Published in final edited form as:
Clin Exp Allergy. 2009 October ; 39(10): 1597–1610. doi:10.1111/j.1365-2222.2009.03302.x.

Inhaled house dust mite induces pulmonary T helper 2 cytokine production

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Abstract

Background—Inhaled house dust mite (HDM) results in T-helper (TH) 2 type pathology in unsensitized mice, in conjunction with airway hyperreactivity and airway remodelling.

However, the pulmonary cytokine and chemokine profile has not been reported.

Methods—We have performed a time course analysis of the characteristic molecular mediators and cellular influx in the bronchoalveolar lavage (BAL) and lung in order to define the pulmonary inflammatory response to inhaled HDM extract. Mice were exposed five times a week to soluble HDM extract for 3 weeks. Lung function was measured in groups of mice at intervals following the final HDM challenge. Recruitment of inflammatory cells and inflammatory mediator production was then assessed in BAL and lungs of individual mice.

Results—We found that Th2 cytokines were significantly increased in BAL and lung after HDM challenge from as early as 2 h post-final challenge. The levels of cytokines and chemokines correlated with the influx of eosinophils and Th2 cells to the different compartments of the lung. However, the production of key cytokines such as IL-4, IL-5 and IL-13 preceded the increase in airways resistance.

Conclusion—Inhaled HDM challenge induces a classical Th2 inflammatory mediator profile in the BAL and lung. These data are important for studies determining the efficacy of novel treatment strategies for allergic airways disease.

Keywords

airway hyperreactivity; allergic inflammation; house dust mite; Th2 cytokines

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Introduction

Asthma is characterized clinically by airway hyperreactivity (AHR) to a variety of stimuli, associated with reversible airway obstruction. Various studies have shown that AHR occurs concomitant with leucocytic infiltration of the airways, as well as structural changes to the airway termed airway remodelling. These airway inflammatory infiltrates are composed predominantly of eosinophils, but there is also a significant lymphocytic component [1]. These lymphocytes are generally of the Th2 phenotype, characteristically releasing cytokines such as IL-4, IL-5 and IL-13 [2, 3]. IL-13 is thought to be central to the development of AHR and mucus production. IL-4 and IL-13 promote immunoglobulin isotype switching to induced production of IgE, while IL-5 is an eosinophil survival and growth factor [4–6]. IL-9 plays a role in mast cell proliferation and differentiation, stimulates the proliferation of activated T cells and enhances B cell immunoglobulin production [7–11]. Chemokines such as CCL11/eotaxin-1, CCL24/eotaxin-2, CCL22/MDC and CCL17/TARC typify a Th2 inflammatory response, and have been shown to promote the recruitment of eosinophils as well as Th2 cells to the lung [12, 13].

Animal models have been used to outline functional roles for Th2 cytokines in the characteristic pathological features of asthma. The vast majority of these studies have used a model of ovalbumin (OVA) sensitization/challenge whereby mice are immunized peripherally with allergen in the presence of the adjuvant alum before local airway challenge with solubilized allergen. It is necessary to immunize mice peripherally with allergen when using OVA since mice become tolerant to inhaled or aerosol challenge with OVA in the absence of systemic sensitization. Clearly sensitization does not occur like this in the human disease and the process may distort the immunological development of disease since the initial contact with the immune system will be in the peritoneum or skin rather than in the lung. Thus, different populations of dendritic and other types of antigen presenting cells will be encountered. However, it was reported recently that continued intranasal delivery of an immunologically more complex allergen, in this case house dust mite (HDM) extract, results in a chronic inflammatory response and structural remodelling of the lung even in naïve, unsensitized mice [14, 15]. This model represents a step forward in that it negates the need for a peripheral sensitization step, uses an environmentally and clinically relevant allergen, and replicates the relevant features of the human disease.

This model has now been used to determine the role of key mediators in the allergic response [16, 17]. However, the temporal relationships between critical inflammatory mediators, inflammatory and regulatory cells and AHR have not yet been explored after inhaled HDM. Previous studies have documented the production of Th2 cytokines by splenocytes restimulated with HDM *in vitro* [14]. However, these levels may not accurately reflect the amount of cytokine in the lung following allergen challenge *in vivo*. The aim of the present study was to investigate the temporal and spatial relationship of leucocytes and Th2-type inflammatory mediators within the lung following inhaled allergen challenge, in association with the development of AHR. In order to do this, the profile of HDM-induced allergic airway disease was compared at each time-point post-final challenge to non-allergic control animals. We have found that the pulmonary response to inhaled HDM in unsensitized mice follows a distinct time course, with Th2 cytokines in particular being produced early after inhaled challenge. Moreover, the peak time to measure changes in lung function does not coincide with the peak production of Th2 cytokines which has important consequences for the study of the molecular mechanisms of tissue pathophysiology in this model of inhaled HDM challenge.

Materials and methods

Animals and Induction of allergic airways disease

Female BALB/c mice were purchased from Harlan Ltd (Bicester, UK). Animals were housed at Imperial College animal facility and used at 6–8 weeks of age. Food and water were supplied *ad libitum*. UK Home Office guidelines for animal welfare based on the Animals (scientific procedures) act 1986 were observed.

Mice were exposed to purified HDM extract (Greer Laboratories, Lenoir, NC, USA; batch number 7500: 21.26 μg derP/mg protein; 13.55 endotoxin U/mg) intranasally (25 μg of protein in 25 μL saline) for 5 days/week for up to 7 weeks. Control animals received 25 μL phosphate-buffered saline (PBS) intranasally.

Measurement of airway hyperreactivity

Airway responsiveness was measured in groups of mice at 2, 4, 8 and 24 h after allergen challenge. Lung resistance (R_L) and compliance (C_{dyn}) were measured in response to increasing doses of methacholine (3–100 mg/mL, Sigma, Poole, UK) in tracheostomized anaesthetized mice using an EMMS system (Electro-Medical Measurement Systems, Bordon, Hants, UK) as described previously [18].

Cell recovery

Airway Lumen—Bronchoalveolar lavage (BAL) was performed by flushing out the lung three times with 0.4 mL PBS via a tracheal cannula. BAL fluid was centrifuged (200×g, 5 min at 4 °C) and cells were resuspended in 0.5 mL complete media [RPMI + 10% fetal calf serum (FCS), 2 mM L-glutamine, 100 U/mL penicillin/streptomycin].

Lung parenchyma—One lobe of lung tissue was mechanically chopped and incubated at 37 °C for 1 h in complete media containing 0.15 mg/mL collagenase (Type D, Roche Diagnostics, Lewes, UK) and 25 μg/mL DNAse (Type 1, Roche Diagnostics). Cells were recovered by filtration through a 70 μm nylon sieve, washed twice and resuspended in 1 mL complete media.

Cytocentrifuge preparation and differential counts of Wright–Giemsa-stained bronchoalveolar lavage and lung cells

Lung and BAL cells were applied to glass slides by centrifugation and stained with Wright–Giemsa (Thermo Fisher Scientific Inc., Waltham, MA, USA). Percentages of macrophages, lymphocytes/mononuclear cells, eosinophils and neutrophils were determined under ×40 magnification by counting cells in eight randomly selected fields and dividing this number by the total number of cells counted. To obtain absolute numbers, this percentage was multiplied by the total number of cells recovered in 1 mL of lavage fluid and lung digest suspension which were normalized for the weight of the lung. All cell counts were performed blind by the same observer.

Staining of bronchoalveolar lavage and lung cells for flow cytometric analysis

Suspensions of BAL and lung tissue cells were stained in staining buffer (PBS containing 1% FCS and 0.01% sodium azide). To reduce non-specific binding, cells were incubated with rabbit serum (Sigma) for 15 min before staining. Cells were stained with the following antibodies APC-labelled anti-mouse CD4, APC-labelled anti-mouse CD8, FITC-labelled anti-mouse γδTCR (BD Pharmingen, Oxford, UK), FITC-labelled anti-mouse T1/ST2 (Morwell Diagnostics, Zurich, CH, Switzerland) or relevant isotype controls for 20 min at 4 °C. Cells were then washed twice and fixed in Cellfix™ (Becton Dickenson, Oxford, UK).

For intracellular cytokine staining, cells were stimulated with PMA/ionomycin (Merck, Whitehouse, NJ, USA) in the presence of Brefeldin A (Sigma) for 6 h before extracellular staining. After extracellular staining and fixing, cells were permeabilized using staining buffer containing 0.5% saponin (Sigma). Cells were then stained with either PE-labelled anti-mouse IL-10, PE labelled anti-mouse IL-17 or appropriate isotype control (BD Pharmingen). Tregs were detected using the $CD4^{+ve}CD25^{+ve}$FoxP3 staining kit available from eBiosciences (San Diego, CA, USA) according to the manufacturer's instructions. Flow cytometric analysis was performed using a FACSCalibar™ (Becton Dickenson) using CellQuest software. Dead cells were excluded on the basis of size (forward scatter) and granularity (side scatter) and lymphocytes gated on size and granularity and investigated for specific markers using fluorescent (FL) channels 1–4 which corresponded to FITC, PE, PerCP and APC.

Analysis of cytokines

Cytokines were analyzed in BAL samples and lung-tissue homogenate supernatants. Lung tissue was homogenized at 50 mg/mL in HBSS containing protease inhibitor tablets (Roche Diagnostics), centrifuged (800×g, 10 min) and the supernatant collected. BAL and lung homogenate chemokine levels were measured using paired antibodies for murine TARC/CCL17, MDC/CCL22, eotaxin-1/CCL11, eotaxin-2/CCL24, MCP-1/CCL2 and RANTES/CCL5 (R&D Systems, Abingdon, UK) in standardized sandwich ELISAs according to the manufacturer's protocol. Kits to measure IL-13 were purchased from R&D Systems. IL-4, IL-5, IFN-γ and KC/CXCL1 were measured in BAL and lung homogenate by MSD multiplex kit for mouse pro-inflammatory cytokines (Mesoscale Discovery, MD, USA), according to manufacturer's instructions.

Eicosanoid quantitation using liquid chromatography/mass spectrometry/mass spectrometry

Ten nanograms 12-HETE-d8 was added to lung homogenate before extraction, as internal standard. Lipid hydroperoxides were reduced to their corresponding stable alcohols by the addition of 1 m_M $SnCl_2$, 10 min, room temperature. Lipids were extracted by adding a solvent mixture [1 M acetic acid, 2-propanol, hexane (2 : 20 : 30, v/v/v)] to the sample at a ratio of 2.5 mL solvent mixture/1 mL sample, vortexing and then adding 2.5 mL hexane. After vortex and centrifugation, lipids were recovered in the upper hexane layer. The samples were then re-extracted by addition of an equal volume of hexane. The combined hexane layers were dried and analyzed for free eicosanoids using liquid chromatography/electrospray ionization tandem/mass spectrometry/mass spectrometry as follows: Lipids were separated on a C18 Spherisorb ODS2, 5 μm, 150×4.6 mm column (Waters Ltd, Elstree, UK) using a gradient of 50–90% B over 10 min, followed by 5 min at 90% B (A, water : acetonitrile : acetic acid, 75 : 25 : 0.1, B, methanol : acetonitrile : acetic acid, 60 : 40 : 0.1) with a flow rate of 1 mL/min. Products were detected using an Applied Biosystems 4000 Q-Trap with specific parent to daughter transitions ($[M-H]^-$) of *m/z* 319.2 → 179 (12-HETE), 319.2 → 115 (5-HETE), 335.2 → 195 (LTB_4) and 327.2 → 184 (12-HETE-d8). Products were identified and quantified using primary standards and 12-HETE d_8 internal standard run in parallel under the same method conditions.

Statistical analysis

Data are expressed as median or mean±SEM unless otherwise stated. All analyses of HDM-treated groups compared with PBS control groups were tested using a Mann–Whitney *U*-Test. A *P*-value of <0.05 was considered significant. Graph generation and statistical analysis were performed using GraphPad Prism software (version 4.00; GraphPad, La Jolla, CA, USA).

Results

Inhaled house dust mite elicits allergic airway inflammation

Exposure of naïve mice to inhaled HDM resulted in changes in lung function and recruitment of inflammatory leucocytes as has been observed previously [14]. During an initial time course study of continuous exposure to HDM for between 1 and 7 weeks, we observed a significant increase in airway resistance from 3 weeks, after 15 doses of inhaled HDM extract compared with mice treated similarly with PBS. This increase in AHR was further elevated at 5 and 7 weeks (Figs 1a and b). The changes in lung function were accompanied by recruitment of inflammatory cells to the airway lumen and tissue. Numbers of eosinophils were significantly enhanced in both the lung and BAL following HDM exposure with peak increased numbers at 2 weeks although eosinophilia was observed as early as week 1 and persisted for the duration of the study (Figs 1c and d). However, when we examined lung tissue and BAL for secretion of Th2 cytokines we were only able to detect significant increases in the prototypical cytokines IL-4, IL-5 or IL-13 at week 2 of challenge, coinciding with peak airway inflammation but before the development of significant AHR. No Th2 cytokines were detected 24 h post-final challenge at 3 weeks even when AHR was observed (Figs 1a and e–i). We hypothesize that levels of Th2 cytokines may peak before 24 h after HDM challenge. Therefore, in order to further characterize the nature of the immunological response to inhaled HDM we set out to analyse the time course of the cellular and mediator responses in the lungs immediately following final HDM challenge of allergic mice in conjunction with the development of AHR. Mice were subjected to 3 weeks of inhaled HDM and then groups of mice were killed 2, 4, 8 and 24 h following the final allergen challenge.

House dust mite exposure results in airway hyperreactivy

In agreement with the data obtained in our preliminary experiments HDM-treated mice demonstrate increased AHR to methacholine challenge compared with PBS controls after 3 weeks. HDM exposure significantly increased airway resistance by 24 h post-challenge (Figs 2a and b), whereas lung compliance was decreased as early as 2 h post-challenge and this level was maintained throughout the 24 h period post-final challenge, compared with PBS controls (Figs 2c and d).

House dust mite results in the early recruitment of inflammatory leucocytes to the lung and airway lumen

Cellular infiltration of the lung was determined in groups of allergic mice killed at intervals following the final inhalation of HDM. As shown in Fig. 3 there was an early recruitment of cells to the lung with total cells peaking at 2 h in the parenchyma. By 4 h post-challenge total cell numbers were significantly reduced compared with 2 h but remained elevated above baseline throughout the 24 h post-challenge period (Fig. 3a). Differential cell counts determined that the majority of these cells were eosinophils (Fig. 3c). In contrast cell recruitment to the airway lumen, as determined in BAL, followed a slower time course, with cell numbers peaking by 4 h and maintained over the 24 h period following allergen inhalation (Figs 3b and d). Infiltrating lymphomononuclear cells and neutrophils were also present in the lung tissue after 2 h and their numbers remained constant for 24 h post-challenge (Figs 3e and g). Likewise in the BAL significant increases in lymphocytes/monocytes and neutrophils were observed at each time-point examined (Figs 3f and h).

In order to characterize the pulmonary T cell response to allergen in more detail, we performed FACS analysis on cells isolated from the lung and BAL (Fig. 4). The majority of T cells in the lung and BAL were of the CD4 phenotype (Figs 4a and b), although CD8 positive cells were also present (Figs 4c and d). In the lung parenchyma, the peak influx was

observed at 2 h after which time cell numbers declined back to baseline values (Figs 4a and c), whereas in the BAL cell numbers continued to increase over the entire period investigated (Figs 4b and d). Around 10% of the CD4 cells were also positive for the surrogate Th2 surface marker T1/ST2. Interestingly numbers of this subset of T helper cells were increased at the earliest time-point investigated and remained elevated for the duration of the study in the lung tissue whereas influx of cells to the airway lumen continued to rise (Figs 4e and f). We also examined levels of non-classical T cells and regulatory T cells in the BAL and lung by flow cytometry (Fig. 5). γδT cells were increased in the lung parenchyma with an early peak at 2 h, while numbers in the BAL peaked at 4 h and remained constant up to 24 h post-challenge (Figs 5a and b). Levels of Th17 cells followed a similar pattern, with an early peak in the lung and maintained increase in the BAL (Figs 5d and e). In both compartments, TH17 cell numbers were still significantly raised at 24 h compared with untreated mice. Interestingly HDM challenge was also associated with the presence of T cells with a regulatory phenotype. We determined numbers of IL-10^{+}CD4 cells as well as FoxP3^{+}CD4 cells (Figs 5g–l). HDM challenge increased levels of both of these populations in the lung tissue 2 h post-challenge and numbers returned to baseline within 24 and 8 h, respectively. In the BAL these regulatory cells remained elevated over the entire time course.

T helper 2 cytokines are produced in the lung following inhaled house dust mite challenge

Production of Th2 cytokines is characteristic of the allergic response. Indeed, Th2 cytokines, such as IL-13 and IL-5 are thought to be critical for the development of pathophysiological features of the allergic response such as AHR and tissue remodelling. However, the production of these cytokines in the lung after inhaled HDM challenge has not previously been reported. Therefore, we measured the concentrations of a range of different cytokines thought to play a role in the development of allergic pathology in both BAL and lungs of PBS- and HDM-treated mice. HDM-treated mice exhibited increased levels of IL-4, IL-5 and IL-13 in the lung and BAL, compared with PBS-treated mice, at all time-points post-challenge (Figs 6a–f). Interestingly, the peak in cytokine production in both BAL and lung was 4h post-challenge for all of the cytokines looked at (between 4 and 8 h for IL-13). In particular, levels in the BAL declined near to baseline by 24 h post-challenge. In contrast, IFN-γ was detectable at very low levels only in the lungs of HDM-treated mice post-challenge but not in the BAL (Figs 6g and h).

Chemokines correlate with cell recruitment and are maintained after allergen challenge

Chemokines promote the recruitment of cells to inflammatory sites. Therefore, we determined the concentration in the lung of particular chemokines that have been shown to be important in the development of allergen induced airway inflammation. We examined the profile of the eosinophil chemokine eotaxin-1/CCL11, as well as the neutrophil chemoattractant KC/CXCL1 and TARC/CCL17, which is associated particularly with Th2 cell migration and activation. Eotaxin-1/CCL11 was significantly elevated in the lung and BAL of HDM-treated mice, compared with PBS controls, at all time-points post-final challenge (Figs 7a and b). Similarly, HDM-treated mice had higher levels of TARC/CCL17, at all time-points compared with PBS-treated mice (Figs 7c and d), although levels declined at the 24 h point, particularly in BAL. Levels of KC/CXCL1 peaked at 4 h in the lung (Fig. 7e), and at 2 h in the BAL (Fig. 7f). We also found significantly raised levels of eotaxin-2/CCL24, MCP-1/CCL2, MDC/CCL22 and RANTES/CCL5 in the lung (Supporting Information, Fig. S1).

House dust mite exposure promotes production of lipid mediators in the lung

Lipid mediators are important components of the inflammatory response and have been implicated in the recruitment of leucocytes to inflammatory sites. We determined the

presence of 5-HETE and LTB_4 in the lung following inhaled HDM challenge. Levels of 5-HETE and LTB_4 were increased early after HDM challenge and were maintained in the lung throughout the 24 h period post-challenge (Figs 8a and b).

Discussion

Asthma is a chronic disease characterized by AHR, lung inflammation and airway remodelling. These pathophysiological phenomena have been modelled in mice in order to determine the molecules responsible for disease pathogenesis. The majority of models rely on peripheral sensitization, usually in conjunction with a powerful adjuvant such as alum, before local challenge with soluble antigen. These models elicit robust Th2-mediated inflammation, AHR and in some cases remodelling [19–21]. More recently a model which induces chronic airway inflammation and remodelling in the absence of sensitization has been described [14, 15]. Continuous exposure of naïve mice to inhaled HDM extract was shown to promote Th2-type allergic disease. Further investigation of the model determined that the Th2-polarized response was important for the development of AHR and airway remodelling [16], however, there have been no reports of lung cytokine production. Cytokine generation has been demonstrated only after *in vitro* restimulation of splenocytes isolated from mice given inhaled HDM [14]. Similarly draining lymph nodes taken from mice after inhaled ragweed challenge and restimulated *in vitro* produced Th2 cytokines [22]. However, allergen challenge in both asthmatic patients and OVA sensitized mice is characterized by AHR and increased production of IL-4, IL-5 and IL-13 in the lung [23, 24]. Therefore, we set out to determine whether key cells and mediators of the allergic response were produced locally in the lungs after exposure to HDM concomitant with AHR.

We chose to characterize the cellular infiltrates together with inflammatory mediator production in the lungs and BAL and correlate this with an assessment of lung function over a time course following the final inhaled HDM challenge after 3 weeks of treatment (the earliest time at which we could demonstrate increased AHR). Preliminary experiments showed that cytokines were detectable in the lungs after between 1 and 3 weeks of treatment at 24 h post-exposure, and that this was dependent on the batch of HDM used. We utilized a commercially available whole HDM preparation as used by others [14] and not purified derp1 in order to mimic human pulmonary exposure to dust mites. We have determined that there is a variation in the absolute amounts of derp1 and endotoxin per milligram of total protein which, although it does not affect the generation of the asthma phenotype (airway inflammation, hyperreactivity and remodelling), may impact the time at which peak inflammation is observed (between 2 and 3 weeks). In our preliminary experiments (Fig. 1) the inflammatory infiltrate in the lungs peaked at week 2 and Th2 cytokines were detectable at 24 h post-HDM challenge with levels of IL-4, IL-5 and IL-13 returning to baseline by week 3, however, in subsequent experiments utilizing a different batch of HDM these inflammatory mediators were still detectable 24 h after the last of 15 challenges.

Significant AHR was not observed before 3 weeks of allergen exposure, but once initiated airway resistance continued to increase over the 7-week protocol of HDM challenge. We examined the lungs and BAL of HDM-treated mice for a range of leucocyte subsets and found that eosinophils were recruited to the lung within 2 h of exposure to HDM and their number then declined, although remained elevated at 24 h compared with untreated mice. In contrast the cellular influx peaked later in the BAL with maximal levels of eosinophils observed at 4 h. Eosinophil numbers in the airway lumen then remained constant over the 24 h study period. Thus, it appears that the eosinophils observed in the lung parenchyma are a transient and mobile population recruited from the bone marrow in response to HDM challenge which then traffic through the lung tissue to the airway lumen. The data presented

in the present study represent a snapshot of this cell movement. In the presence of continued allergen challenge further cells are recruited to the lung.

When we looked at T cell subsets in greater detail we found that numbers of CD4 and CD8 T cells also peaked early in the lung tissue at 2 h presumably moving to the airway lumen where they continued to accumulate post-allergen exposure with the highest levels in the BAL measured at 24 h post-challenge. A similar pattern was observed for γδ T cells. Interestingly, using the surface marker T1/ST2 as a surrogate for Th2 cells [25] we observed that Th2 cell numbers in the lung and BAL remained constant over 24 h. The percentage of $CD4^+T1ST2^+$ cells has been shown to be elevated in the lung in response to 5 weeks of HDM exposure [14]. Upon HDM challenge the percentage of these cells rose to 13.8% from 3.3% in non-allergic animals. It is clear from the present study that T cell subsets and inflammatory leucocytes accumulate rapidly in the lung and precede the increase in airway resistance which, at least at 3 weeks, is only measurable 24 h post-allergen challenge.

Although allergic airway disease is considered a classical Th2 pathway our understanding of the role of T cells in human disease and in particular the pathogenesis of asthma is undergoing revision because of newly discovered T cell phenotypes. The idea is emerging that the fate of CD4+ T cell subsets may be more flexible than previously thought and it seems there is more plasticity between the different T cell subsets *in vivo* [26–28]. For the first time in this model of allergic airways disease we have demonstrated significant increases in regulatory type cells in the lung after exposure to inhaled HDM. $CD4^+IL\text{-}17^+$ (Th17 type), $CD4^+CD25^+FoxP3^+$ and $CD4^+IL\text{-}10^+$ cells were all increased at 2 h post-challenge. In addition to allergen elicited Th2 cells, the presence of both effector and regulatory subsets in the inflammatory infiltrate further serves to illustrate the heterogeneous nature of the pulmonary lymphocyte infiltrate. $CD4^+CD25^+$ regulatory T cells have been shown to play a role in regulating allergic pathology in OVA sensitized and challenged mice [18, 29] and it will be of interest to determine the contribution of these subsets to the immunopathological features of HDM induced allergic airway disease.

Although pulmonary cytokine production has not previously been reported in mice following inhaled HDM challenge, blocking studies have shown that Th2 cytokines play an important role. IL-4 was shown to be absolutely necessary for the development of both airway remodelling and AHR [14]. Similarly blockage of IL-13 using neutralizing antibodies in either a prophylactic or even therapeutic mode was successful in reducing the inflammation, AHR and remodelling characteristic of the inhaled HDM response [30]. Although neither of these studies measured pulmonary Th2 cytokine expression, these systemic blockage studies (gene knock out mouse for IL-4 and neutralizing antibodies for IL-13) indicate that Th2 cytokines are central to the development of both the immunopathological and lung function changes following inhaled HDM challenge.

In our preliminary time course study, we showed that at 24 h post-allergen challenge Th2 cytokines were only detectable at the peak of the inflammatory response. Temporal analysis of lungs showed that Th2 cytokines were indeed measureable in both the lung tissue and BAL after inhaled HDM challenge and production peaked at 4 h post-HDM challenge in both BAL and lung tissue. By 24 h post-challenge levels in the BAL were only just above control levels.

In direct contrast to Th2 cytokine production, levels of chemokines associated with the asthmatic response were maintained throughout the 24 h period directly following challenge. In particular, there was no significant change in the levels of eotaxin-1/CCL11 or TARC/CCL17 in the lung over the 24 h time period. These chemokines have previously been shown to be important in the selective migration of eosinophils and Th2 cells to the lung

following OVA challenge and might be responsible for the continued presence of Th2 cells in the lung and BAL over the 24 h period after challenge [12, 31]. In this model it appears that mediators increase early, with Th2 cytokines decreasing over the 24 h period monitored while levels of chemokines are maintained. Interestingly the decline in cytokine levels at 24 h contrasts sharply with the increase in lung resistance which only reached significance at 24 h post-challenge. This implies that the optimum time-point for cytokine analysis occurs at a time when significant changes in lung resistance cannot yet be measured. IL-13 in particular has been implicated in the development of lung function changes [25, 32]. Interestingly direct instillation of IL-13 to the lungs of naïve mice resulted in development of AHR 72 but not 24 h later [33].

Lipid inflammatory mediators are known to be upregulated during asthma [29, 34] and for the first time we have demonstrated increases in the levels of leukotriene B4 (LTB_4) and 5-HETE in the lung in response to continuous challenge of the airways with HDM. LTB_4 is a potent lipid inflammatory mediator derived from membrane phospholipids and has classically been thought to be an important mediator during the early-phase of the asthmatic response to inhaled allergen [35]. Neutrophils, monocytes, macrophages and mast cells can all synthesize LTB_4 [36, 37] and have been shown to be present in increased numbers in the lung and BALF of HDM-treated mice in the present study. LTB_4 has been identified as being involved in the recruitment of effector T cells into the lung and has also been shown to be essential for selective eosinophil recruitment following allergen challenge of CD4+ cells in a model of chronic eosinophilic inflammation. In addition, it has an autocrine role as a potent chemoatttractant for mast cell progenitors which express the high-affinity LTB_4 receptor, BLT1 [38]. Lipoxins are trihydroxytetraene-containing eicosanoids that are generated at mucosal surfaces *via* leucocyte-epithelial cell interactions and are formed *in vivo* during multicellular responses, such as inflammation and asthma, by the action of lipoxygenases. These novel anti-inflammatory lipid mediators appear to facilitate the resolution of the acute inflammatory response [39] and 5-HETE was upregulated in the present study. The production of these mediators was observed early after inhaled HDM challenge and coordinated with the recruitment of inflammatory leucocytes to the lung. These mediators are likely to contribute to the subsequent production of cytokines and chemokines in the lungs and the ultimate development of altered airway function.

We have shown that inhaled HDM results in a Th2 cytokine profile in the lung with production of the key cytokines associated with the asthmatic response. We have determined for the first time that the peak in cytokine production occurs before significant development of airway resistance. These data have important implications for the analysis of a model that is more closely related to human asthma. These findings are of fundamental importance because mouse models are used to explore the molecular and biochemical pathways leading to immunopathophysiology. In particular, when used for delineation of mechanisms of action of potential novel therapies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by The Wellcome Trust ref #057704 (CML, SAM & LGG) VO'D and CPT are also supported by a Wellcome Trust grant.

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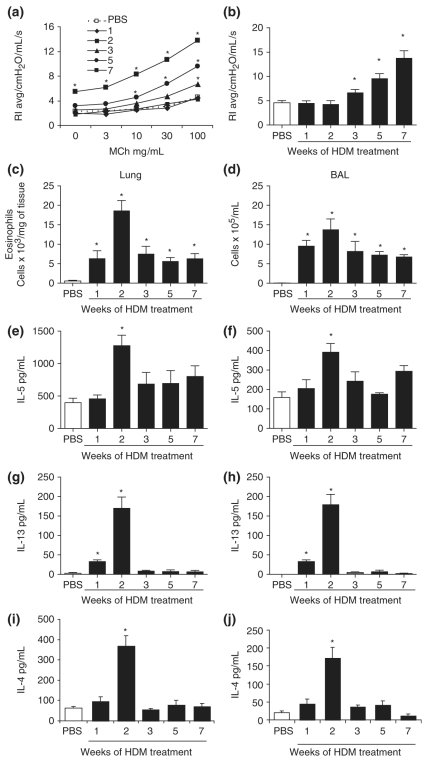

Fig. 1.

House dust mite (HDM)-elicits allergic airway inflammation. Mice were intranasally administered either phosphate-buffered saline (PBS) or 25 μg HDM once daily for 5 days, with 2 days rest, for either 1, 2, 3, 5 or 7 consecutive weeks. Mice were then analysed at these time-points 24 h post-final challenge. (a) Resistance of airways of PBS- and HDM-treated mice. (b) Airway hyperreactivity. RI average was significantly increased 3, 5 and 7 weeks post-challenge compared with PBS, in response to a single dose of methacholine (100 mg/mL). Eosinophils recovered from the (c) lung and (d) bronchoalveolar lavage (BAL) fluid. IL-5 (e and f), IL-13 (g and h) and IL-4 (i and j) measured in the lung (e, g and i) and BALF (f, h and j). Cytokine levels determined by ELISA (n = 18 mice treated with PBS (3–4 mice per group at weeks 1–7). No significant differences were observed between PBS mice at each week therefore data from these control animals were pooled and presented as a single data point for clarity (n = 5–6 mice treated with HDM at each time-point). $P<0.05$; (Mann–Whitney U-test) compared with PBS. Data are mean±SEM.

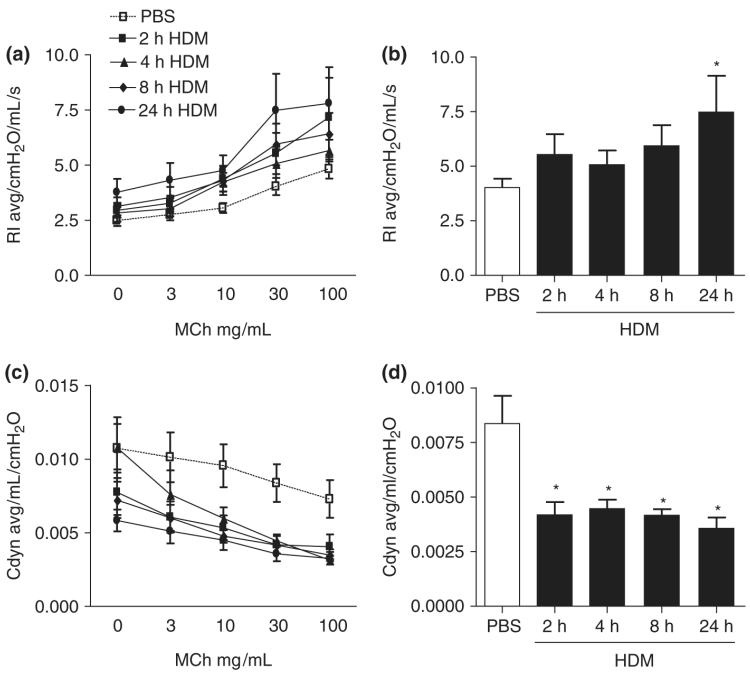

Fig. 2.

Airway hyperreactivity in house dust mite (HDM)-treated mice. (a) Airway resistance of phosphate-buffered saline (PBS)- and HDM-treated mice at 2, 4, 8 and 24 h post-final challenge. (b) Lung resistance (R_L) was significantly increased 24 h post-challenge compared with PBS, in response to 30 mg/mL methacholine. (c) Airway compliance measured at various time-points post-final challenge. (d) Lung compliance (C_{dyn}) was significantly decreased at all time-points post-challenge compared with PBS, in response to a single dose of methacholine (30 mg/mL) (n = 12 mice treated with PBS, n = 5–6 mice treated with HDM at each time-point). *P<0.05 (Mann–Whitney U-test) compared with PBS. Data are mean ±SEM.

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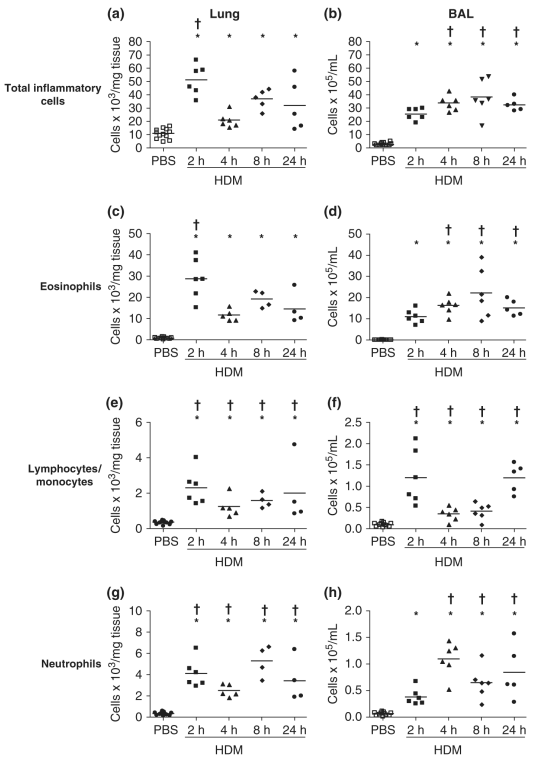

Fig. 3.

Inflammatory cell profile of house dust mite (HDM)-treated mice. Total inflammatory cells, eosinophils, lymphocytes and monocytes and neutrophils recovered from the lung (a, c, e, g) and bronchoalveolar lavage (BAL) (b, d, f, h) 2, 4, 8 and 24 h after the final intranasal challenge (n = 12 mice treated with PBS, n = 5–6 mice treated with HDM at each time-point). *P<0.05; (Mann–Whitney U-test) compared with PBS. †Time-point of peak cellular influx as determined by multiple comparisons between groups. Data averages are median values.

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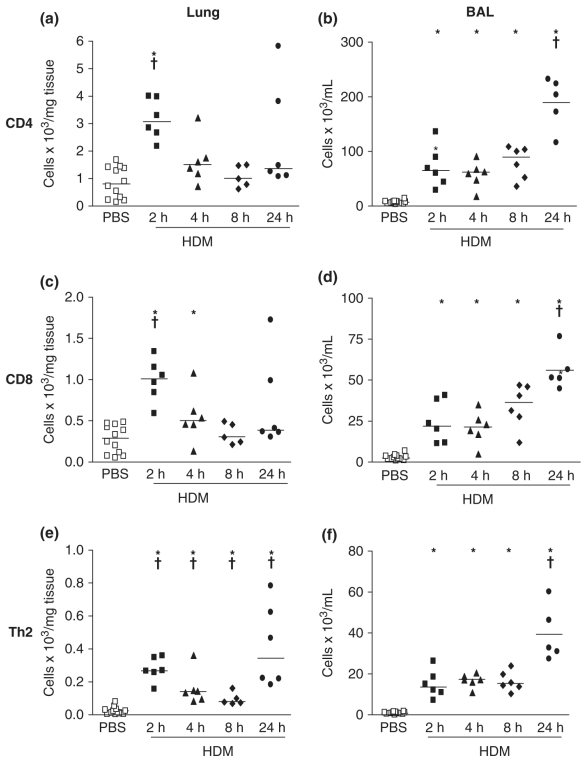

Fig. 4.

Inflammatory T cell profile. CD4, CD8 and T1ST2/CD4 positive T cells recovered from the lung (a, c, e) and BAL (b, d, f) 2, 4, 8 and 24 h after the last intranasal challenge [$n = 12$ mice treated with PBS, $n = 5–6$ mice treated with house dust mite (HDM) at each time-point]. *$P<0.05$; (Mann–Whitney U-test) compared with phosphate-buffered saline (PBS). Data averages are median values. †Time-point of peak cellular influx as determined by multiple comparisons between groups.

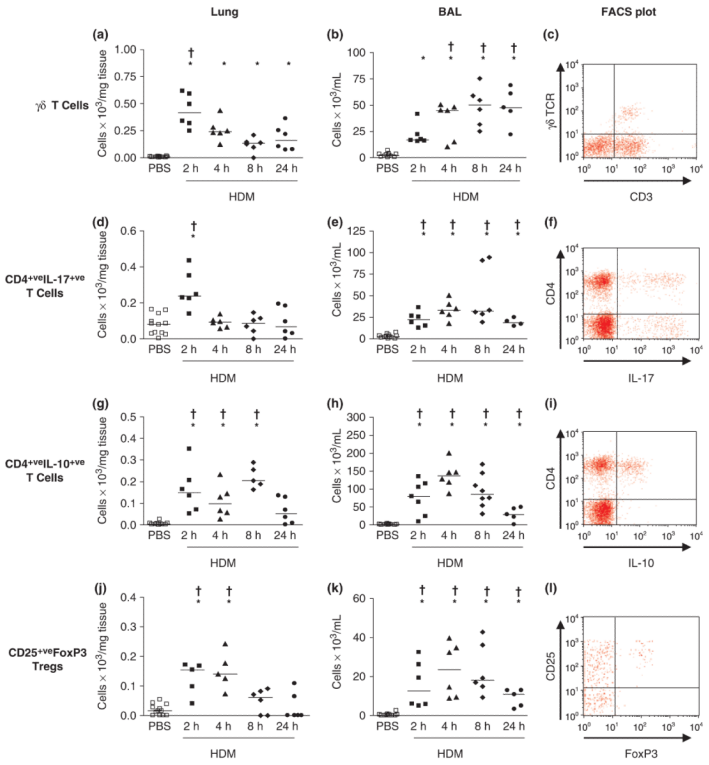

Fig. 5.

Non-classical T cell profile. γδT cells, Th17 cells, CD4, IL-10$^+$ T cells and CD25$^+$ FoxP3$^+$ regulatory T cells recovered from the lung (a, d, g, j) and the bronchoalveolar lavage (BAL) (b, e, h, k) at 2, 4, 8 and 24 h after the final intranasal challenge. A representative FACS plot from the lung cells is shown for each stain (c, CD3+/γδTCR+γδ T cells; f, CD4+/IL-17+T cells; i, CD4+/IL-10+T cells; l, CD25+/FoxP3+Tregs) (n = 12 mice treated with phosphate-buffered saline (PBS), n = 5–6 mice treated with house dust mite (HDM) at each time-point). *P<0.05; (Mann–Whitney U-test) compared with PBS. Data averages are median values. †Time-point of peak cellular influx as determined by multiple comparisons between groups.

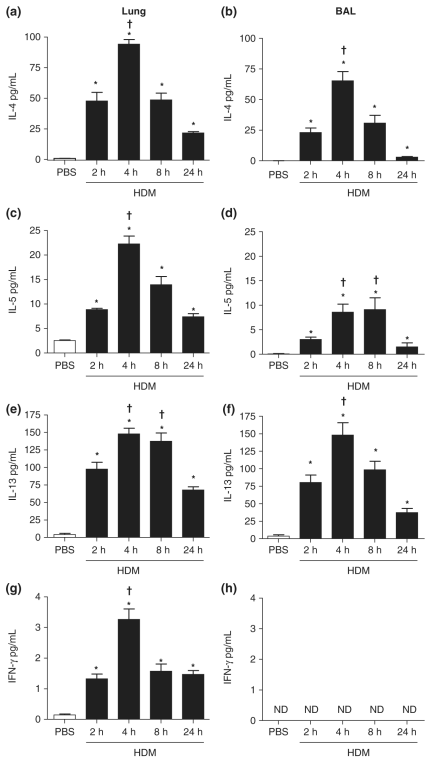

Fig. 6.

Inflammatory cytokine profile of house dust mite (HDM)-treated mice. IL-4, IL-5, IL-13 and IFN-γ measured in the lung (a, c, e, g) and BAL (b, d, f, h) 2, 4, 8 and 24 h after the final intranasal challenge. No IFN-γ was detected in the bronchoalveolar lavage (BAL) fluid. IL-4, IL-5 and IFN-γ levels were determined by MSD and IL-13 levels by ELISA (n = 12 mice treated with PBS, n = 5–6 mice treated with HDM at each time-point). *P<0.05; (Mann–Whitney U-test) compared with phosphate-buffered saline (PBS). Data are mean ±SEM. †Time-point of peak cellular influx as determined by multiple comparisons between groups.

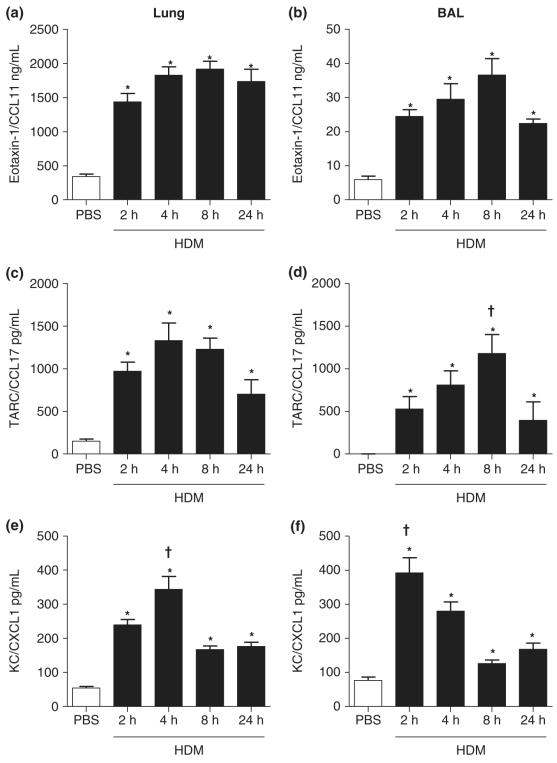

Fig. 7.

Chemokine profile of house dust mite (HDM)-treated mice. Eotaxin-1/CCL11, TARC/CCL17 and KC/CXCL1 levels measured in the lung (a, c, e) and bronchoalveolar lavage (BAL) (b, d, f) 2, 4, 8 and 24 h after the final intranasal challenge. Chemokine levels were determined by ELISA ($n = 12$ mice treated with PBS, $n = 5–6$ mice treated with HDM at each time-point). *$P<0.05$; (Mann–Whitney U-test) compared with phosphate-buffered saline (PBS). Data are mean ±SEM. †Time-point of peak cellular influx as determined by multiple comparisons between groups.

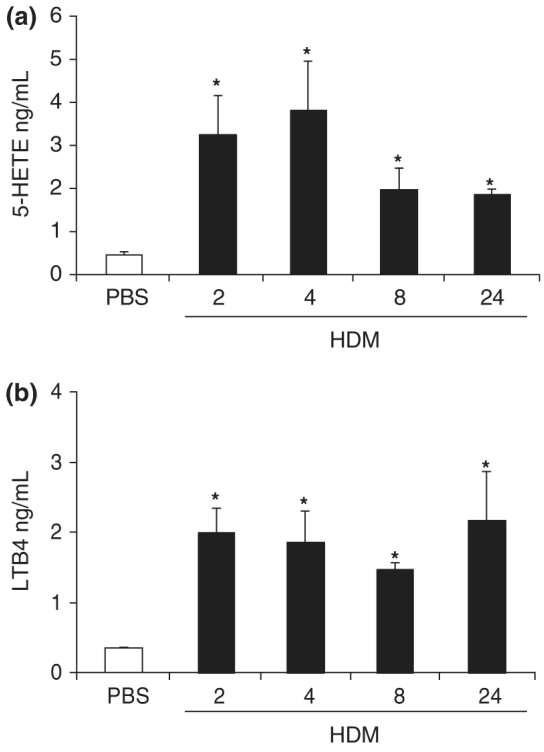

Fig. 8.

Eicosanoids are elevated during induction of house dust mite (HDM). (a) 5-HETE and (b) LTB_4 were extracted and quantified 2, 4, 8 and 24 h after the final intranasal challenge. $n = 6$ *$P<0.05$; (Mann–Whitney *U*-test) compared with phosphate-buffered saline (PBS). Data are mean SEM.

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