SHORT CIGARETTE SMOKE EXPOSURE FACILITATES SENSITIZATION AND

ASTHMA DEVELOPMENT IN MICE

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Abstract

Objective: Epidemiological studies indicate that cigarette smoke (CS) exposure is a risk factor for increased sensitization and asthma development. The aim of the study was to examine the impact of CS on sensitization and allergic airway inflammation, in response to a low dose of house dust mite (HDM), and to obtain potential mechanistic insights.

Methods: Mice were exposed to low doses of HDM extract combined with air or CS exposure, either during allergen sensitization or during development of allergic airway disease.

Results: Mice concomitantly exposed to low dose HDM, combined with CS for 3 weeks, demonstrated an asthmatic phenotype with significantly increased airway eosinophilia, goblet cell metaplasia, airway hyperresponsiveness and a rise in HDM-specific serum IgG1, compared to sole HDM or CS exposure. In addition, short CS inhalation, during the initial contact with HDM allergens, was sufficient to facilitate sensitization and development of a complete asthmatic phenotype after rechallenge with HDM. Mechanistically, short CS exposure amplified DC-mediated transport of FITC-labelled HDM allergens to the intrathoracic lymph nodes and generated a local Th2 response.

Conclusion: Short CS exposure is sufficient to facilitate allergic sensitization and the development of low dose HDM-induced allergic asthma, possibly through affecting dendritic cell function.
Keywords: Airway allergy, dendritic cell, environmental pollutants, murine

Abbreviations:

CS: cigarette smoke
HDM: house dust mite
DC: dendritic cell
mLN: mediastinal lymph nodes
OVA: ovalbumin
BAL: bronchoalveolar lavage
TCM: tissue culture medium
PAS: periodic acid-Schiff
OD: optical density
HRP: horseradish peroxidase
AUC: area under the curve
Introduction

Most asthma begins in early childhood after sensitization and re-exposure to ubiquitous environmental allergens, like house dust mites (HDM), moulds, plant pollen or animal dander. The risk for sensitization is strongly dependent on the level of allergen exposure [1]. Over the last decades, the incidence of asthma has increased worldwide, especially in industrialized countries. This strong rise in asthma prevalence emphasizes an important role for environmental and socio-economic conditions [2].

One of the main environmental risk factors, associated with asthma is the exposure to cigarette smoke (CS) [3]. Epidemiological studies have shown that smoking is responsible for higher asthma severity scores [4,5], diminished lung function [5,6] and poorer asthma control [7,8]. Smoking is even associated with increased sensitization to HDM allergens [9], and appears to be a risk factor for new onset asthma among children and adults [10-12]. The mechanisms mediating the adverse effects of smoking on asthma pathogenesis remain to be elucidated. Most murine models, designed to characterize the complex interaction between smoking and allergic airway inflammation, used to rely on the sensitization to the "surrogate" allergen ovalbumin (OVA) [13-18]. As OVA is an intrinsically inert protein, the role of CS might have been overestimated in the past. Moreover, the differences in biochemical character between OVA and real-life allergens remain an undeniable limitation of the previously used OVA models.

To explain the observed increase in asthma prevalence due to CS exposure, we hypothesized that CS may lower the threshold for sensitization to HDM allergens. We developed a clinically relevant murine model of allergic asthma, using low doses of HDM as real-life aeroallergen, combined with CS. We examined the effect of CS on both sensitization and
ensuing asthma development and checked whether a few days of smoke exposure are sufficient to prime local sensitization in the lymph nodes. Finally, we looked for potential mechanistic insights.
Methods

Mice

Male Balb/c mice (6-8 weeks old) were purchased from Harlan (Zeist, the Netherlands). All in vivo manipulations were approved by the Animal Ethical Committee of the Faculty of Medicine and Health Sciences of Ghent University.

Cigarette smoke (CS) exposure and house dust mite (HDM) administration

Mice (n = 10 per group) were subjected to whole body CS (3R4F Kentucky Reference cigarettes) as described before [19]. Control mice were exposed to air. 30 minutes after the last smoke exposure, 25 μg HDM extract (Dermatophagoides pteronyssinus) (Greer Laboratories, Lenoir, NC, USA) (25,27 μg Der p1/mg protein; 8.43 endotoxin U/mg) or PBS was administered intranasally in isoflurane anesthetized mice on days 0, 7 and 14 and mice were analysed on day 17. To evaluate the impact of CS on the development of HDM-induced allergic asthma, we performed Protocol 1 (Figure 1). To unravel the impact of CS during the sensitization phase, mice were subjected to HDM and CS according to Protocol 2 or 4 (Figure 1). The impact of CS during the challenge phase was evaluated using Protocol 3 (Figure 1).

Bronchoalveolar lavage (BAL) and cytospins

24 hours after the last smoke exposure and 72 hours after the last HDM application, mice were euthanized with an overdose of pentobarbital (Sanofi-Ceva, Paris, France). BAL, cytospins and cell differentiation were performed as described previously [19]. Remaining cells were used for FACS-analysis.
**Lung and mediastinal lymph node (mLN) single-cell suspensions**

Lungs were perfused with saline plus EDTA through the pulmonary artery to remove contaminating blood cells. Lungs and mLN were removed and digested as described before [20].

**Flow cytometry**

Staining procedures, data acquisition and analysis were performed as described previously [19]. Monoclonal antibodies (mAbs) used to identify mouse DC populations were anti-CD11c (clone HL3), anti-MHC class II (I-A/I-E, clone M5/114.15.2) and anti-CD11b (clone M1/70). CD11b+ DCs were defined as CD11c-bright, low autofluorescent cells which strongly express MHC class II and CD11b on their surface. The following mAbs were used to stain mouse T-cell subpopulations: anti-CD3 (clone 145-2C11), anti-CD4 (clone GK1.5), anti-CD8 (clone 53-6.7) and anti-CD69 (clone H1.2F3) (all mAbs from BD Pharmingen, San Diego, CA, USA).

**MLN cell culture**

Paratracheal and parathymic intrathoracic LNs were collected into sterile tubes containing cold (4°C) tissue culture medium (TCM) and digested (see above) to obtain a single cell suspension. TCM was prepared using RPMI 1640 supplemented with 10% fetal bovine serum, L-glutamine, penicillin/streptomycin and β-mercaptoethanol (all from Gibco BRL; Invitrogen Corp). Cells were then transferred in triplicate to round-bottom, 96-well plates (Becton Dickinson (BD), BD, CA, USA) with or without 15 µg HDM extract/ml culture medium, at a density of 2 x 10⁵ cells per well and incubated in a humidified 37°C incubator with 5% CO₂. After 5 days, supernatants were harvested and frozen for cytokine measurements.
**Histology**

The left lung was fixed in 4% paraformaldehyde and embedded in paraffin. Transversal sections of 3 µm were stained with Congo Red (Klinipath, Olen, Belgium) to highlight eosinophils, with periodic acid-Schiff (PAS) to identify goblet cells or immunostained for α-smooth muscle actin to evaluate airway smooth muscle content. Quantitative measurements were performed using a Zeiss KS400 Image Analyzer platform (Oberkochen, Germany) as described earlier [21]. To identify mast cells, lungs were stained with toluidin blue. The number of mast cells was counted per field (whole left lung lobe). Only those located in the airway wall were included for analysis.

**Protein measurements**

Total IgE was measured using coated microtiter plates and biotinylated polyclonal rabbit anti-mouse IgE (S. Florquin, ULB, Brussels, Belgium). For the detection of HDM-specific IgG1, microtiter plates were coated with HDM extract. Serum was added, followed by a horseradish peroxidase (HRP) conjugated polyclonal goat anti-mouse IgG1 antibody (Bethyl Laboratories, Montgomery, USA). Levels of HDM-specific IgG1 were reported in optical densities (OD). MLN supernatants were assayed for IL-4, IL-5, IL-13, IL-10 and IFN-gamma by means of multiplex (Merck Millipore, Brussels, Belgium). In the supernatants of crushed lungs, IL-25, IL-33, TSLP, GM-CSF and IL-1β were measured with ELISA (R&D Systems, Abingdon, UK) following the manufacturer’s instructions.

**Assessment of airway responsiveness**

Airway responsiveness was measured in tracheostomized anaesthetized mice using the FlexiVent System (SCIREQ, Montreal, QC, Canada). Neuromuscular blockade was induced by
injecting pancuronium bromide (1 mg/kg) intravenously. To check for airway hyperresponsiveness, mice were challenged with increasing doses of carbachol (0, 5, 10, 20, 40, 80, 160 and 320 µg/kg). A “snapshot perturbation” manoeuvre was imposed to measure the (dynamic) resistance (R) of the whole respiratory system (airways, lung and chest wall). For each mouse, a dose-response curve was generated and the area under the curve (AUC) was calculated.

**Evaluation of DC migration by intratracheal instillation of fluorescent HDM**

To obtain fluorescently labelled HDM, HDM extract (Greer Laboratories, Lenoir, NC, USA) was dialysed against a carbonate-bicarbonate buffer (pH 9.5), overnight at 4°C. 10mg/ml FITC in DMSO (Sigma Aldrich, Bornem, Belgium) was added to the HDM solution and rotated for 1 hour at room temperature. The whole procedure was performed under dark, sterile conditions. After 4 days of CS exposure, anesthetized mice (i.p. ketamine (80 mg/kg; Ketamine 1000 CEVA; Ceva Sante Animale, Brussels, Belgium) – xylazine (8 mg/kg; Rompun 2%; Bayer AG, Leverkusen, Germany)) were held vertically and 50 µl of FITC conjugated HDM (100 µg) or PBS was pipetted just above their vocal cords. Mice were sacrificed 0, 24 and 48 hours after instillation. MLN were removed and processed as described above. Discrimination in the mLN between the airway derived (AW-DCs) (CD11c<sup>int-high</sup>/MHCII<sup>high</sup>) and non-airway derived DCs (NAW-DCs) (CD11c<sup>+</sup>/MHCII<sup>int</sup>) was performed using the method published by Vermaelen et al [20]. The % of HDM-bearing airway-derived DCs (AW-DCs) was determined by flow cytometry as the fraction of FITC<sup>+</sup> MHCII<sup>high</sup> CD11c<sup>int-high</sup> cells. The results were expressed as the % of FITC<sup>+</sup> DCs regarding to the total DC population.
**Statistical analysis**

Statistical analysis was performed with PASW Statistics 18 using nonparametric tests. The different experimental groups were compared by a Kruskal-Wallis test for multiple comparisons. Pairwise comparisons were made by means of a Mann-Whitney U-test. A p-value $p \leq 0.05$ was considered significant. Reported values are expressed as mean ± SEM.
Results

1. CS facilitates the development of new onset allergic asthma

To evaluate the role of CS in the pathogenesis of HDM-induced allergic asthma, Balb/c mice were exposed to CS or air for 3 weeks and instilled with HDM (25 μg) or PBS once a week (Figure 1 – Protocol 1). We instilled 4 times less HDM compared with our previously described model [22], in order to limit the biological effects of HDM on its own.

Exposure to both HDM and CS aggravates the allergic response in BAL fluid and lung tissue

The intranasal delivery of sole HDM elicited a faint asthmatic phenotype with increased eosinophils, CD11b⁺ DCs and CD4⁺ and CD8⁺ T lymphocytes as compared to PBS exposed control mice (Figure 2). CS exposure as such enhanced the amount of total cells and macrophages (data not shown), neutrophils, CD11b⁺ DCs and CD4⁺ and CD8⁺ T lymphocytes in the BAL fluid compared to air exposed mice (Figure 2). The concomitant exposure to HDM and CS amplified the allergic phenotype considerably. This is reflected by a 10-fold increase in the number of eosinophils and a rise in CD11b⁺ DCs and CD4⁺ and CD8⁺ T lymphocytes in BAL fluid as compared to the other groups. No further increase in macrophages (data not shown) or neutrophils was observed (Figure 2). In line, lung single cell suspensions had more CD11b⁺ DCs and activated CD4⁺CD69⁺ and CD8⁺CD69⁺ T cells as compared to sole HDM or CS (Figure 3a). Histological analysis revealed peribronchovascular eosinophilic inflammation and mucin-producing goblet cells in HDM exposed mice, compared with naive control mice (Figure 3b,c). However, exposure to both CS and HDM, resulted in a further increase of eosinophils, goblet cells and mast cells (Figure 3b,c,d). Other features of airway wall
remodelling, such as quantification of airway smooth muscle content, revealed no differences between the 4 groups (data not shown).

(*Combined exposure to HDM and CS increases HDM-specific IgG1 production in serum*)

Total serum IgE did not differ between the 4 groups (data not shown), but HDM exposed mice showed significantly elevated allergen-specific IgG1 titers in the serum, compared with naive control mice. This level was further increased after combined exposure to HDM and CS (Figure 4a). HDM-specific IgE measurements were below detection limit.

(*Concomitant exposure to HDM and CS amplifies the production of Th2 cytokines*)

HDM restimulated mLN cells from the sole HDM group showed increased production of the inflammatory cytokines IL-4, IL-5, IL-13 and IL-10, together with decreased levels of the typical Th1 cytokine IFN-γ (Figure 4b). The HDM/CS combination yielded in a further increase in IL-4, IL-5, IL-13 and IL-10 (Figure 4b). IFN-γ had a further tendency to decrease (Figure 4b). The role of the epithelium was investigated by measuring innate pro-Th2 cytokines in lung homogenate. Exposure to both HDM and CS, resulted in more IL-25, IL-33 and IL-1β (Figure 4c). TSLP and GM-CSF were below detection limit.

(*CS exposure exacerbates HDM-induced airway hyperresponsiveness*)

Figure 4d shows the dose-response curve of the *in vivo* reactivity of all 4 groups. HDM or CS exposed mice were slightly responsive towards the highest carbachol dose. Mice concomitantly exposed to both stimuli were more responsive (Figure 4d).
2. **CS exposure during sensitization and not during allergen challenge is important for subsequent asthma development**

To investigate the putative role of CS during sensitization, Balb/c mice were briefly exposed to CS (for 4 consecutive days) and instilled with HDM (25 µg) once a week during 3 consecutive weeks (Figure 1 – **Protocol 2**). In the sera of HDM/CS exposed mice, enhanced allergen-specific IgG1 titers were detected (Figure 5a). In contrast to **Protocol 1**, where mice received CS for 3 weeks, we observed no neutrophils after limited CS exposure in **Protocol 2** (Figure 5b). Mice concomitantly exposed to HDM and only 4 days of CS showed more BAL CD11b⁺ DCs, CD4⁺ and CD8⁺ T-lymphocytes in comparison to all other groups and had a 20-fold increase in the number of BAL eosinophils (Figure 5b). Compared to **Protocol 1**, the inflammation in BAL fluid was less pronounced and even in lung single cell suspensions, the number of DCs and T-lymphocytes no longer increased upon combined HDM/CS exposure. (Figure 5c). Histological examination revealed however significantly more eosinophils (Figure 5d) and goblet cells in the airway wall (Figure 5e) together with increased airway hyperresponsiveness in concomitantly exposed mice (Figure 6a). Altogether, these Th2 associated airway responses were accompanied by increases in IL-4, IL-5, IL-13 and IL-10 in the supernatant of restimulated mLN cultures of HDM/CS exposed mice (Figure 6b), but no differences in IL-25, IL-33 and IL-1β production could be observed between all 4 groups (Figure 6c).

On the contrary, exposure to CS exclusively during the HDM challenge phase (Figure 1 – **Protocol 3**) was unable to induce an allergic phenotype and did not show increased HDM specific IgG1 (Figure 7a), nor elevated numbers of eosinophils, CD11b⁺ DCs or T lymphocytes in BAL fluid (Figure 7b) and lung tissue, except for a rise of CD4⁺CD69⁺ T cells in the lung.
Additionally, we found no increase in the number of goblet cells (Figure 7d) or airway hyperresponsiveness in these mice (Figure 7e).

3. **Short exposure to CS enhances HDM uptake and DC migration to the mLN and facilitates sensitization to common aeroallergens**

To investigate if 4 days of smoke inhalation already affects airway DC trafficking and antigen transport to the draining LNs, we delivered fluorescently labelled HDM intratracheally to CS or air exposed mice. FITC⁺ DCs were exclusively found within the population of CD11c<sup>int</sup>-<sup>high</sup>/MHCII<sup>high</sup> AW-DCs. At various time points after HDM instillation (24h, 48h), CS exposed mice showed a marked increase in DC-mediated HDM transport to the draining LNs, compared to air exposed mice (Figure 8a).

To characterize the role of CS during initial sensitization towards common aeroallergens, we exposed Balb/c mice to CS for 3 consecutive days and instilled HDM allergens on the first day (25µg) (Figure 1 – Protocol 4). This short interaction between CS and HDM allergens significantly increased the number of CD11b⁺ DCs in BAL fluid and lung tissue (Figure 8b,c), with enhanced activation of BAL DCs, as read out by the expression of CD86 (Figure 8b). At the functional level, we found that brief exposure to CS during the first contact with HDM allergens was sufficient to induce sensitization in the mLN, characterized by a pronounced Th2 cytokine profile in HDM/CS exposed mice (Figure 8d). To explain this heightened state of allergen-specific sensitization, we measured typical DC-activating cytokines, released by bronchial epithelial cells early in the sensitization process. TSLP and GM-CSF were below detection limit, but IL-25, IL-33 and IL-1β were elevated in CS exposed mice (Figure 8e). IL-1β increased further after concomitant exposure to HDM and CS.
**Discussion**

In this paper, we demonstrate that cigarette smoke (CS) exposure can play a determining role during allergic sensitization and asthma development. (i) We show that concomitant exposure to CS and a low dose of house dust mite (HDM) results in a pronounced Th2-related asthmatic phenotype, which is hardly present when sole HDM is used. In addition, we demonstrate that, (ii) in the presence of allergens, acute CS exposure is sufficient to cause sensitization and subsequent asthma development, (iii) possibly by the amplified allergen transport of airway DCs towards the mLNs.

The strongest predictive factor for asthma development is the sensitization to common environmental allergens, like house dust mites (HDMs), grass pollen or animal dander. Epidemiological studies provide indirect clinical evidence that smoking is associated with increased sensitization to HDM allergens. Smoke exposure is even correlated with higher asthma incidence and severity of the disease. Especially children become more susceptible due to smoke exposure, as illustrated by the increased wheeze and asthma prevalence among children and young adolescents upon passive smoke inhalation [23].

Within our lab, we created a murine model, supporting the findings from epidemiological studies and using HDM as clinically relevant allergen. HDM is the most significant source of indoor allergens, responsible for atopic symptoms in 10% of individuals. It is a complex mixture of various protein allergens and non-protein compounds, with some allergens having the natural capacity to induce mucosal sensitization through the respiratory tract [24]. Although the content of commercially available preparation of HDM extract can vary extensively [25], these extracts might be a good representation of the indoor HDM allergens,
present in our homes. To examine whether CS can lower the threshold for asthma development, we first created a mild murine asthma model by down-titration of the HDM protein content until almost no asthmatic phenotype could be elicited (data not shown). Such models are relevant to evaluate potential synergistic effects upon CS inhalation.

In this paper, we demonstrate that CS exposure facilitates and aggravates the asthmatic disease, as illustrated by the increased eosinophils and neutrophils in BAL. These findings are in agreement with our previous work, investigating the role of CS in asthma development with ovalbumin (OVA) as “surrogate” allergen [14,26]. However, OVA is no naturally occurring allergen and prolonged exposure elicits inhalation tolerance, rather than allergic inflammation in mice [27].

An important novelty of our study, using adolescent mice of 6-8 weeks old, is the striking result that only 3 days of CS exposure during the initial allergen contact are sufficient to prime HDM-specific Th2 cells in the LNs. This process might be driven by the enhanced HDM transport of the airway DCs. Our findings suggest that adolescent smokers and young children, may become more susceptible to allergic sensitization and ensuing asthma development, due to (short) CS inhalation. To our opinion, these results can be extrapolated to humans, since our murine CS exposure protocol reaches carboxyhemoglobin (COHb) levels comparable to those in human smokers [28,29] and since COHb levels of young children (aged 1-2) exposed to parental smoke are similar to those measured in adult smokers [30].

Furthermore, we focussed on the release of innate pro-allergic cytokines, known to instruct DCs to mount Th2-mediated cell responses in the lung [31]. Three weeks of HDM and CS exposure resulted in significantly more IL-25, IL-33 and IL-1β. In contrast, when stopping CS exposure after the initial sensitization, no differences in these cytokines were found 2 weeks
after smoke cessation. This suggests a synergistic role for CS, particularly during the ongoing allergic response and illustrates the direct impact of CS on airway epithelial cells and on the release of innate pro-Th2 cytokines, the driving force in activating DCs and ensuing asthma development. IL-1β and IL-33 were even increased after 1 HDM administration concomitant with 3 days of smoke exposure, suggesting a role for these cytokines during the sensitization phase and in facilitating the sensitization process due to CS. This idea is supported by Willart et al. who recently confirmed a role for these cytokines early in asthma development. By blocking IL-1β and IL-33 signalling during HDM sensitization, they demonstrated that IL-1β was involved in Th2 cytokine production, whereas IL-33 was crucial for BAL inflammation [32].

To our knowledge, this is the first in vivo model showing unambiguous synergy between CS and HDM allergens. In line with our observation, Rusznak et al. found increased inflammatory mediator release from primary in vitro cultures of human bronchial epithelial cells, after exposure to CS and Der p allergens [33]. Blacquiére et al. examined the effect of maternal smoking during pregnancy [34]. Upon HDM exposure, mice offspring of smoking mothers showed increased airway wall remodelling and AHR, but no increase in inflammatory response or elevated Th2 cytokines could be demonstrated in HDM/CS exposed mice. Mitchell et al. investigated the role of progesterone and, or CS in exacerbating allergic airway disease. Although the difference in experimental setup, they found some indications for increased allergic inflammation due to CS, however less pronounced than in our model [35,36]. In contrast to our current findings, a recent study by Botelho et al. [37] using a murine model of established allergic asthma, reported significantly attenuated eosinophilia in BAL fluid of mice exposed to both HDM and CS, together with
decreased mucus production and no difference in CD4$^+$ T cell activation nor AHR between HDM and, or CS exposed mice.

Disparities between our data and the studies mentioned above, might be related to fundamental differences in allergen and CS exposure protocol, such as the use of alum as Th2 skewing adjuvant [35,36] or the timing, duration and intensity of smoke exposure. In addition, the variability between commercially available preparations of HDM extract (e.g. endotoxin content) may affect the development of potential Th2 responses in the lung [37].

Compared to the previous studies, our model is innovative since we show synergy using a mild asthma model and a short CS exposure protocol. The clear distinction between the sensitization and allergen challenge phase, made it possible to examine the impact of CS on different phases of asthma pathogenesis. Because of the diversity of asthma phenotypes, preclinical mouse models of combined exposures to allergens and environmental pollutants will become more important in the future. Combination models mimic more closely the real-life situation in humans and are therefore more reliable to provide mechanistic insights and to test potential therapeutic strategies.

In conclusion and in agreement with epidemiological studies, we provide biological mechanistic data, supporting the hypothesis that environmental factors such as cigarette smoke, are risk factors for sensitization and ensuing asthma development. We found that even short-term cigarette smoke exposure can lower the threshold for allergen sensitization, making individuals more vulnerable to future asthma development.
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Figure legends
**Figure 1: Exposure protocols.** 4 groups were included in our experimental set-up: PBS/air, PBS/CS (sole CS), HDM/air (sole HDM) and HDM/CS. Abbreviations: PBS = phosphate buffered saline, HDM = house dust mite, CS = cigarette smoke
Figure 1

Protocol 1

25 µg HDM or PBS

Day 0
7
14
17
analysis

Protocol 2

25 µg HDM or PBS

Day 0
7
14
17
analysis

Protocol 3

25 µg HDM or PBS

Day 0
7
14
17
analysis

Protocol 4

25 µg HDM or PBS

Day 0
3
analysis

Cigarette smoke or air exposure
Figure 2: Cell differentiation in bronchoalveolar lavage (BAL) fluid from mice simultaneously exposed to phosphate buffered saline (PBS) or house dust mite (HDM), combined with air or cigarette smoke (CS) for 3 weeks. Results are expressed as means ± SEM; n=10 animals/group. *p<0.05; **p<0.01; ***p<0.001.

Figure 3: Cell differentiation in lung tissue and histopathological evaluation from mice simultaneously exposed to phosphate buffered saline (PBS) or house dust mite (HDM), combined with air or cigarette smoke (CS) for 3 weeks. a) Cell differentiation. b) Photomicrographs of eosinophilic peribronchial infiltrates. c) Photographs of goblet cells. d) Total number of mast cells. Results are expressed as means ± SEM; n=10 animals/group. *p<0.05; **p<0.01; ***p<0.001.
Figure 4: Immunoglobulins, cytokines and airway hyperresponsiveness of mice simultaneously exposed to phosphate buffered saline (PBS) or house dust mite (HDM), combined with air or cigarette smoke (CS) for 3 weeks. a) HDM-specific IgG1. b) Protein levels of IL-4, IL-5, IL-13, IL-10 and IFN-γ in the supernatant of HDM restimulated lymph node cells. c) Measurements of IL-25, IL-33 and IL-1β in supernatant of crushed lungs. d) Airway hyperresponsiveness to carbachol. Dose-response curve and area under the curve (AUC). Results are expressed as means ± SEM; n=10 animals/group. *p<0.05; **p<0.01; ***p<0.001.
Figure 5: Immunoglobulins, inflammatory response and histopathological evaluation of mice simultaneously exposed to phosphate buffered saline (PBS) or house dust mite (HDM) and 4 days of air or cigarette smoke (CS). a) HDM-specific IgG1. b) Cell differentiation of bronchoalveolar lavage (BAL) fluid and c) lung digest. d) Quantification of eosinophils. e) Measurement of goblet cells. Results are expressed as means ± SEM; n=10 animals/group. *p<0.05; **p<0.01; ***p<0.001.
Figure 6: Airway hyperresponsiveness to carbachol and cytokine responses of mice simultaneously exposed to phosphate buffered saline (PBS) or house dust mite (HDM) and 4 days of air or cigarette smoke (CS).  a) Dose-response curve to carbachol and area under the curve (AUC). b) Protein levels of IL-4, IL-5, IL-13 and IL-10 in supernatant of HDM restimulated lymph node cells. c) Measurements of IL-25, IL-33 and IL-1β in supernatant of crushed lungs. Results are expressed as means ± SEM; n=10 animals/group. *p<0.05; **p<0.01; ***p<0.001.
Figure 7: Immunoglobulins, inflammatory response, histopathological evaluation and airway hyperresponsiveness of mice simultaneously exposed to phosphate buffered saline (PBS) or house dust mite (HDM) and air or cigarette smoke (CS) during the allergen challenge phase. a) HDM-specific IgG1. b) Cell differentiation of bronchoalveolar lavage (BAL) fluid and c) lung digest. d) Quantification of goblet cells. e) Airway hyperresponsiveness to carbachol. Results are expressed as means ± SEM; n=10 animals/group. *p<0.05; **p<0.01; ***p<0.001.
Figure 8: Migration, recruitment and maturation of pulmonary DCs and corresponding cytokine profiles upon phosphate buffered saline (PBS) or house dust mite (HDM) and acute air or cigarette smoke (CS) exposure. a) Dendritic cell (DC) migration to the mediastinal lymph nodes. b) CD11b+ DCs and expression of CD86 on BAL DCs c) and lung DCs (calculated within the population of low autofluorescent, CD11c+, MHCII+ DCs). d) Protein levels of IL-4, IL-5 and IL-13 in HDM restimulated lymph node cells. e) Measurements of IL-25, IL-33 and IL-1β in supernatant of crushed lungs. Results are expressed as means ± SEM; n=10 animals/group. *p<0.05; **p<0.01; ***p<0.001.