Airborne Indoor Particles from Schools Are More Toxic than Outdoor Particles

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High concentrations of particulate matter (PM_{10}) were measured in classrooms. This study addresses the hazard of indoor particles in comparison to the better-studied outdoor particles. Samples were taken from six schools during teaching hours. Genome-wide gene expression in human BEAS-2B lung epithelial cells was analyzed and verified by quantitative PCR. Polycyclic aromatic hydrocarbons, endotoxin, and cat allergen (Fel d 1) were analyzed by standard methods. Enhancement of allergic reactivity by PM_{10} was confirmed in human primary basophils. Acceleration of human blood coagulation was determined with supernatants of PM_{10}-exposed human peripheral blood monocytes. Indoor PM_{10} induced serine protease inhibitor B2 (involved in blood coagulation) and inflammatory genes (such as CXCL6, CXCL1, IL6, IL8; all $P < 0.001$). Outdoor PM_{10} induced xenobiotic metabolizing enzymes (cytochrome P450 [CYP] 1A1, CYP1B1, TIPARP; all $P < 0.001$). The induction of inflammatory genes by indoor PM_{10} was explained by endotoxin (indoor 128.5 ± 42.2 EU/mg versus outdoor 13.4 ± 21.5 EU/mg; $P < 0.001$), the induction of CYP by outdoor polycyclic aromatic hydrocarbons (indoor 8.3 ± 4.9 ng/mg versus outdoor 16.7 ± 15.2 ng/mg; $P < 0.01$). The induction of serine protease inhibitor B2 was confirmed by a more rapid human blood coagulation ($P < 0.05$). Indoor PM_{10} only affected allergic reactivity from human primary basophils from cat-allergic individuals. This was explained by varying Fel d 1 concentrations in indoor PM_{10} ($P < 0.001$). Indoor PM_{10}, compared with outdoor PM_{10}, was six times higher and, on an equal weight basis, induced more inflammatory and allergenic reactions, and accelerated blood coagulation. Outdoor PM_{10} had significantly lower effects, but induced detoxifying enzymes. Therefore, preliminary interventions for the reduction of classroom PM_{10} seem reasonable, perhaps through intensified ventilation.

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CLINICAL RELEVANCE

Our investigation shows that school indoor air particulate matter is not devoid of biological effects. Indeed, the effects are stronger for indoor particles than for the concomitantly collected outdoor particles. Thus, more attention should be given to the health effects of school indoor air particulate matter. In the meantime, increased ventilation seems a sensible option.

Keywords: children; particulate matter; inflammation; blood coagulation; allergy

Indoor air of school classrooms contains large amounts of airborne particulate matter (PM_{10}), much greater than outdoor or home air (1–5). In Germany, children spend ~5–6 hours per day inside classrooms, and time multiplied by exposure shows that >60% of PM_{10} exposure in children stems from school indoor air (4). Children therefore experience a major part of their exposure to PM in this specific indoor environment. Because children are developing, they are therefore especially vulnerable to the effects of air pollution (6). Much is known about adverse health effects of outdoor PM_{10}, such as its influence on respiratory, cardiovascular, and allergic diseases (7–9), but the effects of indoor PM_{10} are widely unknown (10). We therefore addressed the question whether school indoor particles constitute a health hazard.

Inflammation, oxidative stress, and blood coagulation are discussed as major mechanisms for PM-induced health effects. Inflammation-associated injury may be a predominant mechanism directing PM-induced cardiopulmonary health effects, including the exacerbation of inflammatory disease and increased hospitalization for lung infections (11–13). Oxidative stress mediated by reactive oxygen species is an important contributor to PM-induced lung inflammation (13). Inflammation promotes the development of a prothrombotic state, leading to enhanced blood coagulation (14). PM-induced proinflammatory mediators can elicit local and systemic inflammations in healthy individuals, and can promote ongoing inflammation in the lungs of patients suffering from chronic obstructive pulmonary disease (15). Furthermore, airway inflammation plays an etiologic role in the pathogenesis of childhood asthma and allergic airway disease (16), and can be associated with PM_{10} concentrations (17).

Adverse health effects of PM depend on the particle size, surface, number, and chemical composition (18–21). Thus, higher PM mass concentration does not necessarily mean a higher health risk. Indoor PM_{10} is chemically very different from outdoor PM_{10}, and is characterized by high concentrations of inhalable organic and silicate particles, reported previously to be biologically more active (and cytotoxic) than outdoor PM_{10} (4, 5, 22).

To get a more detailed picture of the effects of classroom versus outdoor PM_{10}, we investigated, within the project Particulate Matter in Indoor and Ambient Environments, genome-wide gene expression in human lung epithelial cells. The differential indoor/outdoor PM_{10} effects were correlated with particle-bound environmental substances (allergen, endotoxin and polycyclic aromatic hydrocarbons [PAHs]) and then confirmed with functional assays, such as blood coagulation and allergic reactions, to assess whether classroom particles evoke health hazards compared with outdoor particles.

MATERIALS AND METHODS

Sampling

Sampling and recovery of PM is described elsewhere (*see* MATERIALS AND METHODS in the online supplement) (4). Particles were recovered from filters after wetting with 0.0125 ml/cm^2 ethanol and 1.7 ml/cm^2 purified water, and sonicated. The particle suspensions were lyophilized and resuspended in water at 1 mg/ml. Motor vehicle traffic density was obtained from the city development department of the city of Munich (Referat für Stadtplanung).

Cell Culture Conditions

Exponentially growing human immortalized bronchial epithelial BEAS-2B cells were cultured in bronchial epithelial cell basal medium according to the suppliers instructions (Lonza Inc., Walkersville, MD; *see* supplemental MATERIALS AND METHODS) at 37°C, 90% relative humidity, and 5% CO_2.

Genome-Wide Gene Expression Analysis

Affymetrix HG U133A 2.0 GeneChips (Affymetrix, Santa Clara, CA) were used to analyze BEAS-2B cells genome wide after being incubated with 10 μg/ml PM_{10} (school 4, indoor and outdoor) for 4, 10, or 24 hours, all in triplicate (*see* supplemental MATERIALS AND METHODS). The microarray data have been deposited in the Gene Expression Omnibus database (http://www.ncbi.nlm.nih.gov/geo/).

Gene expression values of regulated genes were then validated by quantitative RT-PCR on TaqMan Micro Fluidic Cards (Applied Biosystems, Carlsbad, CA). Using this system, gene expression analysis at 4-, 10-, and 24-hour exposure was extended to all available samples (12 classrooms and 6 outdoor sites, pooled from independent triplicates). cDNA was synthesized with the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). Fold regulation was calculated by the $2^{\Delta\Delta CT}$ method. Glyceraldehyde 3-phosphate dehydrogenase and HPRT were used as housekeeping genes.

Analysis of PM-Adsorbed Substances (Endotoxin, PAH, Cat Allergen)

Endotoxin content of PM_{10} suspensions was determined by a kinetic limulus amoebocyte lysate assay (QCL; Lonza Inc.) (23). We applied three dilutions and spiked replicates of all samples. Results were valid if spike recovery was between 50 and 200%. PAHs were measured using high-performance liquid chromatography with fluorescence detection (24) (for details *see* the online supplement). The presence of the cat, dog, cockroach, rat, house dust mite, and mouse allergens (Fel d 1, Can f 1, Bla g 2, Der p 1, Der f 1, Mite group 2, Rat n 1, and Mus m 1, respectively) in PM_{10} was determined with a multiplex allergen-specific ELISA (Indoor Biotechnologies Inc., Charlottesville, VA).

Blood Coagulation Assay

Human peripheral blood monocytes were isolated from healthy volunteers by density gradient centrifugation. After 24 hours of cell culturing, monocytes were exposed to 1, 5, 10, and 50 μg/ml PM_{10} for another 24 hours. Supernatants were tested in a blood coagulation assay that measures the kinetics of thrombin activity colorimetrically via the substrate H-Sar-Pro-Arg-pNA, HCl (no. 539518; Calbiochem, Boston, MA) (25). The time until the half maximal reaction was assessed and related to untreated controls.

Basophil Activation Test

Basophils in whole blood from birch-, grass-, and cat-allergic volunteers (radio allergen sorbent test class > 3 and positive skin prick test) were tested for activation with Basotest (FK-BAT; Bühlmann, Schönebuch, Switzerland) (*see* also supplemental MATERIALS AND METHODS) (26).

Statistical Analysis

Differences were analyzed with a paired Student's t test unless stated otherwise (27). The strength of the relationship between parameters was expressed by the Pearson coefficient of correlation for Gauss-distributed values (r^2) from linear regression (28). A P value less than 0.05 was considered statistically significant.

RESULTS

Sampling

The results and details from sampling of school indoor and outdoor PM_{10} have been published previously (*see* supplemental MATERIALS AND METHODS) (4, 22). School indoor air contained 117 ± 48 μg/m^3 PM_{10}, whereas concomitantly sampled outdoor air contained 21 ± 15 μg/m^3 (5.6 times less; $P < 0.001$). The characteristics of the schools and time of sampling are given in Table 1. No vermin infestation (rat, cockroach) was detected by their allergen as a proxy. The ubiquitous detection of mouse allergen is probably due to ubiquitous mouse presence in combination with the high sensitivity of the ELISA.

Genome-Wide Regulation of Gene Expression by PM_{10}

PM_{10} exposure in the concentration of 10 μg/ml caused relatively small changes in gene expression in BEAS-2B human lung epithelial cells. For most regulated genes, the highest induction was after 4 hours of incubation and decreased at later time points. A total of 153 genes were significantly over 1.5-fold regulated. The highest regulation observed was the 8.6-fold increase in serine protease inhibitor (SERPIN) B2 and CCL20 gene expression, followed by the 8.5-fold up-regulation of cytochrome P450 (CYP) 1A1 gene expression; 95 genes were induced over 1.5-fold (36 of them over twofold), and 58 genes were inhibited (two of them over twofold). As determined by the online resource, DAVID (Database for Annotation, Visualization, and Integrated Discovery; National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD), gene ontology terms concerning inflammation were significantly enriched. The highest induced inflammatory genes were CCL20, CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, IL1A, IL1B, IL6, IL8, LIF, and PTX3. Other gene functions were represented only by single genes or small groups of genes. Five genes of the metabolism of xenobiotics were induced: CYP1A1, CYP1B1, ALDH1A3, NQO1, and TIPARP. Four genes were induced that play a role in tissue processes: MMP1 (degradation of extracellular matrix [29]), GREM1 (induced in cystic fibrosis [30]), IL24 (wound healing [31]), and SERPINB3 (decrease of barrier function [32]). Single regulated gene functions were blood coagulation (SERPINB2 [33]), response to oxidative stress

TABLE 1. CHARACTERISTICS OF THE ELEMENTARY SCHOOLS

	School 1	School 2	School 3	School 4	School 5	School 6
Sampling date	December 2007	February 2008	March 2008	October 2007	June 2008	July 2007
Distance to nearest road, m	80	75	450	460	435	860
Vehicles/day*	15,700	22,000	9,000	23,000	41,000	21,000
Surrounding land use	Countryside	Inner city	Suburban	Suburban	Suburban	Suburban
Heating fuel source	Community†	Community	Gas	Gas	Gas	Gas
Air conditioning	No	No	No	No	No	No
Allergen/location						
Rat n 1‡, ng/m^3						
Indoor	<§	<	<	<	<	<
Outdoor	<	<	<	<	<	<
Mus m 1, ng/m^3						
Indoor	22.1	27.4	30.2	17.8	19.7	20.5
Outdoor	0.4	2.1	1.4	0.9	10.6	5.0
Feld 1, ng/m^3						
Indoor	42.7	94.2	19.6	34.1	6.8	24.9
Outdoor	<	3.1	1.0	<	<	<
Can f 1, ng/m^3						
Indoor	52.2	18.9	54.3	<	14.4	<
Outdoor	<	<	<	<	<	<
Bla g 2, ng/m^3						
Indoor	<	<	<	<	<	<
Outdoor	<	<	<	<	<	<
Der p 1, ng/m^3						
Indoor	<	<	<	<	<	<
Outdoor	<	<	<	<	<	<
Der f 1, ng/m^3						
Indoor	<	<	<	<	<	<
Outdoor	<	<	<	<	<	<
Der f/p 2, ng/m^3						
Indoor	<	<	<	<	<	<
Outdoor	<	<	<	<	<	<

* On the nearest road.

† From a district heating network, delivered by hot water. All schools had central heating.

‡ Determined by multiplex ELISA (Indoor Biotechnologies) according to the manufacturer's instructions.

< indicates below detection.

(SOD2 [34]), ectoderm development (KRT6B; GO term from UniProtKB-GOA database), inhibition of apoptosis (BIRC3; GOA database), toxin binding (NPTX1), calcium homeostasis (STC1), growth inhibition (STC2), stress response (NDRG1; GOA database), epidermal growth factor (EREG; GOA database), calcium signaling (CAMK2B; GOA database), tight junctions (CLDN1; GOA database). Inflammation-related genes were much stronger induced by indoor PM_{10} than by outdoor PM_{10}. For genes of the xenobiotic metabolism, this was the other way round: especially CYP1A1 was more strongly induced by outdoor PM_{10}.

We selected 36 genes that showed the highest regulation in the genome-wide analysis and two housekeeping genes (glyceraldehyde 3-phosphate dehydrogenase, HPRT1). Eight genes were added that were hypothesized to be influenced by environmental particles. These genes were then tested and analyzed with all indoor and outdoor samples using a Taqman low-density array (*see* MATERIALS AND METHODS) at 4-, 10-, and 24-hour incubation in BEAS-2B epithelial cells. All genes found to be positive on the genome-wide array were confirmed with quantitative PCR (data not shown). As was the case with the genome-wide array, indoor PM_{10} preferentially induced SERPINB2 and inflammatory genes and outdoor PM_{10} genes of the xenobiotic metabolism (*see* Figures 1 and 2).

Analysis of PM_{10}

To understand whether specific components of indoor PM_{10} caused different effects than those of outdoor PM_{10}, targeted analyses were performed. General chemical composition of Bavarian school indoor PM_{10} has previously been published (4, 5), but did not explain the observed effects at the genome level (data not shown). Therefore, further analysis focused on specific organics, such as PAH, endotoxin, and allergens attached to environmental particles.

The following PAHs were detected in indoor and outdoor PM_{10} samples: benzo[a]anthracene, chrysene, benzo[e]pyrene, benzoic[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenzo[a,h]anthracene, benzo[g,h,i]perylene, indeno[1,2,3-c,d] pyrene, and coronene. The sum of these PAHs in indoor PM_{10} was 8.3 ± 4.9 ng/mg and in outdoor PM_{10} 16.7 ± 15.2 ng/mg ($P < 0.01$). Samples taken in winter (schools 1–3) contained significantly more PAH than summer samples (schools 4–6; $P < 0.01$) (Figure 3a). The concentration of indoor PAH per milligram PM_{10} was about half of outdoors. However, because ambient particles indoors are 5.6 times the outdoor concentration per cubic meter, the indoor air concentration of PAH was about double that of the of outdoor, indicating an indoor source of PAH.

For endotoxin, our recoveries were 79 ± 15%. There was also a very good correlation between the 1:50 and 1:250 dilutions of the same samples ($y = 0.86x$; $r^2 = 0.97$). Endotoxin concentrations were considerably higher in classroom samples (128.5 ± 42.2 EU/mg PM_{10}) than in outdoor samples (13.4 ± 21.5 EU/mg PM_{10}; $P < 0.001$). A difference between winter and summer samples in endotoxin content was not found (Figure 3b). The high outdoor endotoxin concentration at school 6 was not explained by deviating land use compared with the other schools.

Correlation of Gene Expression with Endotoxin and PAH

For each of the 18 samples (12 indoor, 6 outdoor), the content of PAH and that of endotoxin was compared with its ability to regulate the expression of each of the 46 analyzed genes. Significant correlations were found for the induction of inflammatory genes

Figure 1. Quantitative PCR confirmed data of only those genes with the highest genome-wide particulate matter 10 less than or equal to 10 μm in diameter (PM_{10})–induced expression; relative expression ($2^{-\Delta\Delta CT}$) related to untreated control; housekeeping genes, glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and HPRT (means of 18 PM_{10} samples). Darker shades of *red* indicate higher induction.

and endotoxin ($r^2 = 0.69$ for CXCL6; $P < 0.001$) content and the induction of xenobiotic metabolizing genes and PAH content ($r^2 = 0.75$ for CYP1A1; $P < 0.001$) (Figure 4). Thus, the inflammatory effects of indoor PM_{10} can be at least partly ascribed to the microbiological burden of these samples. On the other hand, the induction of the xenobiotic metabolism seems to be dependent on PAH, attached to a greater extent to outdoor particles than indoor particles. Selective inhibition of Toll-like receptors to inhibit the effects of LPS or selective extraction of PAH might be a possible way to further substantiate our findings.

PM_{10} Accelerated Blood Coagulation

Indoor and outdoor PM_{10} caused an acceleration of blood coagulation in a functional assay (Figure 5). After exposure of human peripheral blood monocytes to indoor PM_{10}, the acceleration was significant already at 5 μg/ml ($P < 0.005$ in Newman-Keuls test), whereas outdoor PM_{10} caused a significant acceleration only at 10 times higher concentrations (50 μg/ml, $P < 0.05$). The regression of the curves of indoor and outdoor PM_{10} was statistically different ($P < 0.05$), with a more rapid coagulation for indoor PM_{10}.

PM_{10} Induced Activation of Human Basophil Granulocytes

Using fluorescence-activated cell sorting (FACS), activation of human primary basophils could be tested within the complex mixture of all human leukocytes without previous stressful purification of these cells. When basophils are activated, they increase the amount of CD63 on their surfaces, as described previously (35, 36). Incubation of human basophils of birch and grass pollen–allergic individuals with their respective allergen caused an activation of basophils (positive control reaction). Neither classroom nor outdoor PM_{10} enhanced the number of activated basophils (*see* Figure 6a, n.s.).

Using basophils of six cat-allergic individuals in the same setup but without addition of any further allergen, we could show basophil activation by indoor, but not outdoor PM, already without allergen coincubation. The sample tested (school 1, classroom 2) with all donors was among the lowest Fel d 1 concentrations of all schools. Still, basophil activation was clear ($P < 0.05$; Figure 6b). ELISA measurements of Fel d 1 confirmed that the applied PM_{10} sample (school 1, classroom 2) contained cat allergen. Altogether, in 6 out of 12 tested classroom PM_{10} samples and 1 out of 6 outdoor PM_{10} samples, Fel d 1 could be detected by ELISA (data not shown). On average, Fel d 1 was present in indoor classroom PM_{10} at 1.06 ± 1.81 ng/mg. In outdoor PM_{10}, 0.04 ± 0.10 ng Fel d 1/mg ($P < 0.001$) was found, which is close to the limit of detection of the assay. Thus, indoor air contained immunologically relevant concentrations of the major cat allergen, Fel d 1.

Testing all indoor PM_{10} samples with basophils of a cat-only–allergic individual showed that CD63 up-regulation was parallel

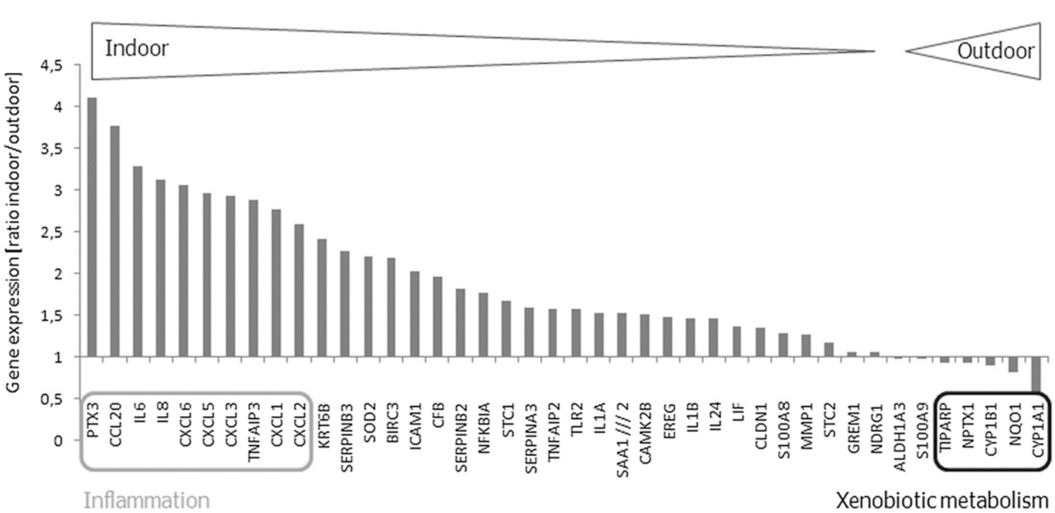

Figure 2. Specificity of indoor and outdoor PM_{10}-induced gene expression; ratio of regulation by indoor over outdoor PM_{10} (average of time points).

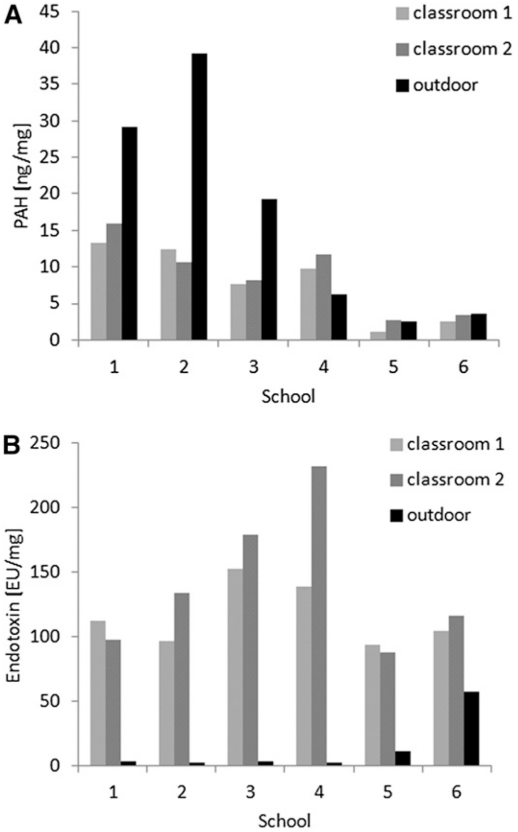

Figure 3. Analysis of particle-adsorbed substances in the PM_{10} suspensions. (*A*) Polycyclic aromatic hydrocarbons (PAH); concentration of Baa, benzo[a]anthracene; Bap, benzo[a]pyrene; Bep, benzo[e]pyrene; Bgh, benzo[g,h,i]perylene; Bkf, benzo[k]fluoranthene; Bpf, benzoic[b]fluoranthene; Cor, coronene; Cry, chrysene; Dba, dibenzo[a,h]anthracene; Ind, indeno[1,2,3-c,d]pyrene. (*B*) Endotoxin.

to the measured Fel d 1 ($r^2 = 0.596$; $P < 0.001$). Some samples showed basophil degranulation in the absence of Fel d 1, which we suspect to be due to the higher sensitivity of the bioassay over ELISA (data not shown).

DISCUSSION

On a genome-wide level, we compared the effects of PM_{10} from one elementary school classroom with those of PM_{10} from concomitantly sampled outdoor air. The 48 most regulated genes were selected and confirmed with all available samples with quantitative PCR. Gene expression was correlated with PM_{10}-adsorbed environmental substances to explain the observed effects. Expression analysis indicated up-regulation of genes involved in blood coagulation, which we confirmed with a functional blood coagulation assay with supernatants from PM_{10}-exposed primary human monocytes. Furthermore, the influence on the elicitation phase of allergic reaction was tested by a basophil activation assay (bioassay), and the allergen that caused positive test results was identified by ELISA.

In all tests, equal amounts of classroom PM_{10} had stronger and different effects compared with outdoor PM_{10} (4). This is in addition to the six-times higher indoor PM_{10} concentrations compared with outdoor PM_{10}. Indoor PM_{10} induced inflammatory genes much more strongly, blood coagulation was more accelerated, and basophils were activated only by indoor PM_{10}. The only effect of outdoor PM_{10} was the induction of genes of the xenobiotic metabolism that are regulated by the aryl hydrocarbon (Ah) receptor (37, 38) and which correlated with the measured PAH content. Indeed, not only CYP1A1, but also other genes under control of the Ah receptor (CYP1B1, NQO1, ALDH1A3), were induced, corroborating our findings. A correlation of CYP1A1 induction and diesel exhaust particles or organic PM constituents was found in many previous studies (39–42). Elemental carbon particles with absorbed PAH stemming from anthropogenic combustion, such as diesel particles, constitute ~8% of outdoor PM_{10}, and are a likely source of the observed effects (43).

The inflammatory effect of PM has been shown in many studies (39, 44–47), and was found to be correlated with endotoxin content (1). The much higher inflammatory effects of indoor PM_{10} compared with outdoor PM_{10} is of special importance, as inflammation-associated injury may be a predominant mechanism directing PM-induced cardiopulmonary health effects, including the exacerbation of inflammatory disease and increased hospitalization for lung infections (11–13), promotion of chronic obstructive pulmonary disease (15), and the pathogenesis of childhood asthma and allergic airway disease (16).

The acceleration of blood coagulation after exposure of monocytes to PM_{10} is in agreement with studies that showed that PM exposure alters hemostasis by promoting clot formation and impeding clot resolution (14, 48–50). PM-induced blood coagulation is often associated with an increase of the plasminogen activator (PA) inhibitor (PAI)-1 in plasma and lung tissue (14, 48, 49). PAI-1 is the major regulator of fibrinolysis (51), and is a member of the SERPIN family. This protein family also contains PAI-2 (SERPINB2), which was the highest induced gene in our study. SERPINB2 was first discovered in the placenta of pregnant women, and is today known to be acutely induced at sites of inflammation or infection (33). In addition to its regulatory role in fibrinolysis, it also has intracellular functions, including immune modulation (suppression of T helper type 1 immunity) (52). Its extracellular activity as PA inhibitor is lower than that of PAI-1 (52), but at sites of inflammation it seems to be the primary PAI (33). The fact that gene expression of SERPINB2 is inducible, on the one hand, by Ah receptor ligation (53, 54), and, on the other hand, by endotoxins (55) might explain why, in our experiments, it was highly induced by both PAH containing outdoor PM_{10} and endotoxin containing indoor PM_{10} (*see* Figures 1 and 4). Thus, in future studies, gene expression of SERPINB2 could be used as a marker for blood coagulation, accelerated by various components of environmental PM.

Organic extracts of urban aerosol particles were reported to influence IgE-mediated allergic diseases by enhancing the allergen-induced activation of human basophils (56). Such adjuvant effect could not be detected with our school indoor and outdoor PM samples. This could be due to the use of organic extracts of outdoor PM, higher in organic compounds in the literature, whereas this study used the PM resuspended in water, with consequently lower amounts of the lipophilic PAH. Alternatively, allergy-inducing effects were reported for diesel exhaust particles (57), which could have been low in our school samples compared with other studies (56). Carbon black, as a marker for combustion particles, was not measured. Although we could show no aggravating effect of school PM on allergic reactions, the assay did function as bioassay for Fel d 1, the major cat allergen, as some classroom PM samples induced basophil activation without additional allergen exposure (*see* Figures 6A and 6B). This activation was only found in cat-allergic individuals, and only with PM samples proven by ELISA to contain the major cat allergen, Fel d 1. Both ELISA and the basophil test were positive, the basophil assay being more sensitive (data not shown). Other studies detected Fel d 1 in settled school dust, but dust is not necessarily inhalable

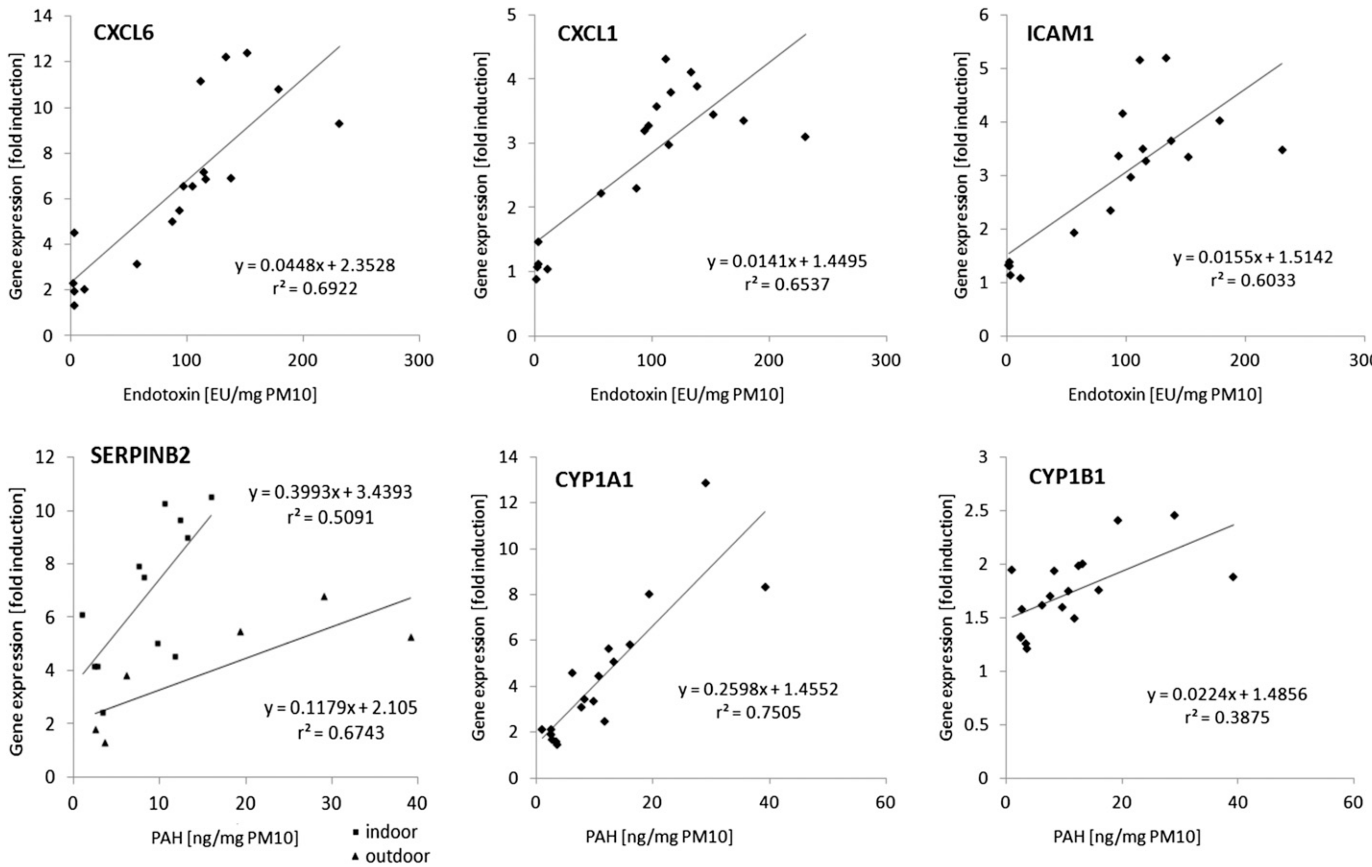

Figure 4. Correlation of gene expression with endotoxin (*upper panel*, 4 h) and PAH (*lower panel*, 24 h) content of the 18 PM_{10} samples. In each case, the three genes with the highest coefficient of determination (r^2) for correlation are shown. Symbols represent outdoor (*triangles*), indoor (*squares*), or both (*diamonds*).

(58, 59). We found Fel d 1 in airborne and the inhalable fraction of ambient air (PM_{10}) of classrooms, but not in outdoor air, at concentrations that were able to activate human basophils, and thus could provoke allergic reactions. In Germany, animals are not allowed in schools, and the allergen must stem from cat skin flakes or hairs—the source of Fel d 1 (60)—brought in. Fel d 1 is a stable protein, and survives longer periods in the environment (61).

On an equal weight basis, indoor PM_{10} induced more inflammatory genes, SERPINB2, a shorter blood coagulation time, and allergic reactions compared with outdoor PM_{10}. Indoor PM_{10} is, in addition, six-times higher than outdoor PM_{10}, and, on an equal respirated air volume (which is the case in humans), the reported differences will be even more dramatic.

CONCLUSIONS

We have shown that school indoor PM during teaching hours, on an equal weight basis, is different from outdoor PM in particle-adsorbed environmental substances, with a lower PAH and

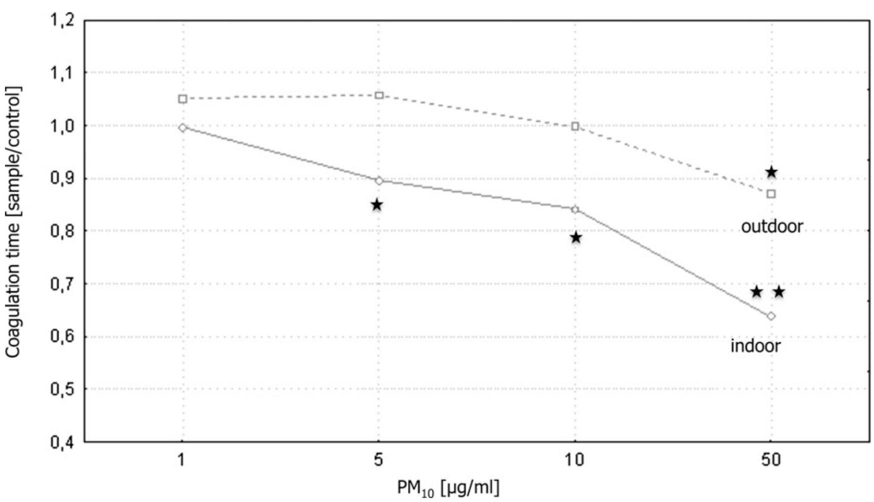

Figure 5. Blood coagulation time after exposure to indoor and outdoor PM_{10} (mean results of 12 indoor and 6 outdoor samples). Both curves were analyzed by repeated-measures ANOVA and *post hoc* tests according to Newman-Keuls: *$P < 0.05$, **$P < 0.01$ against zero effect for the single concentrations. Moreover, curves were compared with each other by ANOVA with the result: $P < 0.001$ for difference in mean level and $P < 0.05$ for a nonparallel course.

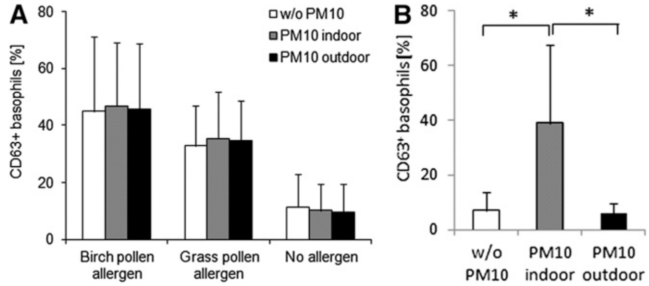

Figure 6. Activation of human primary basophils by indoor/outdoor PM_{10} (school 1). (*A*) Basophils from four birch and grass pollen–allergic individuals. (*B*) Basophils from six cat-monosensitized allergic individuals. Means $\pm$ SD are presented. $^{*}P < 0.05$.

a higher endotoxin content. Human lung epithelial cells especially reacted to indoor particles by expressing genes involved in inflammation, blood coagulation, and xenobiotica metabolism, among others. Endotoxins could partly explain the inflammatory effects, and PAH could partly explain the CYP1A1 induction. Blood coagulation was more strongly induced by indoor PM_{10}. In addition, the major cat allergen, Fel d 1, was demonstrated in the respirable fraction at concentrations that induced basophil degranulation. Our results show that indoor air PM_{10} contains a different biological activity than outdoor air PM_{10}, and that the chemical composition of environmental PM should always be taken into consideration when its health effects are discussed.

These *in vitro* data are only suited to describing a biological activity of classroom PM_{10} and to comparing it with that of outdoor PM_{10} as a known risk factor. The health effects of outdoor air are well known, and this study shows that indoor air could be even more biologically active.

Whether these hazards result in health effects needs to be confirmed in the children themselves. As a first practical implication, we suggest improving ventilation with outdoor air in schools, because outdoor PM_{10} is less concentrated and less biologically active than indoor PM_{10}.

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