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Repeated exposure of bronchial epithelial cells to particular matter increases allergen-induced cytokine release and permeability

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ABSTRACT

Long term particulate matter (PM) exposure has been associated with an increased incidence of respiratory diseases. Here, an *in vitro* model was developed to study how long term diesel exhaust particle (DEP) exposure might predispose to the development of allergic reactions. Airway epithelial (16HBE) cells were exposed to low concentrations of diesel exhaust particle (DEP) for 4 days after which they were challenged with house dust mite (HDM) extract (24 h). Compared to acute exposure (24 h), 4 days DEP exposure to 16HBE cells further reduced the transepithelial electrical resistance (TEER) and increased CXCL-8 release. DEP pre-exposure aggravated HDM-induced loss of TEER, increased tracer flux across the barrier and reduced CLDN-3 expression in these 16HBE cells. HDM-induced cytokine (IL-6, CCL-22, IL-10 and CXCL-8) release was significantly increased after DEP pre-exposure. In the current study an *in vitro* model with long term PM exposure was presented, which might be helpful for further understanding the interplay between long term PM exposure and allergic responses.

1. Introduction

According to the World Health Organization (WHO) estimation in 2016, more than 339 million individuals were globally affected by asthma. The global prevalence of asthma in children and adults has increased rapidly during the last decades [2,5]. Long-term air pollution exposure is one of the leading causes for the development and aggravation of many respiratory diseases, like allergic asthma [33,34] and chronic obstructive pulmonary disease (COPD) [19]. Chronic exposure to air pollutants leads to the development of inflammatory reactions to allergens and clinical manifestations of allergic diseases [13,79]. In line with human studies, several animal studies also demonstrate that exposure to diesel exhaust particles and co-exposure with an allergen result in inflammatory responses in the lung as observed by increased key characteristics of allergic sensitization and asthma [30,35,40,58,60,69]. Air pollution represents a variable and complex mixture of gases and particles, including ozone (O3), nitrogen dioxide (NO_2), and particulate matter (PM) [31]. PM, especially particles with a diameter smaller than 2.5 μm (PM2.5), penetrate deeply into the respiratory system, reaching the small airways and alveoli, which results into blood-air barrier disruption [62,76].

The structural and functional alteration of airway epithelium is a key player in the pathogenesis of major lung diseases, including asthma [17,26]. An important driving force for airway remodeling is the existence of chronic inflammation as the result of continuous exposure to pollutants [24]. Since airway epithelial cells are the first and major site of deposition of PM in the lung, it is important to study how long term PM exposure might impact sensitization to potential allergens or the exacerbation and severity of allergic responses [15].

During the last decades, animal models have been used extensively to understand the etiology of lung diseases. However, Replacement, Refinement and Reduction alternatives to avoid or replace the use of experimental animals are high on the political agenda. To get a better understanding of the molecular pathogenesis of lung diseases, new and appropriate *in vitro* human airway models have to be developed [47]. Investigating the mechanistic effects of PM exposure on the development of airway diseases, such as asthma and allergy, requires an airway epithelial cell culture model demonstrating as closely as possible the (*in*

Abbreviations: PM, particulate matter; HDM, house dust mite; DEP, diesel exhaust particle.

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vivo) characteristics of human airway epithelium (first line defense).

Therefore, this study aimed to develop and implement an *in vitro* model of PM exposure using human bronchial airway epithelial cells mimicking more closely real-life long term exposure conditions (4 days) and studying the integrity of the epithelial barrier and cytokine release. To mimic an allergic respiratory challenge, long term PM-exposed bronchial epithelial cells were challenged with HDM, which is one of the most common respiratory allergens, and the effect on epithelial barrier function and the inflammatory response was measured.

2. Methods

2.1. Diesel exhaust particles (DEP) preparation and size examination

Diesel clay loam was obtained from Sigma, USA. Stock suspension of 10 mg/ml of particles was prepared in PBS. Subsequently, particles were vortexed for 3 min and sonicated at 50–60 Hz for 30 min to disperse the particles [14,72]. Particle distribution was measured using dynamic light scattering using the PSS AccuSizer 708 APS (Soliton, Germany) and showed 95% of the particles at a size below 2.5 μm (Fig S1).

2.2. Cell culture

The SV40-transformed and immortalized human bronchial airway epithelial cell line 16HBE14o- (16HBE) was kindly provided by the University Medical Center Utrecht (Utrecht, The Netherlands). Cells were maintained and passaged in Minimum Essential Medium Eagle (MEM) supplemented with 10% (v/v) inactivated fetal calf serum (FCS) (Gibco) and penicillin (100 U/ml) and streptomycin (100 μg/ml) (Biocambrex) at 37 °C in a humidified atmosphere at 5% CO_2. Sub-confluent cells were passaged using 0.05% trypsin (Gibco, Thermo Fisher Scientific, Wilmington, DE, USA) and 0.54 mM ethylene diamine tetra acetic acid (EDTA).

2.3. Cell viability assay

Cytotoxicity induced by DEP exposure at 24 h, 48 h, 72 h and 96 h was measured using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. In short, cells were seeded in a 48-well plate and were exposed to DEP. At the end of each timepoint, MTT (5 mg/ml) was added to the wells and incubated for 3 h. Thereafter, medium containing MTT was removed and cells were lysed in 200 μl DMSO. Optical density at 595 nm was measured using the ELISA reader GloMax (Promega, USA).

2.4. 16HBE cell monolayer on transwell insert

All *in vitro* experiments were performed with 16HBE cells grown on 0.3 cm^2 high-pore density transwell membrane inserts with 0.4 μm pores (Falcon, BD Biosciences, Franklin Lakes, NJ, USA) placed in a 24-well plate. Cells were seeded at a density of $0.4*10^5$ cells per insert and incubated for 11–14 days to achieve a confluent monolayer with a mean transepithelial electrical resistance (TEER) exceeding 350 $\Omega.cm^2$. Confluent 16HBE cell monolayers were apically exposed to different DEP concentrations (12.5, 25, 50, 100, 200, 400 and 800 μg/ml) for 24–96 h with and without a subsequent challenge with HDM (50 μg/ml) Greer Laboratories, Lenoir, USA) or vehicle (PBS) for 24 h. The HDM concentration used in the current study is in the same range as described in previous *in vitro* studies [50]. In addition, the concentration range of DEP is in agreement with other *in vitro* studies where airway epithelial cells were exposed to DEP particles in the range of 2.5–2500 μg/ml [51,71]. Prior to these experiments concentration–response curves of HDM were obtained and TEER, paracellular tracer flux and CXCL-8 were measured (Fig S2).

2.5. Trans epithelial electrical resistance (TEER)

To evaluate the integrity of the epithelial barrier, TEER values of the confluent 16HBE cell monolayer were measured using a Millicell-ERS volt-ohm meter system (Millipore, Temecula, CA, USA) at 24, 48, 72, 96 and 120 h after DEP exposure (Fig. 1).

2.6. Paracellular tracer flux assay

Paracellular permeability across the 16HBE cell monolayer was determined by measuring the lucifer yellow (LY, molecular mass of 0.457 kDa, Sigma chemical Co., St Luis, MO, USA) flux from the apical compartment to the basolateral compartment. At the end of the experiment, the membrane-impermeable molecule, LY (20 μg/ml), was added to the apical compartment of the transwell inserts for 4 h and the paracellular flux was determined by measuring fluorescence intensity of medium collected from the basolateral compartment (transferred to a 96 well plate) with the fluorometer at excitation and emission wavelengths of 410 nm and 520 nm (Fluoroskan Ascent FL; Thermo Labsystems, Waltham, MA, USA).

2.7. Western blot analysis

The 16HBE cells were rinsed with ice-cold PBS and lysed using 50 μl RIPA lysis buffer (Thermo Scientific, Rockford, IL, USA) containing protease inhibitor (Roche Applied Science, Penzberg, Germany). Total protein concentration was measured using a BCA protein assay kit (Thermo Scientific, Rockford, IL, USA). An equal amount of protein was separated by electrophoresis (Criterion™ Gel, 4–20% Tris-HCl, Bio-Rad Laboratories, Hercules, CA, USA) and transferred onto Trans-Blot Turbo Midi PVDF Transfer Packs (Bio-Rad Laboratories, Hercules, CA, USA). Membranes were blocked using PBS supplemented with 0.05% Tween-20 (PBST) and 5% milk proteins (Protifar, Nutricia, The Netherlands). Thereafter, membranes were incubated with the primary antibodies for zonula occludens-1 (ZO-1), claudin 3 (CLDN-3) (402200 and 341700, Invitrogen, Carlsbad, CA, USA) overnight at 4°C, followed by incubation with Goat Anti-Rabbit Immunoglobulins/HRP secondary antibody (Dako, Agilent, USA). Blots were washed and developed with ECL reagents mix (Amersham Biosciences, Roosendaal, The Netherlands) and the digital images were obtained using ChemiDoc™ XRS+ System (Bio-Rad Laboratories, Hercules, CA, USA). The intensity of the bands was quantified with Image Lab software (version 5.2, 2014, Bio-Rad Laboratories, Hercules, CA, USA) and normalized to anti-human β-actin (Cell Signaling, Danvers, MA, USA).

2.8. Immunofluorescence staining

To localize ZO-1 protein expression, an immunofluorescence staining was conducted. The 16HBE cells were washed with ice-cold PBS, fixed in 10% formalin and permeabilized with PBS containing 0.1% (v/v) Triton X-100 for 5 min. Following 3 times washing with PBS, cells were incubated with 5% serum in PBS containing 1% (w/v) bovine serum albumin (BSA) for 1 h at room temperature. Subsequently, cells were incubated with primary antibody against ZO-1 (402200, Invitrogen, Carlsbad, CA, USA) for 2 h at room temperature. Thereafter, cells were washed and incubated with Alexa Fluor 488-conjugated anti-rabbit IgG (Invitrogen, Carlsbad, CA, USA at 1:50 in PBS with 1% BSA) for 1 h at room temperature. After washing, nuclear counterstaining was conducted using Hoechst (1:3000, Invitrogen) and inserts were washed and mounted with ProLong Gold anti-fade reagent (Invitrogen). Immunofluorescence images were taken with Microscope Leica TCS SP8 X.

2.9. Cytokine and chemokine measurement

CXCL-8 and IL-6 concentrations were measured in the 16HBE culture supernatants collected from the apical compartment of the transwell

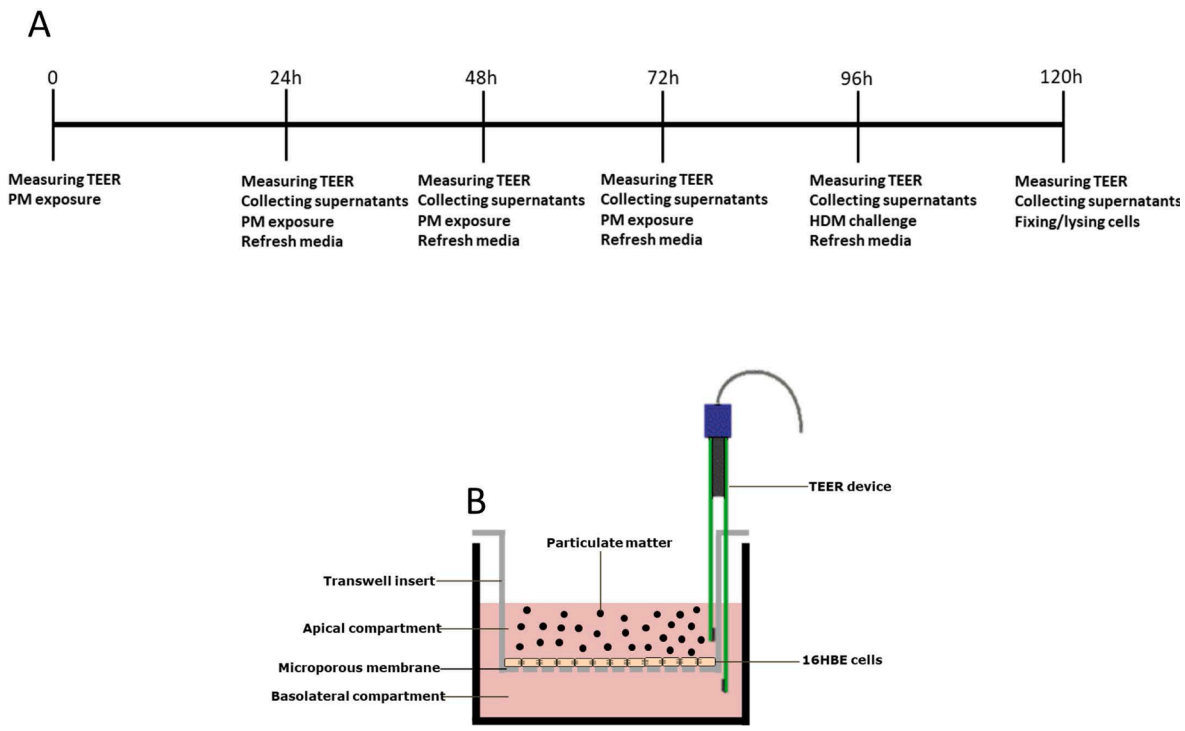

Fig. 1. Time schedule of *in vitro* DEP exposure model. 16HBE cells were exposed for 96 h to DEP. At each time point (24 h, 48 h, 74 h and 96 h) TEER was measured, medium was refreshed and cells were exposed to fresh DEP/medium. After 96 h exposure to DEP, cells were exposed for an additional 24 h to HDM (50 μg/ml) or medium.

inserts after exposure to different DEP concentrations using human CXCL-8 DuoSet ELISA kit (R&D Systems Europe, Abingdon, UK) and IL-6 ELISA kit (Invitrogen, San Diego, CA, USA). Moreover, the IL-6, CCL-22, IL-10, CXCL-8, GM-CSF and CCL-20 release into the apical compartment by 16HBE cells after DEP, HDM and combined exposures was determined by IL-6 ELISA kit (Invitrogen, San Diego, CA, USA), CCL-22 ELISA kit (R&D Systems Europe, Abingdon, UK), IL-10 ELISA kit (R&D Systems Europe, Abingdon, UK), CXCL-8 DuoSet ELISA kit (R&D Systems Europe, Abingdon, UK) GM-CSF ELISA kit (R&D Systems Europe, Abingdon, UK) and CCL-20 ELISA kit (R&D Systems Europe, Abingdon, UK) according to the manufacturer's guidelines. The detection limits of the assay were as follows: IL-6, 3.1 pg/ml; CCL-22, 7.81 pg/ml; IL-10, 31.2 pg/ml; CXCL-8, 31.2 pg/ml; GM-CSF, 15.6 pg/ml and CCL-20, 15.6 pg/ml.

2.10. Statistical analysis

All experiments were performed in triplicates (three independent biological experiments), and each replicate included three technical replicates (3 wells/plate). Data analysis was performed using GraphPad Prism (version 7.04, 2017, GraphPad, La Jolla, CA, USA). Experimental results are expressed as mean ± SEM and differences between experimental groups were assessed by using One-way or Two-way analysis of variance (ANOVA) followed by Bonferroni's post-hoc testing for multiple comparisons. For all statistical analysis, P-values lower than 0.05 were considered statistically significant.

3. Results

3.1. Long term DEP exposure disrupts bronchial epithelial barrier integrity

To mimic long term exposure *in vitro* (Fig. 1), 16HBE cells were allowed to reach confluence and TEER values of at least 350 Ω.cm2. On day 0 the supernatant was collected, the cells were washed and received fresh medium containing increasing concentrations of DEP. After day 1 (24 h), medium was collected, the cells were washed and received fresh medium with increasing concentrations of DEP. This procedure was repeated on day 2 (48 h) and 3 (72 h) (See Fig. 1). On day 4 (96 h), medium was collected, the cells were washed and lysed/fixed for further analysis. TEER values were measured before each change of culture medium. At the end of the experiment, cells were either fixed or lysed to determine the effect DEP exposure on tight-junction protein expression. 16HBE cell were exposed to increasing concentrations of DEP and cell viability was assessed over a period of 4 days (Fig. 2a).

DEP concentrations below 200 μg/ml did not show a reduction in cell viability in contrast to DEP concentrations of 200–800 μg/ml which showed a reduction in cell viability already after 48 h. The impact of DEP exposure on barrier integrity was assessed in a similar way and indicated that for all concentrations of DEP, TEER values showed a concentration-dependent decrease over time (Fig. 2B). To visualize the consequence of loss of barrier integrity, cells were apically exposed to the membrane impermeable lucifer yellow (LY, 0.457kDA) and the LY flux to the basolateral compartments was assessed (Fig. 2c). Although loss of TEER was observed at DEP concentrations of 12.5 and 25 μg/ml, no significant LY flux to the basal compartment was observed. However, DEP concentrations ≥ 50 significantly increased LY flux.

Reductions of 16HBE TEER values and the corresponding increased flux of LY are indicators for a loss of barrier function. To evaluate the effect on tight junction proteins, the expression and localization of the tight junction protein zonula occludens-1 (ZO-1) was measured. ZO-1 protein expression did not significantly change after 96 h exposure to different concentrations of DEP (Fig. 2D). However, the immunofluorescence ZO-1 staining showed a clear concentration-dependent loss of ZO-1-stained epithelial borders, indicative of ZO-1 redistribution and loss of tight junction formation (Fig. 2E).

3.2. Long term DEP exposure induces cytokine production by 16HBE cells

To evaluate the effect of long term DEP exposure on the inflammatory response16HBE cells were seeded on transwells and exposed for 4

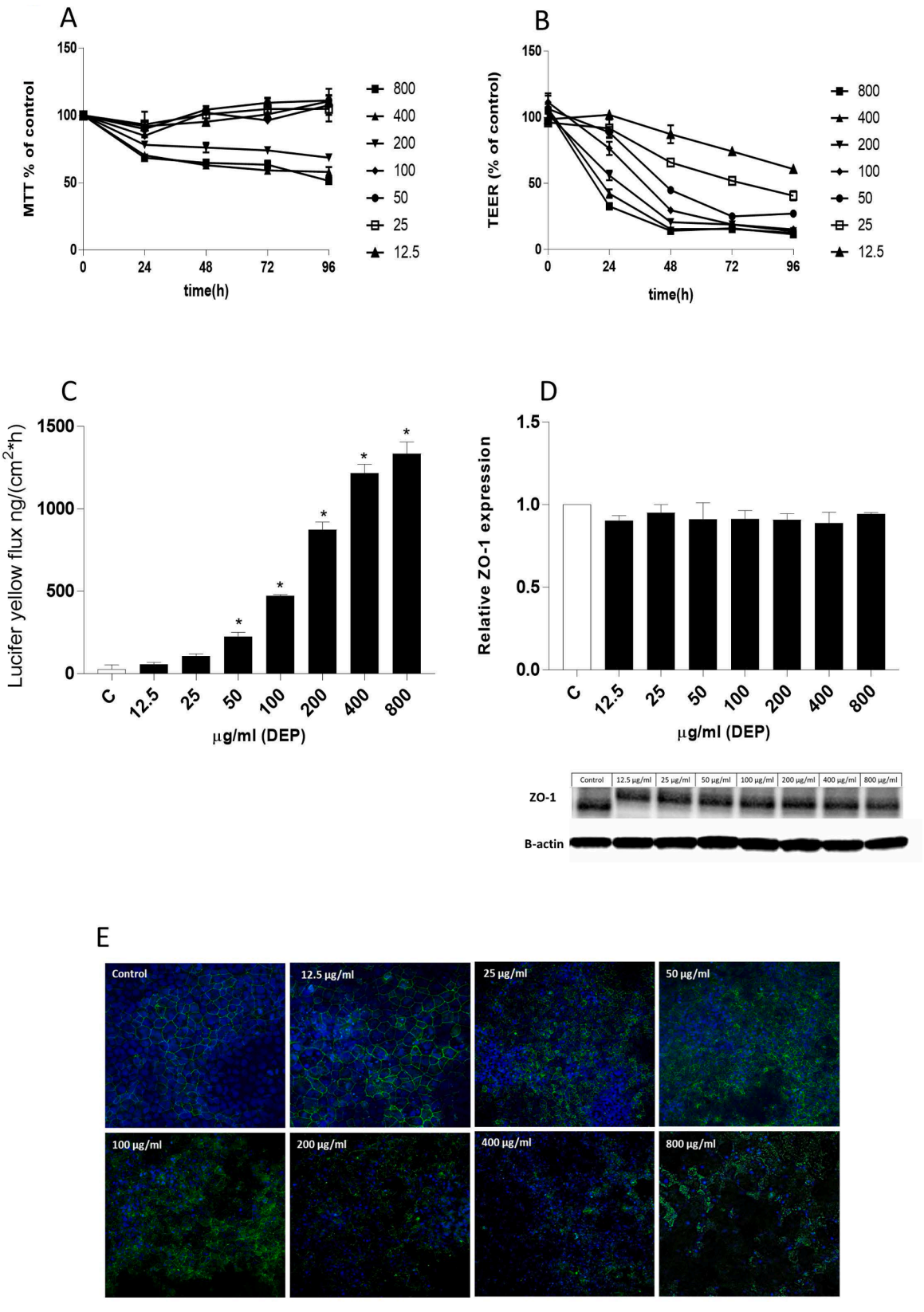

Fig. 2. Long term DEP exposure disrupts bronchial epithelial barrier integrity. Cell viability (MTT) (A) and TEER values (B) were measured. The LY translocation, ZO-1 protein analysis (D) and IF staining (E) were investigated at 96 h (C). Data are presented as means ± SEM (N = 3), *P < 0.05, significantly different versus control (C). Three independent experiments (each performed in triplicate).

days according to the scheme presented in Fig. 1. IL-6 and CXCL-8 release were measured in the apical compartment of transwell inserts at 24 h and 96 h. As shown in Fig. 3A, 24 h exposure to increasing concentrations of DEP (100–400 μg/ml) significantly increased IL-6 release. 96 h exposure to increasing concentrations of DEP (except 12.5 μg/ml) induced a significant increase in IL-6 release (Fig. 3B). At 24 h exposure, 200 and 400 μg/ml DEP induced a significant CXCL-8 release (Fig. 3C). At 96 h exposure, increasing concentrations of DEP (50–400 μg/ml) induced a more pronounced increase in CXCL-8 compared to 24 h DEP exposure (Fig. 3D). Moreover, 16HBE cells exhibited increased basal production of CXCL-8 and IL-6 after 96 h culture compared to 24 h (Fig S3).

3.3. Effect DEP pre-exposure aggravates HDM-induced loss of barrier integrity

Next, the long term DEP exposure model (4 days DEP exposure) was used to study the effect of 16HBE exposure to an extract of HDM, a well-known aeroallergen for causing allergic airway diseases. 16HBE cells were exposed according to the above described method for 96 h, after which the cells were washed and challenged for a subsequent 24 h to HDM. Only those concentrations of DEP were tested that did not induce cell cytotoxicity. Exposure to HDM itself significantly reduced TEER values compared to control cells (Fig. 4A). Pre-exposure to DEP (12.5 and 25 μg/ml) aggravated the HDM-induced loss of TEER compared to HDM or DEP alone. This additional effect was not observed at higher DEP concentrations.

Exposure of 16HBE cells to HDM also induced an increased flux of LY to the basolateral compartment (Fig. 4B). Pre-exposure to DEP at 12.5 and 25 μg/ml additionally increased HDM-induced LY flux, but this was only significant for 12.5 μg/ml DEP. Higher concentrations of DEP did not show this additive effect. To investigate whether the loss of barrier integrity was related to changes in tight junction protein expression, ZO-1 and CLDN-3 protein expression was measured in these 16HBE cells. ZO-1 expression was not changed following DEP exposure, however, HDM exposure tended to increase the ZO-protein expression (Fig. 4C). DEP pre-exposure seemed to further increase the HDM-induced ZO-1 expression, however, this did not reach significance. Moreover, there was no difference observed between ZO-1 expression levels related to different DEP concentrations. While HDM seemed to increase ZO-1 expression, CLDN-3 expression tended to reduce after HDM incubation. Exposure to DEP did not show a significant difference in CLDN-3 expression compared to control. However, pre-incubation with DEP (12.5, 25 and 50 μg/ml) in combination with HDM exposure significantly reduced CLDN-3 expression compared to DEP alone.

3.4. DEP pre-exposure aggravates HDM-induced cytokine release by bronchial epithelial cells

To investigate the effects of DEP pre-exposure on HDM-induced

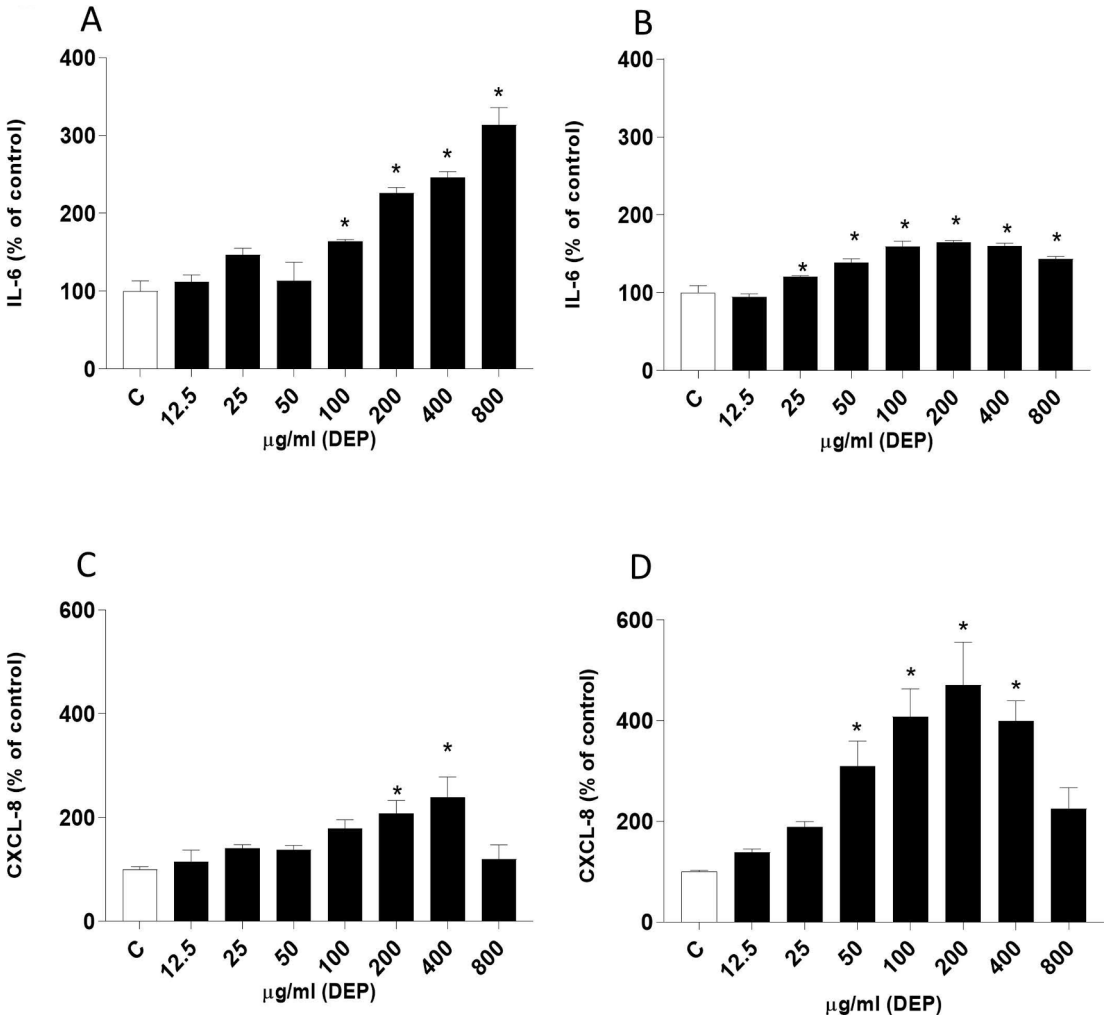

Fig. 3. Long term DEP exposure induces cytokine production by 16HBE cells. IL-6 and CXCL-8 secretion into the apical compartment was measured at 24 h (A,C) and 96 h (B,D). Results are presented as mean ± SEM (N = 3), *P < 0.05; significantly different versus control. Three independent experiments (each performed in triplicate).

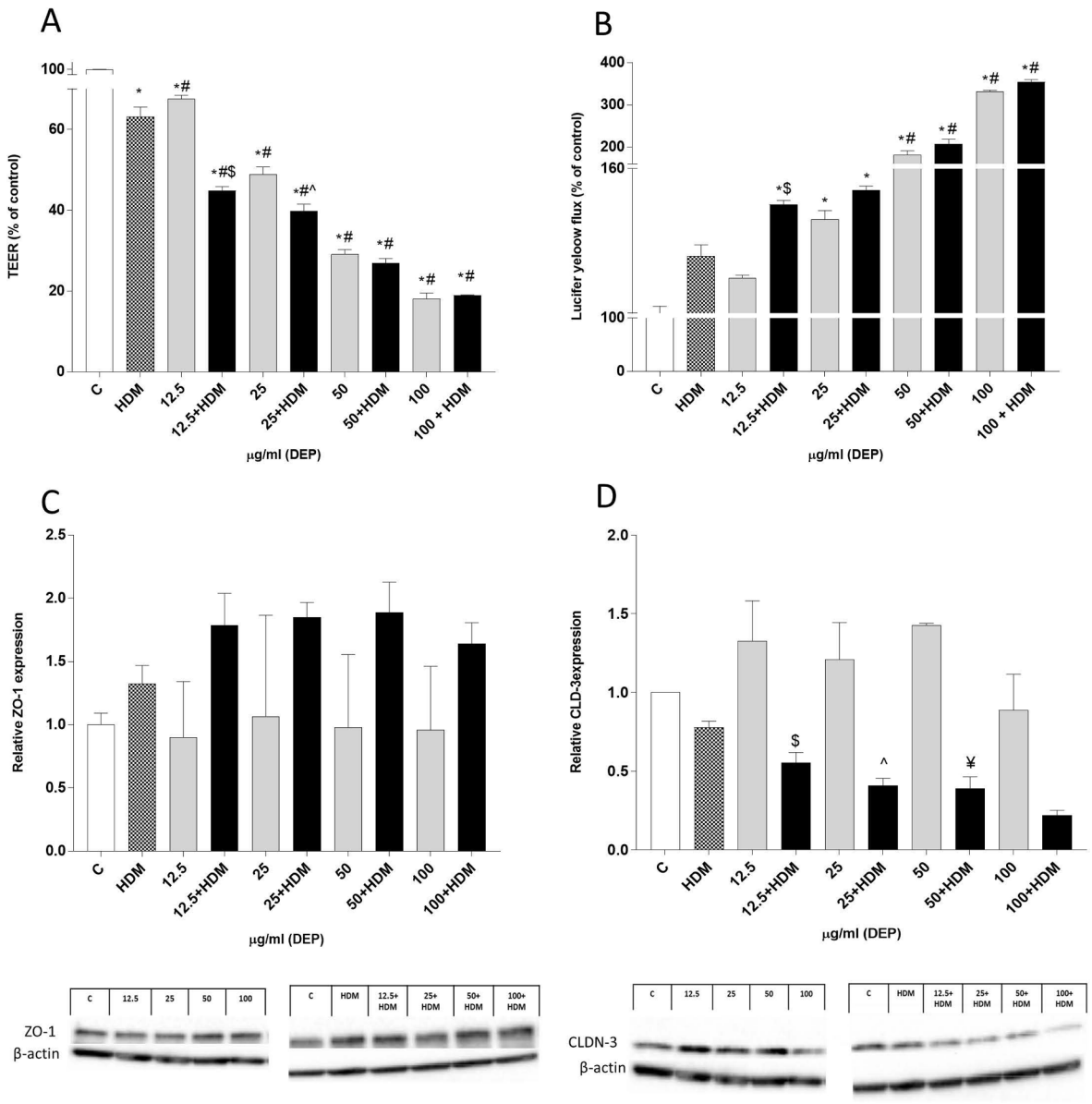

Fig. 4. DEP pre-exposure aggravates HDM-induced loss of barrier integrity. TEER (A), LY translocation (B), relative ZO-1 (C) and CLDN-3 protein expression (D) based on Western Blot analysis (optical density normalized with β-Actin). Data are presented as mean ± SEM (N = 3), *P < 0.05; significantly different versus control, #P < 0.05; versus HDM (50 µg/ml), $P < 0.05; versus DEP (12.5 µg/ml), P < 0.05; versus DEP (25 µg/ml), ¥P < 0.05; versus DEP (50 µg/ml). Three independent experiments (each performed in triplicate).

cytokine and chemokine release, IL-6, CCL-22, IL-10, CXCL-8, GM-CSF and CCL-20 concentrations in the apical compartment (Fig. 5 A-F) were analyzed. HDM exposure for 24 h alone increased all measured cytokines and chemokines, however, this effect was only significant for IL-10 and CCL-22. Pre-exposure to increasing concentrations of DEP (12.5–50 µg/ml) followed by HDM challenge significantly increased the IL-6 and CCL-22 release. Pre-exposure to DEP at 12.5, 25 and 50 µg/ml additionally increased HDM-induced IL-10 release, but this was only significant for 12.5 µg/ml DEP compared to only HDM-challenged cells. Pre-exposure to increasing concentrations of DEP (except 12.5 µg/ml) with a subsequent challenge to HDM, significantly increased CXCL-8 release compared to HDM and DEP. Although, DEP pre-exposure increased the GM-CSF release, the subsequent challenge with HDM did not lead to additional GM-CSF release. Moreover, pre-treatment of the cells with increasing concentrations of DEP or HDM exposure for 24 tended to increase CCL-20 levels. However, CCL-20 levels were significantly increased when both were combined. Furthermore, the actual concentrations of aforementioned cytokines released by 16HBE cells are depicted in supplementary Fig. 4 (Fig S4).

4. Discussion

Indoor and outdoor air pollution exposure have been linked with asthma and asthma symptoms for a long time. For instance, a recent epidemiological study demonstrated an association between exposure to PM2.5 and bronchial and asthmatic symptoms [29]. Although murine models have been used extensively to investigate the effect of PM on airways, the mechanism(s) by which PM exposure enhances the bronchial epithelial cell responses to inhaled allergens remains largely unknown. An *in vitro* model might be helpful for further understanding of the interplay between chronic PM exposure and allergens.

The current study showed that short term exposure (24 h) to 200–800 ug/ml DEP particles leads to airway barrier disruption as observed by a significant decrease in TEER values and increase in LY

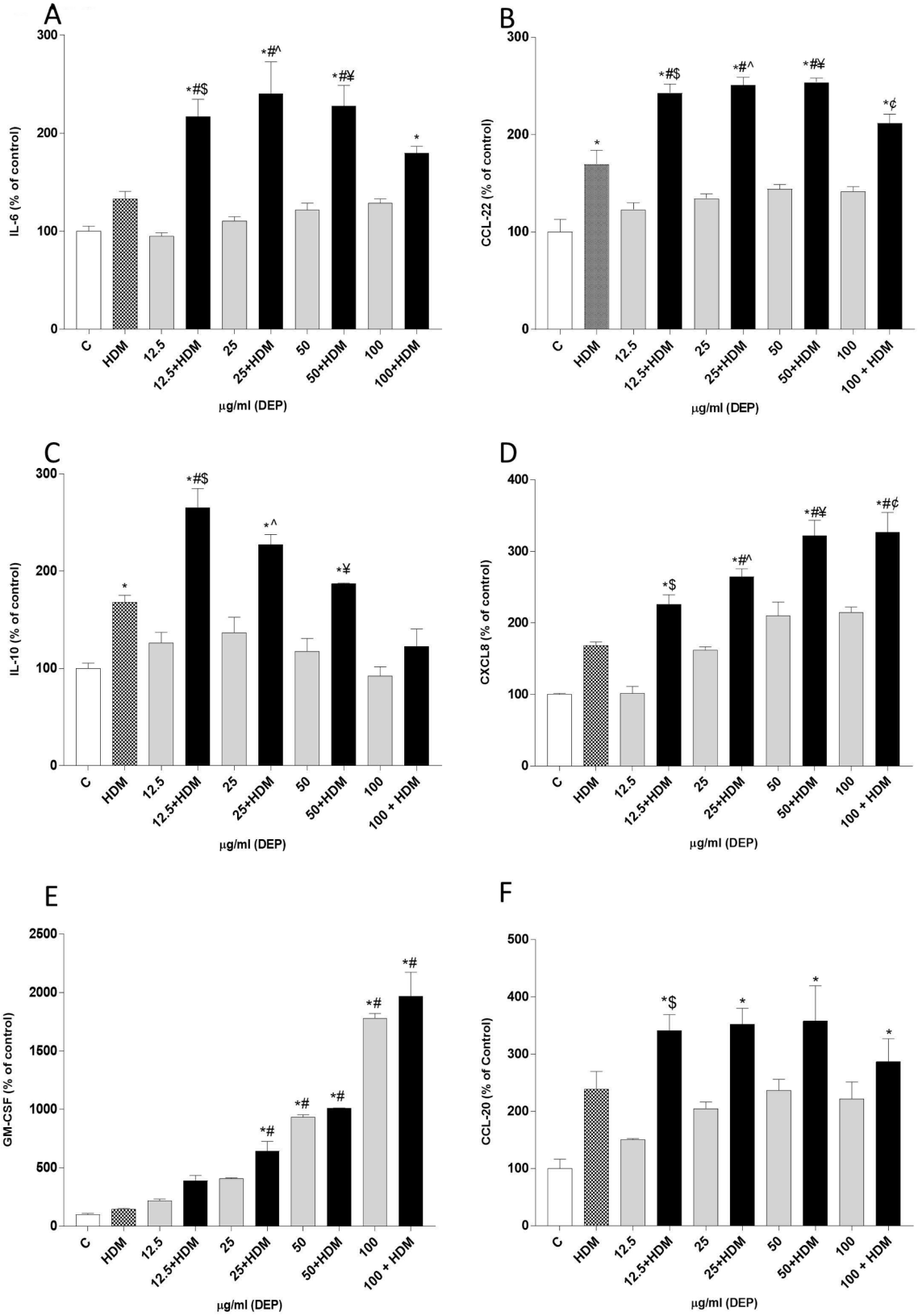

Fig. 5. DEP pre-exposure aggravates HDM-induced cytokine release by bronchial epithelial cells. IL-6(A), CCL-22(B), IL-10(C), CXCL-8(D), GM-CSF(E) and CCL-20(F) were measured. Data are presented as mean ± SEM (N = 3). *P < 0.05; significantly different versus control, #P < 0.05; versus HDM (50 µg/ml), $P < 0.05; versus DEP (12.5 µg/ml), P < 0.05; versus DEP (25 µg/ml), ¥P < 0.05; versus DEP (50 µg/ml), ∅P < 0.05 versus DEP (100 µg/ml). Three independent experiments (each performed in triplicate).

translocation. It has been reported that exposure of 16HBE cells to 25 and 50 ug/ml DEP (for 8.5 h) leads to reduced TEER values along with reduced tight junction (tricellulin) expression [64], while mice exposed to DEP for a short time (2 h per day for 5 days) also exhibited lung barrier disruption as observed by reduced tricellulin expression [64]. Moreover, an *in vitro* study of Liu et al. further demonstrated that short term exposure to high concentrations of PM results in reduced TEER values. Exposure to 200 ug/ml PM for 24 h significantly decreased TEER values in a human bronchial epithelial cell line (BEAS-2B) [43]. Although in the current study we did not detect any changes in TEER values and LY translocation after exposure to lower DEP concentrations (12.5 and 25 μg/ml) for 24 h, we found that long term exposure (96 h) of human bronchial epithelial cells to a low DEP concentration (12.5 ug/ml DEP) suffices to induce a loss of airway barrier integrity and enhance the inflammatory response. Although the lowest concentrations of DEP (12.5 and 25 ug/ml) resulted in significant reduction in TEER values, no significant increase in LY translocation was detected. Both parameters, the measurement of electrical resistance across a cellular monolayer, known as TEER, and paracellular translocation of LY, are regarded as a sensitive and reliable method for assessing the integrity and permeability of the epithelial barrier function [65]. However, it has been described that TEER measurement is more sensitive as it captures the ionic transfer and can be altered by very small changes in the cell monolayer [65], while LY translocation reflects the paracellular water flow and pore size of tight junction proteins [80]. In addition, TEER and cell viability seem to be closely inversely correlated, and data would suggest that cellular viability is compromised before paracellular transport is affected [38]. Based on the added toxin, Caco-2 TEER values seem to be inversely correlated with permeability of small molecules such as mannitol. However, for larger molecules such as LY there seems to be no clear correlation with TEER changes. In general, it could be argued that TEER drops always precede LY transport, and, depending on the toxin, TEER drops might be observed without changes in LY permeability [38]. In our experiments 16HBE cells were used and it remains to be determined if the same principles apply here. Nevertheless, it could be argued that low-concentration DEP exposure to 16HBE cells is enough to cause significant drops in TEER which would allow for the permeability of smaller molecules while larger molecules, the size of LY, could still be retained.

To further investigate the effect of DEP on barrier function, we performed western blot analysis of ZO-1, an important tight junction protein. Apical localization of tight junctions has been shown as a main contributor to maintain the airway epithelial resistance and limiting the paracellular permeability. This assembly is made up of intercellular proteins, including claudins, and are attached to the cytoskeleton by the scaffolding protein ZO-1 [25]. Therefore, changes in the localization of ZO-1, could lead to a reduced epithelial resistance and increased paracellular permeability. A direct association between decreased TEER and delocalized ZO-1 has been shown in nasal epithelial cells exposed to Staphylococcus aureus [10]. In the current study, the immunofluorescence staining for ZO-1 protein demonstrated an altered localization, while we did not find significant changes in ZO-1 protein expression after 96 h DEP exposure. Several studies did show that DEP exposure results in junctional dysfunction by reducing junction proteins in primary rat alveolar epithelial cells [9], human RPMI 2650 cells [20] and human nasal epithelium cells [36].

Although the exact mechanism responsible for the altered barrier function after DEP exposure is not known, increased immune responses and corresponding cytokine release, such as enhanced IL-6 and CXCL-8 (pro-inflammatory cytokine) release measured in our study, might play a role. We observed an almost similar pattern for IL-6 and CXCL-8 release after exposure to 12.5–400 ug/ml DEP for 24 h and 96 h. However, it seems that exposure to 800 ug/ml DEP leads to higher IL-6 responses compared to CXCL-8. Moreover, we observed that the DEP-associated increase in IL-6 is lower than the DEP-associated increase in CXCL-8 after 96 h. However, we have to take into account that DEP concentrations exceeding 200 μg/ml become toxic to the cells which might influence IL-6 and CXCL-8 release and activity differently [21,63,73]. At the moment, there is no clear explanation why DEP exposure causes a difference between IL-6 and CXCL-8 release over time and more research is needed to unravel the underlying mechanism. Regardless and in agreement with our observations, Steerenberg et al, also showed differential effects between IL-6 and CXCL-8 production by human bronchial epithelial (BEAS-2B) cells after DEP exposure for 24 and 48 h [67].

It is becoming increasingly clear that cytokines, in addition to their known pro-inflammatory function, have a significant physiological and pathological impact on the tight junction barrier [1]. Indeed, exposure of the intestinal epithelial cell line (Caco-2) to IL-6 induced a significant reduction in TEER values [70], while IL-6 treatment caused increased permeability in 16HBE cells observed as an increase in transepithelial leakage of C-D-mannitol [8]. In a study of Desai et. al, the IL-6-induced increase in permeability in endothelial cells was associated with a redistribution of ZO-1 [18]. Furthermore, mice treated with an anti-IL-6 antibody maintained ZO-1 and occludin localization followed by ethanol exposure [78]. In addition to IL-6, CXCL-8 has also been shown to affect epithelial barrier function. Endothelial cells stimulated with CXCL-8 for 2, 4, 6 and 8 h resulted in barrier dysfunction as observed by reduced TEER values [77].

To investigate the effect of air pollution exposure on increased risk of allergic asthma development, we further developed our *in vitro* model to investigate the hypothesis that long term exposure to DEP can aggravate the immune responses against an allergen which consequently leads to allergic sensitization or exacerbation. We found that HDM treatment of long-term pre-exposed 16HBE cells to 12.5 ug/ml DEP leads to higher IL-6, CCL-22, IL-10 and CXCL-8 release compared to either HDM or DEP exposure alone suggesting exposure to DEP could enhance the risk of the allergic immune reactions. Elevated allergic immune responses after air pollution exposure have also been demonstrated by human and animal studies [3,11,48,51]. For instance, a prospective cohort study revealed a positive association of traffic-related air pollution exposure during middle age and increased risk of allergen sensitization, asthma, and lower levels of lung function [6]. Moreover, Carlsten et al, showed that 2 h exposure to DEP followed by an allergen challenge (birch, Pacific grasses and HDM) lead to higher airway eosinophilia, and IL-5 levels in the human lung [11]. Pre-exposure of BALB/c mice to fine PM combined with ovalbumin (OVA) followed by an additional OVA challenge for three times induced a significant increase in plasma IgE and pulmonary IL-25 and TNF-α release [12]. Moreover, several animal studies have shown that exposure to DEP followed by HDM treatment leads to higher Th2 immune responses as the main contributor to allergic asthma development [7,32,69]. For example, mice co-exposed to PM and HDM exhibited a significant increase in Th2-immune responses as observed by enhanced pulmonary IL-4, IL-5, IL-6 and IL-13 release [13]. Although studies have shown the critical role of IL-4, IL-5, IL-13 and TSLP in driving the allergic responses, we were not able to detect the release of these cytokines by 16HBE cells. Overall, It can be hypothesized that the aggravated cytokine release following HDM challenge in DEP-pre-exposed cells could be an important factor to explain the increased incidences of asthma and allergies as a consequence of rising air pollution levels [61].

Several studies have revealed the crucial role of exacerbated cytokine release in allergic asthma development. CXCL-8 has been shown to play an important role in facilitating airway remodeling and hyperresponsiveness, which leads to the development of asthma [42,52]. There is a correlation between IL-6 and asthma in humans, suggesting a primary role for IL-6 in lung function abnormalities in asthma [59]. IL-10 exerts immunomodulatory and proinflammatory effects in the lung [39] and primary human bronchial epithelial cells have the capacity to produce IL-10 [4,75], which is in agreement with the observed results in our study by using 16HBE cells. Although IL-10 is important in the reduction or prevention of the allergic immune responses, it has also

been shown that IL-10 is involved in promoting the development of airway hyperresponsiveness during allergic responses [46]. In the present study, a HDM challenge after DEP pre-exposure resulted in a significant increase in CCL-22 release compared to HDM-exposed airway epithelial cells. CCL-22 plays a functional role in diverse immunological diseases, including allergic reactions and autoimmunity with elevated CCL-22 levels detected during allergic responses [54]. CCL-22 overproduction is a leading factor in allergen-driven Th2 cell accumulation in asthmatic airways [74]. Human peripheral blood analysis of 144 adult asthma cases and 199 controls revealed that asthmatic patients exhibit elevated CCL-22 levels compared to the control group [49]. In addition, it was demonstrated that DEP pre-exposure of 16HBE cells followed by a HDM challenge leads to an additional increase in CCL-20 release. Airway epithelial cells are a major source of CCL-20 secretion [57] and there is a higher CCL-20 release observed in asthmatic patients [53]. Moreover, Hastie et al., demonstrated that severe asthma patients release higher CCL-20 levels in sputum than no severe asthma patients [23].

Accumulating evidence indicates that airway allergic diseases are not only caused by a dysregulated immune response, but also via a disrupted airway epithelial barrier, which facilitates allergen sensitization and inflammation [22,28]. In the current study, we demonstrated that that 24 h exposure of HDM significantly increases the permeability of the 16HBE cell monolayer. This is in line with previous *in vitro* studies where exposure of airway epithelial cells (16HBE14o and primary nasal epithelial cells) to HDM increases the permeability and reduces TEER values [27,56,55,66]. Moreover, we found that HDM treatment of cells pre-exposed to 12.5 ug/ml DEP significantly reduces the TEER values and increases the LY translocation compared to HDM only or 12.5 ug/ml DEP-exposed groups. Changes in TEER values and permeability points to alterations in airway epithelial tight junction expression. To best of our knowledge, there is no *in vitro* study available indicating the effect on tight junction expression after HDM treatment in DEP pre-exposed cells. We showed that HDM, but not DEP exposure, tends to reduce CLDN-3 protein expression. In cells pre-exposed to DEP and challenged with HDM, CLDN-3 expression was concentration-dependently reduced, while ZO-1 expression was not significantly affected. The difference in CLDN-3 expression between high and low DEP concentrations might be explained by the IL-10 release from 16HBE cells. We reported that exposure to 12.5, 25 and 50 ug/ml DEP followed by additional HDM treatment significantly increased IL-10 production, while no significant increase in IL-10 production was observed for 100 ug/ml DEP with an additional HDM treatment. IL-10 could have a protective effect on airway barrier function as IL-10 plays crucial role in maintaining barrier homeostasis [41,44,45]. *In vitro* treatment of IL-10 significantly restored the IFN-y-induced decrease in TEER values in intestinal epithelial cells [37]. This effect was confirmed in mice treated with exogenous IL-10 showing a restored intestinal epithelial barrier function after a crystalloid treatment [68]. In addition to IL-10, in the present study, the additional HDM trigger after DEP exposure significantly increased the CXCL-8 production by 16HBE cells compared to HDM- and DEP-exposed cells. Yu et. Al (2013) demonstrated that CXCL-8 modulates the endothelial cell permeability by reduced protein expression of tight junctions protein, such as claudin-5 [77].

Although the exact underlying mechanism how DEP pre-exposure leads to increased sensitivity of airway epithelial cells to a specific allergen challenge is not yet entirely understood, stimulated immune responses and altered cytokine release in combination with increased epithelial paracellular permeability and disruption of airway barrier function might play an important role [1,16].

5. Conclusion

In conclusion, this study showed that long term exposure (4 days) to DEP particles leads to a reduction in TEER values and an increase in cellular permeability, already at low concentrations. Higher concentrations of PM were previously reported in literature to induce similar effects, however, effects were observed in an acute setting with concentrations that might not physiologically relevant. The tested concentrations used in this study are probably more physiologically relevant and indicate that long term exposure could be more devastating to lung health compared to acute high concentrations. Additionally, these novel data support a mechanism whereby low long term DEP exposure would stimulate the immune response against specific aeroantigens and possibly modulate airway allergic sensitization and (asthma) exacerbation. However, future studies using primary human bronchial epithelial cells cultured in an air–liquid interface setup are necessary to further validate our observations as complex morphological and functional characteristics of the *in vivo* human airways may not be adequately captured by using 16HBE cells in a submerged culture system.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Conflict of Interest: JvB and JG are employees of DNR. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cyto.2022.155878.

References

[1] R. Al-Sadi, M. Boivin, T. Ma, Mechanism of cytokine modulation of epithelial tight junction barrier, Front. Biosci. (Landmark Ed.) 14 (2009) 2765–2778, https://doi.org/10.2741/3413.

[2] H.R. Anderson, G. Favarato, R.W. Atkinson, Long-term exposure to air pollution and the incidence of asthma: meta-analysis of cohort studies, Air Qual. Atmos. Heal. 6 (1) (2013) 47–56, https://doi.org/10.1007/s11869-011-0144-5.

[3] S. Baldacci, S. Maio, S. Cerrai, G. Sarno, N. Baïz, M. Simoni, I. Annesi-Maesano, G. Viegi, Allergy and asthma: Effects of the exposure to particulate matter and biological allergens, Respir. Med. 109 (2015) 1089–1104, https://doi.org/10.1016/j.rmed.2015.05.017.

[4] T.L. Bonfield, M.W. Konstan, M. Berger, Altered respiratory epithelial cell cytokine production in cystic fibrosis, J. Allergy Clin. Immunol. 104 (1) (1999) 72–78, https://doi.org/10.1016/S0091-6749(99)70116-8.

[5] G. Bowatte, C. Lodge, A.J. Lowe, B. Erbas, J. Perret, M.J. Abramson, M. Matheson, S.C. Dharmage, The influence of childhood traffic-related air pollution exposure on asthma, allergy and sensitization: a systematic review and a meta-analysis of birth cohort studies, Allergy 70 (3) (2015) 245–256, https://doi.org/10.1111/all.12561.

[6] G. Bowatte, C.J. Lodge, L.D. Knibbs, A.J. Lowe, B. Erbas, M. Dennekamp, G. B. Marks, G. Giles, S. Morrison, B. Thompson, P.S. Thomas, J. Hui, J.L. Perret, M. J. Abramson, H. Walters, M.C. Matheson, S.C. Dharmage, Traffic-related air pollution exposure is associated with allergic sensitization, asthma, and poor lung function in middle age, J. Allergy Clin. Immunol. 139 (2017) 122–129.e1, https://doi.org/10.1016/j.jaci.2016.05.008.

[7] E.B. Brandt, M.B. Kovacic, G.B. Lee, A.M. Gibson, T.H. Acciani, T.D. Le Cras, P. H. Ryan, A.L. Budelsky, G.K. Khurana Hershey, Diesel exhaust particle induction of IL-17A contributes to severe asthma, J. Allergy Clin. Immunol. 132 (2013) 1194–1204.e2, https://doi.org/10.1016/j.jaci.2013.06.048.

[8] P.J. Callaghan, E. Rybakovsky, B. Ferrick, S. Thomas, J.M. Mullin, M. Koval, Retinoic acid improves baseline barrier function and attenuates TNF-α-induced barrier leak in human bronchial epithelial cell culture model, 16HBE 14o-, PLoS One 15 (12) (2020) e0242536, https://doi.org/10.1371/journal.pone.0242536.

[9] J.C. Caraballo, J. Borcherding, P.S. Thorne, A.P. Comellas, Protein kinase C-ζ mediates lung injury induced by diesel exhaust particles, Am. J. Respir. Cell Mol. Biol. 48 (3) (2013) 306–313, https://doi.org/10.1165/rcmb.2012-0056OC.

[10] F.M. Carlier, C. de Fays, C. Pilette, Epithelial Barrier Dysfunction in Chronic Respiratory Diseases, Front. Physiol. 12 (2021), 691227, https://doi.org/10.3389/fphys.2021.691227.
[11] C. Carlsten, A. Blomberg, M. Pui, T. Sandstrom, S.W. Wong, N. Alexis, J. Hirota, Diesel exhaust augments allergen-induced lower airway inflammation in allergic individuals: a controlled human exposure study, Thorax 71 (1) (2016) 35–44.
[12] A.R. Castañeda, K.J. Bein, S. Smiley-Jewell, K.E. Pinkerton, Fine particulate matter (PM(2.5)) enhances allergic sensitization in BALB/c mice, J. Toxicol. Environ. Health. A 80 (4) (2017) 197–207, https://doi.org/10.1080/15287394.2016.1222920.
[13] A.R. Castañeda, C.F.A. Vogel, K.J. Bein, H.K. Hughes, S. Smiley-Jewell, K. E. Pinkerton, Ambient particulate matter enhances the pulmonary allergic immune response to house dust mite in a BALB/c mouse model by augmenting Th2- and Th17-immune responses, Physiol. Rep. 6 (2018), https://doi.org/10.14814/phy2.13827 e13827.
[14] M.-W. Chao, C.-H. Yang, P.-T. Lin, Y.-H. Yang, Y.-C. Chuang, M.-C. Chung, C.-Y. Tseng, Exposure to PM(2.5) causes genetic changes in fetal rat cerebral cortex and hippocampus, Environ. Toxicol. 32 (4) (2017) 1412–1425, https://doi.org/10.1002/tox.22335.
[15] D.M. Cooper, M. Loxham, Particulate matter and the airway epithelium: the special case of the underground? Eur. Respir. Rev. 28 (153) (2019) 190066, https://doi.org/10.1183/16000617.0066-2019.
[16] C.B. Coyne, M.K. Vanhook, T.M. Gambling, J.L. Carson, R.C. Boucher, L. G. Johnson, D. Goodenough, Regulation of airway tight junctions by proinflammatory cytokines, Mol. Biol. Cell 13 (9) (2002) 3218–3234, https://doi.org/10.1091/mbc.e02-03-0134.
[17] R.G. Crystal, S.H. Randell, J.F. Engelhardt, J. Voynow, M.E. Sunday, Airway epithelial cells: current concepts and challenges, Proc. Am. Thorac. Soc. 5 (7) (2008) 772–777, https://doi.org/10.1513/pats.200805-041HR.
[18] T.R. Desai, N.J. Leeper, K.L. Hynes, B.L. Gewertz, Interleukin-6 Causes Endothelial Barrier Dysfunction via the Protein Kinase C Pathway, J. Surg. Res. 104 (2002) 118–123, https://doi.org/10.1006/jsre.2002.6415.
[19] A. Faustini, M. Stafoggia, P. Colais, G. Berti, L. Bisanti, E. Cadum, A. Cernigliaro, S. Mallone, C. Scarnato, F. Forastiere, Air pollution and multiple acute respiratory outcomes, Eur. Respir. J. 42 (2) (2013) 304–313, https://doi.org/10.1183/09031936.00128712.
[20] A. Fukuoka, K. Matsushita, T. Morikawa, H. Takano, T. Yoshimoto, Diesel exhaust particles exacerbate allergic rhinitis in mice by disrupting the nasal epithelial barrier, Clin. Exp. Allergy 46 (2016) 142–152, https://doi.org/10.1111/cea.12597.
[21] H.R. Gatla, N. Muniraj, P. Thevkar, S. Yavvari, S. Sukhavasi, M.R. Makena, Regulation of Chemokines and Cytokines by Histone Deacetylases and an Update on Histone Decetylase Inhibitors in Human Diseases, Int. J. Mol. Sci. 20 (2019) 1110, https://doi.org/10.3390/ijms20051110.
[22] S.N. Georas, F. Rezaee, Epithelial barrier function: at the front line of asthma immunology and allergic airway inflammation, J. Allergy Clin. Immunol. 134 (3) (2014) 509–520, https://doi.org/10.1016/j.jaci.2014.05.049.
[23] A.T. Hastie, W.C. Moore, D.A. Meyers, P.L. Vestal, H. Li, S.P. Peters, E.R. Bleecker, Analyses of asthma severity phenotypes and inflammatory proteins in subjects stratified by sputum granulocytes, J. Allergy Clin. Immunol. 125 (5) (2010) 1028–1036.e13, https://doi.org/10.1016/j.jaci.2010.02.008.
[24] F. He, B. Liao, J. Pu, C. Li, M. Zheng, L. Huang, Y. Zhou, D. Zhao, B. Li, P. Ran, Exposure to Ambient Particulate Matter Induced COPD in a Rat Model and a Description of the Underlying Mechanism, Sci. Rep. 7 (2017) 45666, https://doi.org/10.1038/srep45666.
[25] I.H. Heijink, S.M. Brandenburg, J.A. Noordhoek, D.S. Postma, D.-J. Slebos, A.J. M. van Oosterhout, Characterisation of cell adhesion in airway epithelial cell types using electric cell-substrate impedance sensing, Eur. Respir. J. 35 (4) (2010) 894–903, https://doi.org/10.1183/09031936.00065809.
[26] I.H. Heijink, V.N.S. Kuchibhotla, M.P. Roffel, T. Maes, D.A. Knight, I. Sayers, M. C. Nawijn, Epithelial cell dysfunction, a major driver of asthma development, Allergy 75 (2020) 1902–1917, https://doi.org/10.1111/all.14421.
[27] I.H. Heijink, A. van Oosterhout, A. Kapus, Epidermal growth factor receptor signalling contributes to house dust mite-induced epithelial barrier dysfunction, Eur. Respir. J. 36 (5) (2010) 1016–1026, https://doi.org/10.1183/09031936.00125809.
[28] P.W. Hellings, B. Steelant, Epithelial barriers in allergy and asthma, J. Allergy Clin. Immunol. 145 (6) (2020) 1499–1509, https://doi.org/10.1016/j.jaci.2020.04.010.
[29] G.J. Holst, C.B. Pedersen, M. Thygesen, J. Brandt, C. Geels, J.H. Bønløkke, T. Sigsgaard, Air pollution and family related determinants of asthma onset and persistent wheezing in children: nationwide case-control study, BMJ 370 (2020), m2791, https://doi.org/10.1136/bmj.m2791.
[30] K.-I. Inoue, E. Koike, H. Takano, R. Yanagisawa, T. Ichinose, T. Yoshikawa, Effects of diesel exhaust particles on antigen-presenting cells and antigen-specific Th immunity in mice, Exp. Biol. Med. (Maywood) 234 (2) (2009) 200–209, https://doi.org/10.3181/0809-RM-285.
[31] X.-Q. Jiang, X.-D. Mei, D. Feng, Air pollution and chronic airway diseases: what should people know and do? J. Thorac. Dis. 8 (2016) E31–E40, https://doi.org/10.3978/j.issn.2072-1439.2015.11.50.
[32] H.J. Jung, Y.-K. Ko, W.S. Shim, H.J. Kim, D.-Y. Kim, C.-S. Rhee, M.K. Park, D. H. Han, Diesel exhaust particles increase nasal symptoms and IL-17A in house dust mite-induced allergic mice, Sci. Rep. 11 (2021) 16300, https://doi.org/10.1038/s41598-021-94673-9.
[33] A. Karakatsani, A. Analitis, D. Perifanou, J.G. Ayres, R.M. Harrison, A. Kotronarou, I.G. Kavouras, J. Pekkanen, K. Hämeri, G.P. Kos, J.J. de Hartog, G. Hoek, K. Katsouyanni, Particulate matter air pollution and respiratory symptoms in individuals having either asthma or chronic obstructive pulmonary disease: a European multicentre panel study, Environ. Health 11 (2012) 75, https://doi.org/10.1186/1476-069X-11-75.
[34] F.J. Kelly, J.C. Fussell, Air pollution and airway disease. Clin. Exp. allergy, J. Br. Soc. Allergy Clin. Immunol. 41 (2011) 1059–1071, https://doi.org/10.1111/j.1365-2222.2011.03776.x.
[35] J. Kim, S. Natarajan, L.J. Vaickus, J.C. Bouchard, D. Beal, W.W. Cruikshank, D. G. Remick, Diesel exhaust particulates exacerbate asthma-like inflammation by increasing CXC chemokines, Am. J. Pathol. 179 (6) (2011) 2730–2739, https://doi.org/10.1016/j.ajpath.2011.08.008.
[36] N. Kim, D.H. Han, M.-W. Suh, J.H. Lee, S.-H. Oh, M.K. Park, Effect of lipopolysaccharide on diesel exhaust particle-induced junctional dysfunction in primary human nasal epithelial cells, Environ. Pollut. 248 (2019) 736–742, https://doi.org/10.1016/j.envpol.2019.02.082.
[37] D.J. Kominsky, E.L. Campbell, S.F. Ehrentraut, K.E. Wilson, C.J. Kelly, L.E. Glover, C.B. Collins, A.J. Bayless, B. Saeedi, E. Dobrinskikh, B.E. Bowers, C.F. MacManus, W. Müller, S.P. Colgan, D. Bruder, IFN-γ-mediated induction of an apical IL-10 receptor on polarized intestinal epithelia, J. Immunol. 192 (3) (2014) 1267–1276, https://doi.org/10.4049/jimmunol.1301757.
[38] R. Konsoula, F.A. Barile, Correlation of in vitro cytotoxicity with paracellular permeability in Caco-2 cells, Toxicol. In Vitro 19 (5) (2005) 675–684, https://doi.org/10.1016/j.tiv.2005.03.006.
[39] F.N. Lauw, D. Pajkrt, C.E. Hack, M. Kurimoto, S.J.H. van Deventer, T. van der Poll, Proinflammatory Effects of IL-10 During Human Endotoxemia, J Immunol 165 (5) (2000) 2783–2789.
[40] N. Li, J.R. Harkema, R.P. Lewandowski, M. Wang, L.A. Bramble, G.R. Gookin, Z. Ning, M.T. Kleinman, C. Sioutas, A.E. Nel, Ambient ultrafine particles provide a strong adjuvant effect in the secondary immune response: implication for traffic-related asthma flares, Am. J. Physiol. Lung Cell. Mol. Physiol. 299 (3) (2010) L374–L383, https://doi.org/10.1152/ajplung.00115.2010.
[41] R. Lin, F. Chen, S. Wen, T. Teng, Y. Pan, H. Huang, Interleukin-10 attenuates impairment of the blood-brain barrier in a severe acute pancreatitis rat model, J. Inflamm. 15 (2018) 4, https://doi.org/10.1186/s12950-018-0180-0.
[42] C. Liu, X. Zhang, Y. Xiang, X. Qu, H. Liu, C. Liu, M. Tan, J. Jiang, X. Qin, Role of epithelial chemokines in the pathogenesis of airway inflammation in asthma (Review), Mol Med Rep 17 (2018) 6935–6941, https://doi.org/10.3892/mmr.2018.8739.
[43] J. Liu, X. Chen, M. Dou, H. He, M. Ju, S. Ji, J. Zhou, C. Chen, D. Zhang, C. Miao, Y. Song, Particulate matter disrupts airway epithelial barrier via oxidative stress to promote Pseudomonas aeruginosa infection, J. Thorac. Dis. 11 (2019) 2617–2627, https://doi.org/10.21037/jtd.2019.05.77.
[44] V. Lorén, E. Cabré, I. Ojanguren, E. Domènech, E. Pedrosa, A. García-Jaraquemada, M. Mañosa, J. Manyé, R. Schneider-Stock, Interleukin-10 Enhances the Intestinal Epithelial Barrier in the Presence of Corticosteroids through p38 MAPK Activity in Caco-2 Monolayers: A Possible Mechanism for Steroid Responsiveness in Ulcerative Colitis, PLoS One 10 (6) (2015) e0130921, https://doi.org/10.1371/journal.pone.0130921.
[45] K.L. Madsen, S.A. Lewis, M.M. Tavernini, J. Hibbard, R.N. Fedorak, Interleukin 10 prevents cytokine-induced disruption of T84 monolayer barrier integrity and limits chloride secretion, Gastroenterology 113 (1) (1997) 151–159.
[46] M.J. Mäkelä, A. Kanehiro, L. Borish, A. Dakhama, J. Loader, A. Joetham, Z. Xing, M. Jordana, G.L. Larsen, E.W. Gelfand, IL-10 is necessary for the expression of airway hyperresponsiveness but not pulmonary inflammation after allergic sensitization, Proc. Natl. Acad. Sci. U.S.A. 97 (11) (2000) 6007–6012.
[47] A.J. Miller, J.R. Spence, In Vitro Models to Study Human Lung Development, Disease and Homeostasis. Physiology (Bethesda) 32 (3) (2017) 246–260, https://doi.org/10.1152/physiol.00041.2016.
[48] R.L. Miller, D.B. Peden, Environmental effects on immune responses in patients with atopy and asthma, J. Allergy Clin. Immunol. 134 (5) (2014) 1001–1008, https://doi.org/10.1016/j.jaci.2014.07.064.
[49] N. Mostafavi, A. Jeong, J. Vlaanderen, M. Imboden, P. Vineis, D. Jarvis, M. Kogevinas, N. Probst-Hensch, R. Vermeulen, The mediating effect of immune markers on the association between ambient air pollution and adult-onset asthma, Sci. Rep. 9 (2019) 8818, https://doi.org/10.1038/s41598-019-45327-4.
[50] A.T. Nathan, E.A. Peterson, J. Chakir, M. Wills-Karp, Innate immune responses of airway epithelium to house dust mite are mediated through beta-glucan-dependent pathways, J. Allergy Clin. Immunol. 123 (2009) 612–618, https://doi.org/10.1016/j.jaci.2008.12.006.
[51] T. Ohtoshi, H. Takizawa, H. Okazaki, S. Kawasaki, N. Takeuchi, K. Ohta, K. Ito, Diesel exhaust particles stimulate human airway epithelial cells to produce cytokines relevant to airway inflammation in vitro, J. Allergy Clin. Immunol. 101 (6) (1998) 778–785, https://doi.org/10.1016/S0091-6749(98)70307-0.
[52] J.E. Pease, I. Sabroe, The role of interleukin-8 and its receptors in inflammatory lung disease: implications for therapy, Am. J. Respir. Med. 1 (1) (2002) 19–25, https://doi.org/10.1007/BF03257159.
[53] M. Pichavant, A.-S. Charbonnier, S. Taront, A. Brichet, B. Wallaert, J. Pestel, A.-B. Tonnel, P. Gosset, Asthmatic bronchial epithelium activated by the proteolytic allergen Der p 1 increases selective dendritic cell recruitment, J. Allergy Clin. Immunol. 115 (4) (2005) 771–778, https://doi.org/10.1016/j.jaci.2004.11.043.
[54] V. Pinho, S.H. Oliveira, D.G. Souza, D. Vasconcelos, A.L. Alessandri, N.W. Lukacs, M.M. Teixeira, The role of CCL22 (MDC) for the recruitment of eosinophils during allergic pleurisy in mice, J. Leukoc. Biol. 73 (2003) 356–362, https://doi.org/10.1189/jlb.0502243.
[55] S. Post, M.C. Nawijn, T.L. Hackett, M. Baranowska, R. Gras, A.J.M. van Oosterhout, I.H. Heijink, The composition of house dust mite is critical for mucosal barrier

dysfunction and allergic sensitisation, Thorax 67 (6) (2012) 488–495, https://doi.org/10.1136/thoraxjnl-2011-200606.
[56] S. Post, M.C. Nawijn, M.R. Jonker, N. Kliphuis, M. van den Berge, A.J.M. van Oosterhout, I.H. Heijink, House dust mite-induced calcium signaling instigates epithelial barrier dysfunction and CCL20 production, Allergy 68 (2013) 1117–1125, https://doi.org/10.1111/all.12202.
[57] J. Reibman, Y. Hsu, L.C. Chen, B. Bleck, T. Gordon, Airway epithelial cells release MIP-3alpha/CCL20 in response to cytokines and ambient particulate matter, Am. J. Respir. Cell Mol. Biol. 28 (2003) 648–654, https://doi.org/10.1165/rcmb.2002-0095OC.
[58] M. Riedl, D. Diaz-Sanchez, Biology of diesel exhaust effects on respiratory function, J. Allergy Clinical Immunology 115 (2) (2005) 221–228.
[59] M. Rincon, C.G. Irvin, Role of IL-6 in asthma and other inflammatory pulmonary diseases, Int. J. Biol. Sci. 8 (9) (2012) 1281–1290, https://doi.org/10.7150/ijbs.4874.
[60] A.T. Saber, N.R. Jacobsen, J. Bornholdt, S.L. Kjaer, M. Dybdahl, L. Risom, S. Loft, U. Vogel, H. Wallin, Cytokine expression in mice exposed to diesel exhaust particles by inhalation. Role of tumor necrosis factor, Part. Fibre Toxicol. 3 (2006) 4, https://doi.org/10.1186/1743-8977-3-4.
[61] A. Saxon, D. Diaz-Sanchez, Air pollution and allergy: you are what you breathe, Nat. Immunol. 6 (3) (2005) 223–226, https://doi.org/10.1038/ni0305-223.
[62] F. Schulze, X. Gao, D. Virzonis, S. Damiati, M. Schneider, R. Kodzius, Air Quality Effects on Human Health and Approaches for Its Assessment through Microfluidic Chips, Genes (Basel). 8 (10) (2017) 244, https://doi.org/10.3390/genes8100244.
[63] B. Singha, H.R. Gatla, S. Manna, T.-P. Chang, S. Sanacora, V. Poltoratsky, A. Vancura, I. Vancurova, Proteasome inhibition increases recruitment of IκB kinase β (IKKβ), S536P–p65, and transcription factor EGR1 to interleukin-8 (IL-8) promoter, resulting in increased IL-8 production in ovarian cancer cells, J. Biol. Chem. 289 (5) (2014) 2687–2700, https://doi.org/10.1074/jbc.M113.502641.
[64] T. Smyth, J. Veazey, S. Eliseeva, D. Chalupa, A. Elder, S.N. Georas, Diesel exhaust particle exposure reduces expression of the epithelial tight junction protein Tricellulin, Part. Fibre Toxicol. 17 (2020) 52, https://doi.org/10.1186/s12989-020-00383-x.
[65] B. Srinivasan, A.R. Kolli, M.B. Esch, H.E. Abaci, M.L. Shuler, J.J. Hickman, TEER measurement techniques for in vitro barrier model systems, J. Lab. Autom. 20 (2) (2015) 107–126, https://doi.org/10.1177/2211068214561025.
[66] B. Steelant, R. Farré, P. Wawrzyniak, J. Belmans, E. Dekimpe, H. Vanheel, L. Van Gerven, I. Kortekaas Krohn, D.M.A. Bullens, J.L. Ceuppens, C.A. Akdis, G. Boeckxstaens, S.F. Seys, P.W. Hellings, Impaired barrier function in patients with house dust mite-induced allergic rhinitis is accompanied by decreased occludin and zonula occludens-1 expression, J. Allergy Clin. Immunol. 137 (4) (2016) 1043–1053.e5, https://doi.org/10.1016/j.jaci.2015.10.050.
[67] P.A. Steerenberg, J.A.J. Zonnenberg, J.A.M.A. Dormans, P.N.T. Joon, I.M. Wouters, L.V. Bree, P.T.J. Scheepers, H.V. Loveren, Diesel exhaust particles induced release of interleukin 6 and 8 by (primed) human bronchial epithelial cells (BEAS 2B) in vitro, Exp. Lung Res. 24 (1) (1998) 85–100, https://doi.org/10.3109/01902149809046056.
[68] X. Sun, H. Yang, K. Nose, S. Nose, E.Q. Haxhija, H. Koga, Y. Feng, D.H. Teitelbaum, Decline in intestinal mucosal IL-10 expression and decreased intestinal barrier function in a mouse model of total parenteral nutrition, Am. J. Physiol. Gastrointest. Liver Physiol. 294 (1) (2008) G139–G147, https://doi.org/10.1152/ajpgi.00386.2007.
[69] G. Takahashi, H. Tanaka, K. Wakahara, R. Nasu, M. Hashimoto, K. Miyoshi, H. Takano, H. Yamashita, N. Inagaki, H. Nagai, Effect of Diesel Exhaust Particles on House Dust Mite-Induced Airway Eosinophilic Inflammation and Remodeling in Mice, J. Pharmacol. Sci. 112 (2) (2010) 192–202, https://doi.org/10.1254/jphs.09276FP.
[70] Y. Tazuke, R.A. Drongowski, D.H. Teitelbaum, A.G. Coran, Interleukin-6 changes tight junction permeability and intracellular phospholipid content in a human enterocyte cell culture model, Pediatr. Surg. Int. 19 (5) (2003) 321–325, https://doi.org/10.1007/s00383-003-1003-8.
[71] A.I. Totlandsdal, F.R. Cassee, P. Schwarze, M. Refsnes, M. Låg, Diesel exhaust particles induce CYP1A1 and pro-inflammatory responses via differential pathways in human bronchial epithelial cells, Part. Fibre Toxicol. 7 (1) (2010) 41, https://doi.org/10.1186/1743-8977-7-41.
[72] C.-Y. Tseng, M.-C. Chung, J.-S. Wang, Y.-J. Chang, J.-F. Chang, C.-H. Lin, R.-S. Hseu, M.-W. Chao, Potent In Vitro Protection Against $PM_{2.5}$-Caused ROS Generation and Vascular Permeability by Long-Term Pretreatment with Ganoderma tsugae, Am. J. Chin. Med. 44 (2016) 355–376, https://doi.org/10.1142/S0192415X16500208.
[73] M.D. Turner, B. Nedjai, T. Hurst, D.J. Pennington, Cytokines and chemokines: At the crossroads of cell signalling and inflammatory disease, Biochim. Biophys. Acta - Mol. Cell Res. 1843 (2014) 2563–2582, https://doi.org/10.1016/j.bbamcr.2014.05.014.
[74] P. Vijayanand, K. Durkin, G. Hartmann, J. Morjaria, G. Seumois, K.J. Staples, D. Hall, C. Bessant, M. Bartholomew, P.H. Howarth, P.S. Friedmann, R. Djukanović, Chemokine receptor 4 plays a key role in T cell recruitment into the airways of asthmatic patients, J. Immunol. 184 (8) (2010) 4568–4574, https://doi.org/10.4049/jimmunol.0901342.
[75] X. Wu, Y. Mi, H. Yang, A. Hu, Q. Zhang, C. Shang, The activation of HMGB1 as a progression factor on inflammation response in normal human bronchial epithelial cells through RAGE/JNK/NF-κB pathway, Mol. Cell. Biochem. 380 (1-2) (2013) 249–257, https://doi.org/10.1007/s11010-013-1680-0.
[76] Y.-F. Xing, Y.-H. Xu, M.-H. Shi, Y.-X. Lian, The impact of PM2.5 on the human respiratory system, J. Thorac. Dis. 8 (2016) E69–E74, https://doi.org/10.3978/j.issn.2072-1439.2016.01.19.
[77] H. Yu, X. Huang, Y. Ma, M. Gao, O.u. Wang, T. Gao, Y. Shen, X. Liu, Interleukin-8 regulates endothelial permeability by down-regulation of tight junction but not dependent on integrins induced focal adhesions, Int. J. Biol. Sci. 9 (9) (2013) 966–979, https://doi.org/10.7150/ijbs.6996.
[78] A. Zahs, M.D. Bird, L. Ramirez, M.A. Choudhry, E.J. Kovacs, Anti-IL-6 antibody treatment but not IL-6 knockout improves intestinal barrier function and reduces inflammation after binge ethanol exposure and burn injury, Shock 39 (2013) 373–379, https://doi.org/10.1097/SHK.0b013e318289d6c6.
[79] Q. Zhang, Z. Qiu, K.F. Chung, S.-K. Huang, Link between environmental air pollution and allergic asthma: East meets West, J. Thorac. Dis. 7 (2015) 14–22, https://doi.org/10.3978/j.issn.2072-1439.2014.12.07.
[80] F. Zucco, A.-F. Batto, G. Bises, J. Chambaz, A. Chiusolo, R. Consalvo, H. Cross, G. Dal Negro, I. de Angelis, G. Fabre, F. Guillou, S. Hoffman, L. Laplanche, E. Morel, M. Pinçon-Raymond, P. Prieto, L. Turco, G. Ranaldi, M. Rousset, Y. Sambuy, M.L. Scarino, F. Torreilles, A. Stammati, An inter-laboratory study to evaluate the effects of medium composition on the differentiation and barrier function of Caco-2 cell lines, Altern. Lab. Anim. 33 (6) (2005) 603–618, https://doi.org/10.1177/026119290503300618.