dynex Technologies, Berlin, Germany) was used.

Statistical analysis. Data from the mouse model were checked for normal distribution and subsequently analyzed by the unpaired *t*-test. Graph Pad Prism Software (Version 5) was used for the analysis. To adjust for multiple comparisons Bonferroni correction was performed. Therefore, statistical significant differences were assumed for the 70 analytes detected in the BALF headspace at $P < 0.0007$ and for the 53 analytes detected in the breath at $P < 0.0009$.

RESULTS

Analysis of volatile metabolites in the BALF headspace. The BALF headspace from untreated animals and from sensitized and challenged animals was analyzed by MCC/IMS. A total of 70 different signals were identified in the mice of either group. Twenty-three signals showed a difference in intensity between mice with and without airway inflammation. Some of these signals were identified as the aldehydes: hexanal, heptanal, octanal (monomer and dimer), nonanal (monomer and dimer), and decanal; the alcohols: 1-hexanol, 1-heptanol, 2-ethyl-1-hexanol (monomer and dimer); and the ketone 2-octanone. Others could not be identified by searching the ISAS database (all signals showing differences in the signal intensity are shown in Fig. 4 and Table 1).

For instance, two of them, namely nonanal and decanal, were identified in the BALF headspace, as shown in Fig. 2. Nonanal and decanal showed a higher concentration in the BALF headspace of OVA/OVA than in healthy mice. The latter had at least a 10 times lower concentration.

Additionally, the BALF headspace was examined by GC/MS through SPME. We identified higher concentrations of octanal, 2-octenal, nonanal, and 2-nonenal in mice with airway inflammation than in healthy mice (Fig. 3). The metabolites octanal and nonanal were also identified by MCC/IMS. How-

Table 1. Correlation analysis between the signal intensities identified by ion mobility spectrometer and parameters of allergic inflammation

	Correlation Between Signal Intensity and:					
	Eosinophilis		IgE concentration		IL-5 concentration	
	Spearman <i>r</i>	<i>P</i> value	Spearman <i>r</i>	<i>P</i> value	Spearman <i>r</i>	<i>P</i> value
P_0570-049	0.8899	0.0005	0.578	0.0656	0.7065	0.0182
P_0572-023	0.9358	0.0001	0.6514	0.0336	0.7523	0.0098
P_0611-047	0.6789	0.0251	0.5046	0.1142	0.6147	0.0478
P_0634-010 hexanal	0.4863	0.1294	0.3303	0.3132	0.6514	0.0336
P_0650-013 1-hexanole	0.7432	0.0112	0.3119	0.3415	0.578	0.0656
P_0652-018	0.6055	0.0519	0.3762	0.2483	0.6055	0.0519
P_0652-040	0.6239	0.044	0.4312	0.1826	0.5964	0.0562
P_0660-026 2-octanone	0.6881	0.0226	0.5597	0.0761	0.6881	0.0226
P_0668-013 heptanal	0.5964	0.0562	0.3762	0.2483	0.789	0.0055
P_0687-023 1-heptanole	0.6422	0.0368	0.422	0.1928	0.7523	0.0098
P_0688-092	0.7615	0.0086	0.5505	0.0818	0.8257	0.0027
P_0696-033 2-ethyl-1-hexanole	0.6881	0.0226	0.4954	0.1217	0.7707	0.0074
P_0701-025 octanal	0.6331	0.0402	0.5046	0.1142	0.7982	0.0047
P_0720-049	0.6881	0.0226	0.4954	0.1217	0.7523	0.0098
P_0725-023	0.5138	0.1072	0.3119	0.3415	0.6422	0.0368
P_0727-223	0.6422	0.0368	0.578	0.0656	0.789	0.0055
P_0734-055 nonanal	0.6514	0.0336	0.5138	0.1072	0.8349	0.0022
P_0741-025	0.5597	0.0761	0.1927	0.5765	0.6606	0.0306
P_0762-129 decanal	0.6514	0.0336	0.3945	0.225	0.6514	0.0336
P_0775-055	0.5872	0.0609	0.2202	0.5208	0.6789	0.0251
P_0892-026 octanal dimer	0.6422	0.0368	0.5872	0.0609	0.8257	0.0027
P_0892-039 2-ethyl-1-hexanole dimer	0.6514	0.0336	0.5964	0.0562	0.7432	0.0112
P_0949-056 nonanal dimer	0.6698	0.0277	0.5872	0.0609	0.8165	0.0033

A correlation analysis between the signal intensities and eosinophilic granulocyte counts, IgE concentration of bronchoalveolar lavage fluid as well as IL-5 concentration of splenic-cell-culture was performed. Left column shows $1/K_0$ value and retention time of each signal.

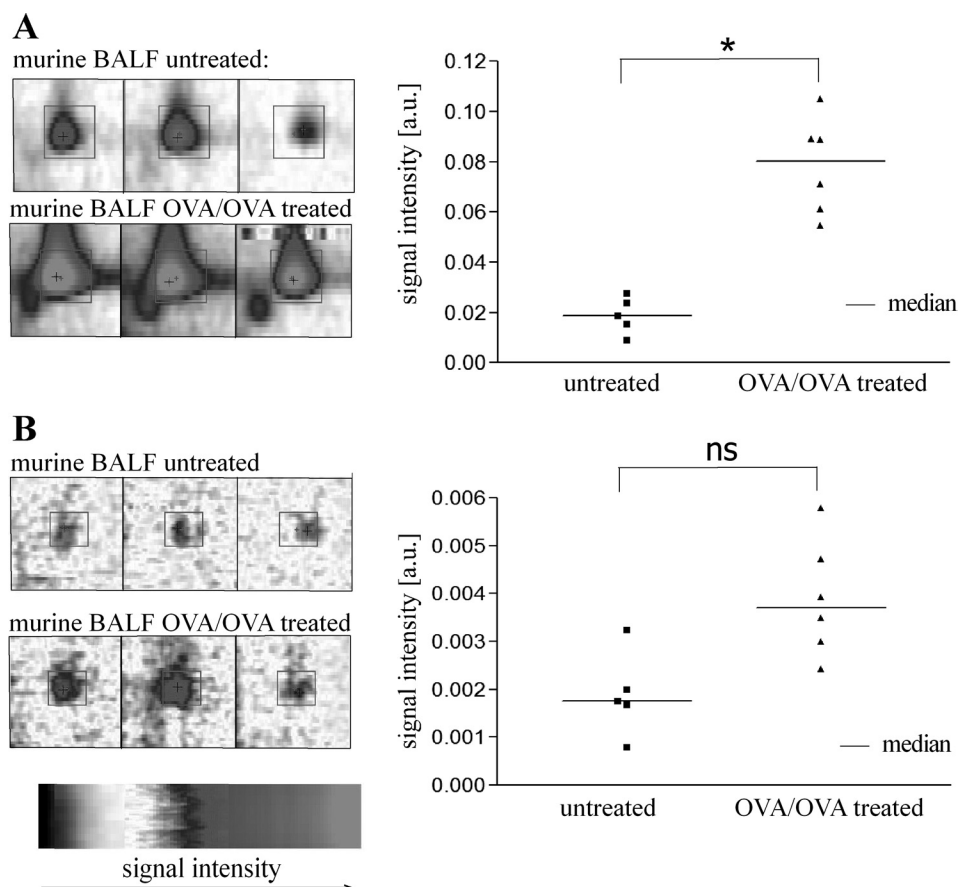
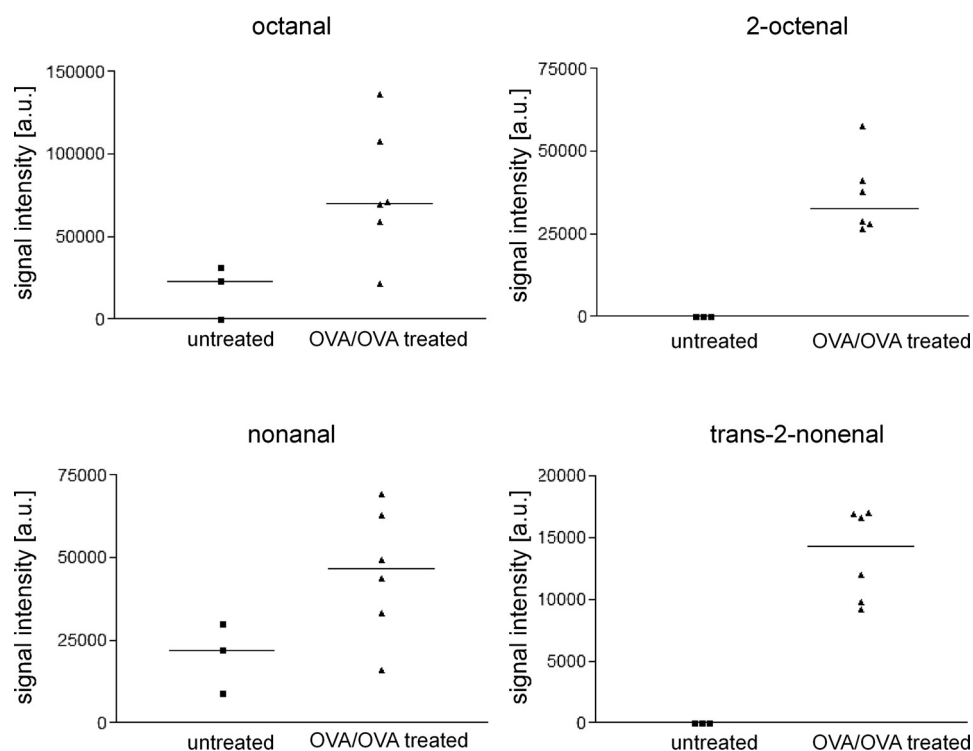


Fig. 2. MCC/IMS signal intensity of nonanal and decanal in the bronchoalveolar lavage fluid (BALF) headspace. The BALF headspace was measured by MCC/IMS of mice that were sensitized and challenged with ovalbumin (OVA) or left untreated. Illustration of the specific position of nonanal (A) and decanal (B) in the chromatogram. A significant difference between signal intensity of untreated and OVA/OVA mice was found for nonanal (* $P < 0.0007$).

Fig. 3. Comparison of the BALF headspace measured by GC/MS. Volatile compounds of the BALF headspace were adsorbed by solid phase microextraction (SPME; PDMS 100 μ m) and conducted to GC/MS through thermo desorption. Headspace of BALF of 3 healthy mice and 6 mice with airway inflammation was analyzed. Substance identification was made by NIST database.



ever, 2-octenal and trans-2-nonenal were not available in the MCC/IMS database and, therefore, could not be identified by this method.

Specific metabolites showing a different concentration between the two groups are summarized in Fig. 4. Interestingly, all compounds were found in a higher concentration in the BALF headspace of mice with established airway inflammation than in that of healthy mice, yet some of them, namely 2-ethyl-1-hexanol, nonanal, heptanal, 1-heptanol, unidentified signal "P_0720-049," and octanal reached statistical significance ($P < 0.0007$). For the remaining substances not shown in Fig. 4, no differences between the analyzed groups were found.

Interestingly, concentration (signal intensity) of many of these volatile compounds correlated with the number of eosinophils in the BALF and the amount of IL-5 produced by the

lymphocytes of the spleen after restimulation with OVA. In contrast, no significant correlation between IgE concentration and signal intensity was found (Table 1).

Analysis of volatile metabolites in the exhaled breath of mice. To study whether differences found in the BALF headspace between mice with and without airway inflammation are also reflected by the molecular pattern of breath, we subjected sensitized and challenged mice to breath analysis by MCC/IMS.

A total of 53 different signals were identified in the breath of mice. Seven of these substances had already been identified in the BALF headspace: for instance, nonanal (Fig. 5A) was found in a similar amount as in the BALF headspace of healthy mice. However, in contrast to the results from the BALF analysis, in exhaled breath, no difference between the amount of nonanal in OVA/PBS and OVA/OVA mice was found. Similarly with deca-

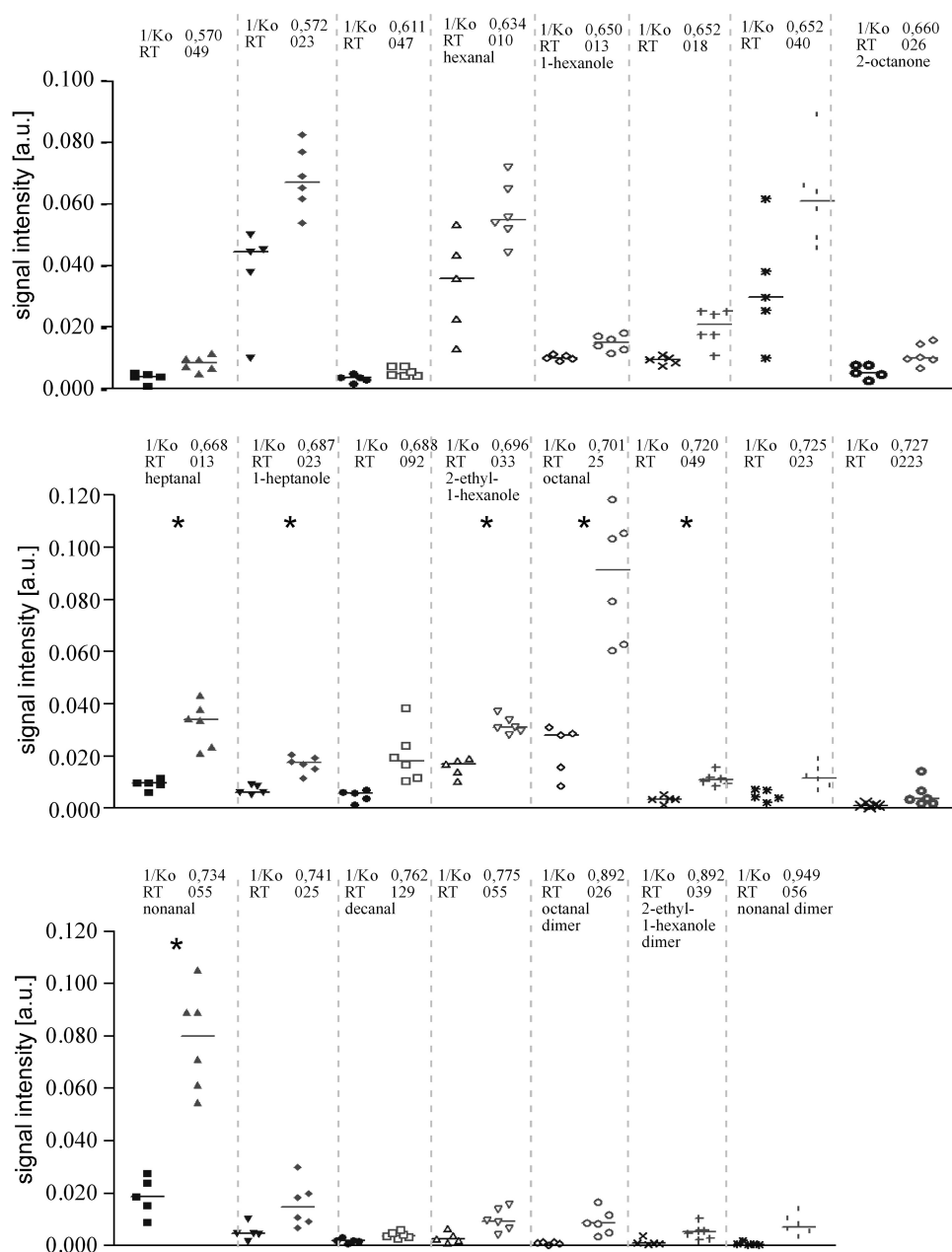


Fig. 4. Comparative illustration of BALF signals. The BALF headspace of 5 healthy and 6 OVA/OVA mice was compared. All presented signals showing a significant difference between headspace of BALF of healthy and OVA/OVA mice were labeled by an asterisk ($P < 0.0007$). For each signal the left scatter column displays healthy mice and the right the OVA/OVA-treated mice, respectively.

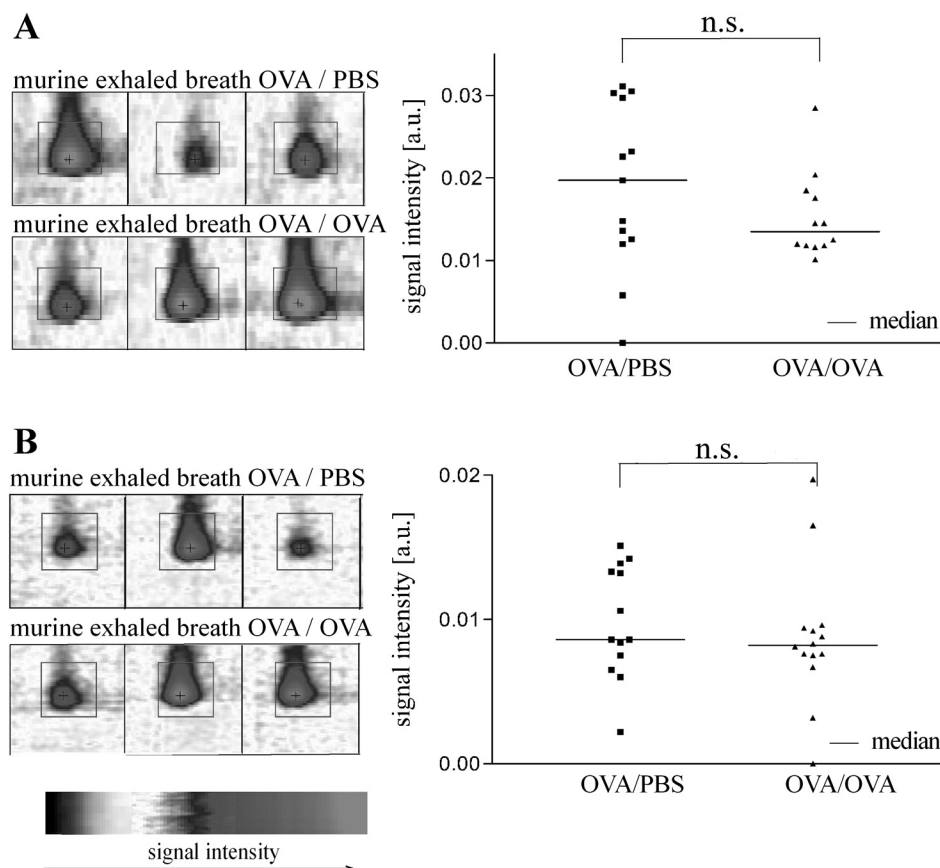


Fig. 5. MCC/IMS signal intensity of nonanal and decanal in the exhaled breath. The exhaled breath of mice was analyzed by MCC/IMS. Ten PBS/OVA and 10 OVA/OVA mice were examined. Illustration of the specific position of nonanal (A) or decanal (B) in the chromatogram. Significant differences between signal intensities of the exhaled breath and baseline measurements were found for both nonanal [OVA/PBS vs. OVA/OVA: not significant (n.s.), exhaled breath vs. baseline measurement: $P < 0.0001$] and decanal (OVA/PBS vs. OVA/OVA: n.s., exhaled breath vs. baseline measurement: $P < 0.0001$).

nal, no significant difference in signal intensity was found between the two different groups treated (Fig. 5B).

Figure 6 shows a comparison of all signals that were detected in the BALF headspace, as well as in the breath of mice. This comparison revealed that several signals show a higher concentration in the BALF headspace than in the breath of mice, with the exception of decanal, which behaves oppositely. Although several substances were found in the breath as well as in the BALF headspace, statistical differences in concentration were only found in the BALF headspace.

Measurement of substances characteristic for airway inflammation by MCC/IMS. To find out whether substances already known to be involved in allergic airway inflammation

are detectable by MCC/IMS, we determined histamine, nitric oxide, and the prostaglandin precursor arachidonic acid by MCC/IMS.

A dilution series of histamine was measured in vitro by MCC/IMS. Histamine is detectable with a lower detection limit of 150 mM with dose dependently increasing signal intensity, as shown in Fig. 7A.

Furthermore, nitric oxide is detectable by MCC/IMS, as shown in Fig. 7B. Both signals detected at 0.5073 Vs/cm² and 0.5490 Vs/cm² can be observed after applying NO in a concentration of 200 ppb to the IMS.

In contrast, arachidonic acid was not observed, at least in the tested concentration of 100 mM.

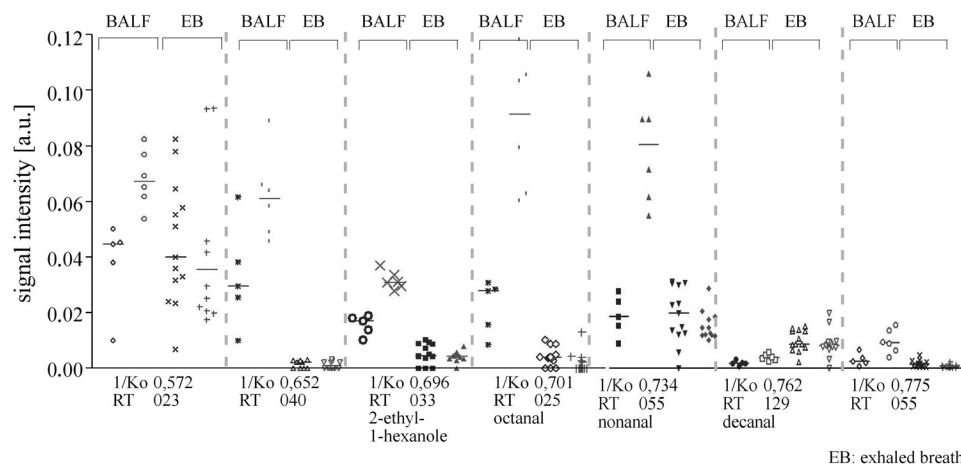
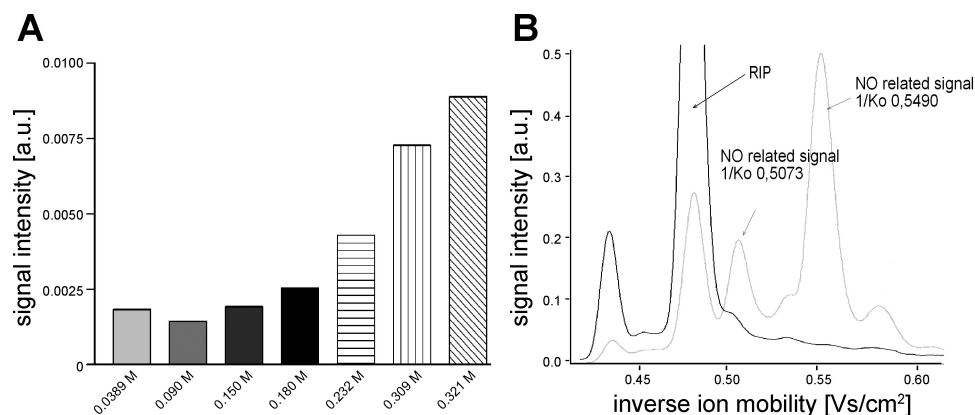


Fig. 6. Comparison of signal intensities detected in headspace of BALF and exhaled breath. The BALF headspace of 5 healthy and 6 OVA/OVA mice was compared. For sampling of the exhaled breath, 10 OVA/OVA and 10 OVA/PBS mice were measured. Mice with airway inflammation are displayed on the left and those without airway inflammation on the right of each group comparison.

Fig. 7. Histamine and NO measurement by MCC/IMS. The headspace of a solution of histamine at a concentration between 0.0389 M and 0.321 M was measured without previous incubation in a glass bottle with 100 ml volume (A). NO was analyzed by IMS without prepreparation through MCC at a concentration of 200 ppb (B). Baseline measurement is shown in black and nitric oxide sample in gray.



DISCUSSION

The aim of this study was to investigate whether it is possible to detect volatile molecules indicating airway inflammation by means of MCC/IMS in vitro and in vivo. In vivo measurements were performed in a mouse model of asthma.

We were able to measure histamine and nitric oxide by MCC/IMS in vitro. However, these molecules were neither detected in the BALF headspace nor in the exhaled breath of mice. Regarding histamine, the reason for this might be a concentration of 150 mM that is needed to detect this molecule via MCC/IMS. It is unlikely that this high concentration is reached under physiological conditions in vivo. Nitric oxide is known to react rapidly with oxygen to produce nitrogen dioxide (NO₂) and with water to produce nitrite. Thus the sampling procedure in mice might take too long to detect the intact molecule. However, since the sampling procedure in human breath would be faster, NO might be measureable by MCC/IMS in this case. In contrast, prostaglandins were generally not detectable either in vitro or in vivo. One explanation for this observation could be their high molecular weight and high boiling point leading to long retention time on the MCC. This is one limitation of IMS, that large molecules cannot be detected by this technique. For instance, it is impossible to measure proteins by IMS as recently demonstrated for EBC collection with successive proteome analysis (1). This limitation may be overcome in the future by modifying the experimental setup. For instance, electro-spray ionization may be used instead of β -radiation to enable the detection of proteins.

In contrast to those relatively large molecules that were not detectable via MCC/IMS in the murine lung, more than 60 small, volatile compounds were measured in the BALF headspace of mice of both groups. Even more interesting was the fact that 23 of these molecules were detected in the BALF headspace of mice with airway inflammation at a higher concentration than in the BALF headspace of healthy mice, with six of the detected differences reaching statistical significance ($P < 0.0007$). The majority of molecules detected were simple aliphatic aldehydes likely to be degradation products of lipids. Brussino et al. (30) showed that allergen challenge of patients with asthma is accompanied by oxidative stress. The reactive oxygen species produced by activated macrophages, mast cells, and eosinophils (5, 16) attack cell membrane lipids releasing oxidized alkanes. In our experiments, these volatile degradation products were detected by MCC/IMS in the BALF headspace as well as in the breath of mice. Nonanal, for

example, was found, which has already been described as being generated during lipid peroxidation in models of ozone exposure (24). Moreover, we observed that the concentration of volatile substances, e.g., nonanal and decanal, correlated well with parameters of allergic diseases such as the counts of eosinophilic granulocytes and production of IL-5. Therefore, it is likely that these substances were derived from inflammatory processes accompanied by oxidative stress, for instance, the generation of nitric oxide or oxygen radicals. Yet the actual biological origin of these molecules is still speculative. However, the fact that we identified these molecules as part of the inflammatory process in bronchial asthma opens the opportunity to study their mechanistic significance in asthma, i.e., by the use of gene-deficient mice that are more and more available. For instance, mice deficient for inducible NO synthase, a deficiency known to result in an alleviated allergic airway inflammation, may be used in the established model to evaluate its influence on the concentration of volatile compounds in the BALF headspace (12).

However, one important question still remains, namely why inflammation could be detected in the BALF headspace but not in the exhaled breath. Even substances identified in the BALF headspace as specific markers for airway inflammation were not increased in the exhaled breath of OVA/OVA mice. A possible explanation for this might be that due to mucus deposition in the airways of OVA/OVA mice, the release of volatile compounds from inflamed compartments of the lung into the airstream is reduced. By performing lavage, mucus is washed from the airways and concomitantly volatile compounds were released. A similar discrepancy was found in human disease when adenosine triphosphate (ATP) concentration was determined in either the EBC or BALF of persons with asthma. On the one hand, Idzko et al. (11) showed that ATP in the BALF of persons with asthma is increased after segmental allergen challenge. Lázár et al. (9), on the other hand, found no significant difference in ATP concentration by comparing the EBC of healthy patients and patients with asthma. Other authors have also described that there is no correlation between biomarker concentration determined in the BALF and EBC from humans (10). Noninvasive approaches for the evaluation of lung disease status are more and more in the focus of modern diagnostics. It will thus be important to reveal the origin of the discrepancy observed between biomarker concentration in breath and BALF. Since the mouse model reflects the absent correlation between the biomarker

concentration in BALF and breath observed, we suggest the use of this model for further investigations in humans.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

REFERENCES

1. Bloemen K, Van Den Heuvel R, Govarts E, Hooyberghs J, Nelen V, Witters E, Desager K, Schoeters G. A new approach to study exhaled proteins as potential biomarkers for asthma. *Clin Exp Allergy* 41: 346–356, 2011.
2. Brown RH, Walters DM, Greenberg RS, Mitzner W. A method of endotracheal intubation and pulmonary functional assessment for repeated studies in mice. *J Appl Physiol* 87: 2362–2365, 1999.
3. Brussino L, Badiu I, Sciascia S, Bugiani M, Heffler E, Guida G, Malinovschi A, Bucca C, Rolla G. Oxidative stress and airway inflammation after allergen challenge evaluated by exhaled breath condensate analysis. *Clin Exp Allergy* 40: 1642–1647, 2010.
4. Bunkowski A, Bödeker B, Bader S, Westhoff M, Litterst P, Baumbach JI. MCC/IMS signals in human breath related to sarcoidosis—results of a feasibility study using an automated peak finding procedure. *J Breath Res* 3: 1–10, 2009.
5. Calhoun WJ, Reed HE, Moest DR, Stevens CA. Enhanced superoxide production by alveolar macrophages and air-space cells, airway inflammation, and alveolar macrophage density changes after segmental antigen bronchoprovocation in allergic subjects. *Am Rev Respir Dis* 145: 317–325, 1992.
6. Corradi M, Folesani G, Andreoli R, Manini P, Bodini A, Piacentini G, Carraro S, Zanconato S, Baraldi E. Aldehydes and glutathione in exhaled breath condensate of children with asthma exacerbation. *Am J Respir Crit Care Med* 167: 395–399, 2003.
7. Corradi M, Pignatti P, Manini P, Andreoli R, Goldoni M, Poppa M, Moscato G, Balbi B, Mutti A. Comparison between exhaled and sputum oxidative stress biomarkers in chronic airway inflammation. *Eur Respir J* 24: 1011–1017, 2004.
8. Hartmann M, Zimmermann D, Nolte J. Changes of the metabolism of the colon cancer cell line SW-480 under serum-free and serum-reduced growth conditions in vitro. *Cell Dev Biol Anim* 44: 458–463, 2008.
9. Idzko M, Hammad H, van Nimwegen M, Kool M, Willart MA, Muskens F, Hoogsteden HC, Luttmann W, Ferrari D, Di Virgilio F, Virchow JC Jr, Lambrecht BN. Extracellular ATP triggers and maintains asthmatic airway inflammation by activating dendritic cells. *Nat Med* 13: 913–919, 2007.
10. Jackson AS, Sandrini A, Campbell C, Chow S, Thomas PS, Yates DH. Comparison of biomarkers in exhaled breath condensate and bronchoalveolar lavage. *Am J Respir Crit Care Med* 175: 222–227, 2007.
11. Lázár Z, Cervenak L, Orosz M, Gálffy G, Komlósi ZI, Bikov A, Losonczy G, Horváth I. Adenosine triphosphate concentration of exhaled breath condensate in asthma. *Chest* 138: 536–542, 2010.
12. Naura AS, Zerfaoui M, Kim H, Abd Elmageed ZY, Rodriguez PC, Hans CP, Ju J, Errami Y, Park J, Ochoa AC, Boulares AH. Requirement for inducible nitric oxide synthase in chronic allergen exposure-induced pulmonary fibrosis but not inflammation. *J Immunol* 185: 3076–3085, 2010.
13. Perl T, Carstens E, Hirn A, Quintel M, Vautz W, Nolte J, Jünger M. Determination of serum propofol concentrations by breath analysis using ion mobility spectrometry. *Br J Anaesth* 103: 822–827, 2009.
14. Peters M, Dudziak K, Stiehm M, Bufe A. T-cell polarization depends on concentration of the danger signal used to activate dendritic cells. *Immunol Cell Biol* 88: 537–544, 2010.
15. Peters M, Kauth M, Schwarze J, Korner-Rettberg C, Riedler J, Nowak D, Braun-Fahrlander C, von Mutius E, Bufe A, Holst O. Inhalation of stable dust extract prevents allergen induced airway inflammation and hyperresponsiveness. *Thorax* 61: 134–139, 2006.
16. Sanders SP, Zweier JL, Harrison SJ, Trush MA, Rembish SJ, Liu MC. Spontaneous oxygen radical production at sites of antigen challenge in allergic subjects. *Am J Respir Crit Care Med* 151: 1725–1733, 1995.
17. Soyer OU, Dizdar EA, Keskin O, Lilly C, Kalayci O. Comparison of two methods for exhaled breath condensate collection. *Allergy* 61: 1016–1018, 2006.
18. Vautz W, Baumbach JI. Exemplar application of multi-capillary column ion mobility spectrometry for biological and medical purpose. *Int J Ion Mobility Spectrom* 11: 35–41, 2008.
19. Vautz W, Baumbach JI, Jung J. Beer fermentation control using ion mobility spectrometry—Results of a pilot study. *J Inst Brewing* 112: 157–164, 2006.
20. Vautz W, Nolte J, Bufe A, Baumbach JI, Peters M. Analyses of mouse breath with ion mobility spectrometry: a feasibility study. *J Appl Physiol* 108: 697–704, 2010.
21. Vautz W, Nolte J, Fobbe R, Baumbach JI. Breath analysis performance and potential of ion mobility spectrometry. *J Breath Res* 3: 1–8, 2009.
22. Vautz W, Zimmermann D, Hartmann M, Baumbach JI, Nolte J, Jung J. Ion mobility spectrometry for food quality and safety. *Food Addit Contam* 23: 1064–1073, 2006.
23. Westhoff M, Litterst P, Freitag L, Urfer W, Bader S, Baumbach JI. Ion mobility spectrometry for the detection of volatile organic compounds in exhaled breath of patients with lung cancer: results of a pilot study. *Thorax* 64: 744–748, 2009.
24. Wynalda KM, Murphy RC. Low-concentration ozone reacts with plas-malogen glycerophosphoethanolamine lipids in lung surfactant. *Chem Res Toxicol* 23: 108–117, 2010.