

IL-33 promotes the induction of immunoglobulin production after inhalation of house dust mite extract in mice

D. Canbaz^{1,*}, L. Utsch^{1,*}, A. Logiantara¹, R. van Ree^{1,2} & L. S. van Rijt¹

¹Department of Experimental Immunology, AMC; ²Department of Otorhinolaryngology, AMC, University of Amsterdam, Amsterdam, The Netherlands

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Correspondence

Leonie S. van Rijt, Department of Experimental Immunology, Academic Medical Centre, Room KO-104, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands.
Tel.: +31205668044
Fax: +31205669756
E-mail: l.s.vanrijt@amc.uva.nl

*Both authors contributed equally to this study.

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Abstract

Background: The initial immune response to house dust mite (HDM) is orchestrated by an interplay between epithelial cells (ECs) and dendritic cells (DCs). Innate cytokines released by HDM-exposed ECs activate airway DCs and effector inflammatory cells, which together induce a HDM-specific Th2 cell response. Here, we investigate the respective roles of DCs and IL-33 in sensitization to HDM.

Method: Balb/c mice were exposed via the airways to different HDM extracts, differing in at least endotoxin levels [Lotox (LT) and HiTox (HT)]. Alternatively, HDM-pulsed DCs in the presence or absence of additional LT-HDM, or administration of LT-HDM plus recombinant IL-33, were intratracheally (i.t.) administered to induce allergic airway inflammation. Eosinophil recruitment, cytokine production, serum immunoglobulins, and airway histology were analyzed.

Results: Direct exposure of airways with HT-HDM induced an eosinophilic airway inflammation, Th2 cytokine production, and an increase in total IgE and HDM IgG1, while LT-HDM was not able to do so. In contrast, i.t. instillation of LT-HDM-pulsed DCs induced a similar airway inflammation, mucus production, and cytokine production, but IgE or HDM IgG1 was not induced. Administration of HDM-pulsed DCs together with LT-HDM, to supply B cells with unprocessed antigen, was not sufficient to induce antibody production. Simultaneous administration of recombinant IL-33 with LT-HDM induced an antibody response, besides a cellular immune response.

Conclusion: These results demonstrate that HDM-pulsed DCs were able to drive a Th2 response but that IL-33 was needed to induce a humoral immune response to a single inhalational challenge to HDM.

Allergic asthma is a Th2-driven disease, which causes bronchoconstriction, airway hyper-responsiveness, mucus cell hyperplasia, elevated IgE production, and eosinophilic airway inflammation. Exposure to allergens derived from house dust mite (HDM) is a risk factor for this severe lung disease. It is estimated that 10–20% of the population is allergic to HDM (1). The underlying immunological mechanisms that are involved in the development of HDM-induced airway inflammation have been the focus of many recent studies.

The interplay between dendritic cells (DCs) and epithelial cells (ECs) has been shown to be crucial for the initial immune response to HDM (2). TLR4 signaling in ECs by

lipopolysaccharide (LPS), originating from the gastrointestinal tract of the HDM, has been demonstrated to contribute to Th2-type adaptive immune responses via activation of mucosal DCs (2–4). Activation of ECs by exposure to HDM results in the release of innate cytokines such as IL-33, TSLP, GM-CSF, and IL-25 (2). Recent evidence demonstrated that IL-33 is crucial for development of allergic airway inflammation to HDM (5–10). IL-33 has a superior ability to induce maturation and upregulation of the Th2 skewing costimulatory molecule OX40L on DCs (8). IL-33-activated DCs were shown to be more potent in inducing eosinophilic airway inflammation and mucus production in an OVA DC-driven asthma model (11). However, IL-33 is a pluripotent cytokine,

which activates other inflammatory cells, such as eosinophils, mast cells, basophils, and Th2 cells, and expands type 2 innate lymphoid cells (ILC2s), which are able to secrete IL-5 and IL-13 via ST2 receptor signaling (12).

Interestingly, administration of IL-33 is sufficient to induce eosinophil recruitment, mucus production, and airway hyper-reactivity in an immune system without T and B cells (9). Genomewide studies investigating asthma-associated alleles identified only eight alleles, which includes IL-33 receptor, IL-33, and IL-13 (13, 14). Increased levels of IL-33 are found in the plasma of patients with asthma and in the lungs of allergic mice (15, 16). The respective roles of DCs and IL-33 in the immune response to inhaled HDM still need to be further elucidated.

Here, we compared a DC-driven immune response to HDM with an IL-33-driven immune response in a mouse model for HDM-induced allergic airway inflammation. We demonstrate that a DC-driven immune response induced a clear Th2-mediated airway inflammation, but that IL-33 was needed to induce a humoral immune response after a single HDM inhalational exposure.

Materials and methods

Mice

Female Balb/c mice (6–8 weeks; Harlan, Horst, the Netherlands) were housed under specific pathogen-free conditions at the animal care facility of the AMC. All experiments were approved by the AMC Animal Ethics Committee, the Netherlands.

HDM extracts

A spent mite medium extract (*Dermatophagoides pteronyssinus*, LoTox™ LTN-DPE-4, lotnr 33019; INDOOR Biotechnologies, Cardiff, UK) and a crushed whole-body HDM extract (*Dermatophagoides pteronyssinus*, XPB82D3A2.5, lotnr 136401; Greer Laboratories, Lenoir, NC, USA) were used. The first extract will be referred to as LoTox HDM (LT-HDM) with an endotoxin level of ≤ 0.061 EU/ μ g Der p 1 and the second will be referred to as HiTox HDM (HT-HDM) containing 1.2 EU endotoxins/ μ g Der p 1, according to the manufacturer's instructions. In all experiments, 1 μ g Der p 1, which is equivalent to 6.1 μ g protein of LT-HDM and 31 μ g protein of HT-HDM, was used.

Generation, HDM pulsing, and phenotyping of bone marrow-derived DCs

Bone marrow-derived DCs were obtained as described earlier (17). On day 9 of culture, cells were pulsed overnight with 31 μ g protein/ml HT-HDM or 6.1 μ g protein/ml LT-HDM (equivalent to 1 μ g Der p 1/ml for both extracts). At day 10 of DC culture, cells were stained with MHCII-FITC, CD11c-APC, in combination with PE-labeled CD80, CD86, CD40, OX40L, or Rat IgG2a and Ham IgG (eBioscience Inc., San Diego, CA, USA). To prevent nonspecific antibody binding,

anti-FcR γ II/III antibody (2.4G2; provided by Louis Boon, Bioceros, Utrecht, the Netherlands) was added to the monoclonal antibody mixture. Propidium iodide (Sigma-Aldrich Corp., St. Louis, MO, USA) was used to distinguish between live and dead. Airway DCs were phenotyped as described before (18). Expression of costimulatory molecules were analyzed using FlowJo software (Tree Star Inc, Ashland, OR, USA). IL-6 and IL-10 production by HDM-pulsed DCs was determined in supernatants obtained at day 10 (stored at -20°C) by ELISA (Ready-SET-Go ELISA KIT; eBioscience) according to manufacturer's instructions.

Mouse models of HDM-induced eosinophilic airway inflammation

Mice were intratracheally (i.t.) exposed at day 0 to 31 μ g (protein) HT-HDM or 6.1 μ g (protein) LT-HDM (equivalent to 1 μ g Der p 1 in both extracts) and challenged intranasally (i.n.) at day 10 with the same concentration HDM extract. As a control, mice received i.t. PBS at day 0 and 31 μ g HT-HDM or PBS at day 10 (Fig. 1A). Alternatively, mice were instilled i.t. with 1×10^6 *in vitro* HT-HDM- (Fig. 1B) or LT-HDM-pulsed DCs (Fig. 1C) or as a control unpulsed DCs at day 0. One group received additionally to LT-HDM-pulsed DCs 6.1 μ g LT-HDM i.t. (Fig. 1C). To investigate the effect of IL-33, mice received i.t. either (i) LT-HDM-pulsed DCs + LT-HDM, (ii) LT-HDM-pulsed DCs + LT-HDM + 0.5 μ g IL-33 (eBioscience), (iii) LT-HDM + 0.5 μ g IL-33, or (iv) PBS (Fig. 1D). At day 10, all mice were challenged i.n. with 31 μ g HT-HDM or 6.1 μ g LT-HDM, accordingly to the extract used for sensitization. At day 13, mice were killed.

Collection and analysis of cells and tissues

Bronchoalveolar lavage fluid

Bronchoalveolar lavage fluid inflammatory cells were obtained by airway lumen lavage with 3×1 ml PBS supplemented with 0.1 mM EDTA. Cell differentiation was made by flow cytometric analysis as described earlier (19).

Ex vivo restimulation of lung-draining lymph node cells

Lung-draining lymph node cell suspensions were plated in 96-well round-bottom plates at a density of 2×10^5 cells per well and were restimulated for 4 days with 31 μ g/ml HT-HDM or 6.1 μ g/ml LT-HDM (equivalent to 1 μ g Der p 1/ml). Supernatants were analyzed for IL-4, IL-5, IL-13, IFN γ , and IL-17A production by ELISA (Ready-SET-Go ELISA KIT; eBioscience) according to manufacturer's instructions.

Serum immunoglobulins

Serum was analyzed for the level of total and HDM-specific IgE and IgG1 by ELISA (Opteia; BD, San Diego, CA, USA). A standard curve of murine IgE or IgG1 was used as a quantitative reference (20).

Airway histology

Frozen sections (5 μ m) were stained with periodic acid Schiff's reagent (Sigma-Aldrich). Degree of inflammation and

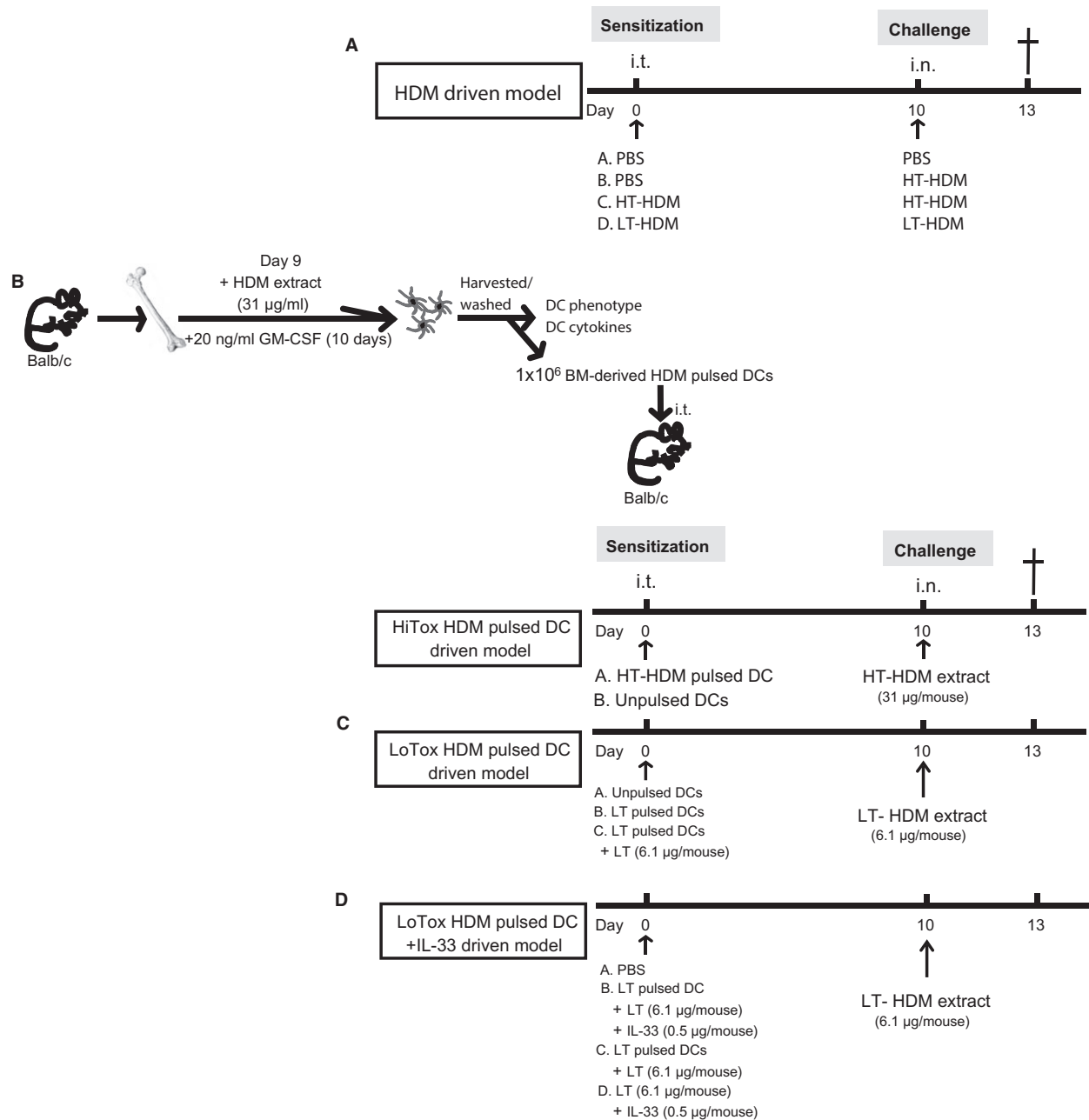


Figure 1 Study designs to investigate the immune response to house dust mite (HDM) after (A) inhalation of HT-HDM (crushed whole-body extract) or LT-HDM (spent mite medium extract), (B) after adoptive transfer into the airways of HT-HDM-pulsed BMDCs, (C) after adoptive transfer of LT-HDM-pulsed BMDCs with or without

additional LT-HDM extract, and (D) adoptive transfer of LT-HDM-pulsed BMDCs with additional LT-HDM extract with or without IL-33 in comparison with inhalational exposure to LT-HDM and IL-33 or with PBS as a control. In all models, mice were challenged with the corresponding HDM extract at day 10 and killed at day 13.

mucus-producing goblet cells were semi-quantified as described earlier (18).

Real-time PCR on lung tissue

Mice were exposed i.t. to HT-HDM, LT-HDM, HT-HDM-pulsed DCs or PBS, and 24 h later, lung tissue was macerated in Trizol (Invitrogen Life Technologies, Carlsbad, CA,

USA) to isolate total RNA. After the synthesis of cDNA using RevertAid H Minus First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, Vilnius, Lithuania), real-time PCR was performed using SYBR Green PCR Master Mix (Applied Biosystems, Warrington, UK) and 200 nM of each primer:

IL-33 FW: 5'-CTGCTTGCTTTCCTTATGCACACGT-3';

IL-33 RV: 5'-AAGAACCAAAGAAGGGGCAGAA GCT-3';

HPRT FW: 5'-AGTCCTGTGGCCATCTGCCTAGT-3';

HPRT RV: 5'-CTGGGGACGCAGCAACTGACA-3'.

All reactions were performed in optical 96-well reaction plates using StepOne Plus RealTime PCR System (Applied Biosystems). IL-33 mRNA concentrations from each sample were calculated based on the standard curve as previously described and were normalized to the concentration of the housekeeping gene HPRT (21).

Protein detection in lung tissue

Mice were exposed i.t. to HT-HDM, LT-HDM (equivalent to 1 µg Der p 1 of both extracts), 1×10^6 HT-HDM-pulsed DCs, or PBS, and 24 h later, lungs were harvested and lung homogenate was lysed by adding 1 ml Greenberger's buffer (300 mM NaCl, 15 mM Tris HCl, 2 mM MgCl₂, 2 mM, Triton (X-100), pepstatin A, leupeptin, aprotinin (all 20 ng/ml), pH 7.4) after 30 min incubating on ice. Then, the lysate was centrifuged at 500×g for 10 min and the supernatant was stored at -20°C until further use. The concentration (mg/ml) of total lung protein was measured using BCA Protein Assay Kit according to manufacturer's instruction (Pierce-Thermo Scientific, N.Meridian Rd., Rockford, USA). IL-33 protein concentration (ng/mg total protein) was measured by ELISA (Ready-SET-Go ELISA KIT; eBioscience).

Statistical analysis

For statistical analysis, the Mann-Whitney *U*-test was performed using GRAPHPAD PRISM 5 software (GraphPad Software Inc., La Jolla, CA, USA). All experiments were performed at least two times with 4–6 animals per group. Differences were considered to be significant at a *P*-value of <0.05.

Results

Differential capacity of HDM extracts to induce an immune response after inhalational challenge

The immune response of Balb/c mice upon inhalation of two different HDM extracts was compared. A crushed whole-body extract (HT-HDM) and a spent mite medium extract (LT-HDM) were used at an equal concentration of Der p 1 (1 µg), but HT-HDM contained a 20-fold higher endotoxin level. As shown by us and others before (20), mice exposed at day 0 (i.t.) and 10 (i.n.) to HT-HDM (HT/HT) developed eosinophilic airway inflammation, Th2 cytokine production, mucus production, and elevated levels of HDM-specific IgG₁, total IgE, and HDM-specific IgE production, although the last did not reach significance (Fig. 2). In contrast, exposure of the airways to LT-HDM (LT/LT) induced a milder inflammation (Fig. 2) without visible peribronchial inflammatory infiltrates, and no detectable IL-4 or antibody production. IL-5 and IL-13 production, eosinophil recruitment, and mucus production were detectable, but significantly less compared with HT-HDM exposure. A single airway exposure

(i.n.) to HT-HDM at day 10 (PBS/HT) induced a limited recruitment of eosinophils to the BAL compartment, induced IL-4, IL-5, and IL-13 production, and increased IFNγ production. PBS/HT did not result in inflammatory infiltrates, nor in mucus and antibody production (Fig. 2).

Different capacity of HDM extracts to activate BMDCs

To determine the role of DCs in the immune response to inhaled HDM, *in vitro* HDM-pulsed bone marrow-derived DCs (BMDCs) were administered i.t. to mice. First, we determined expression of costimulatory molecules by BMDCs after pulsing with HT-HDM or LT-HDM. The expression of the costimulatory molecules CD80, CD86, CD40, and to a lesser extent OX40L was increased in both LT-HDM-pulsed and HT-HDM-pulsed BMDCs compared with unpulsed BMDCs. The induction by LT-HDM was, however, less pronounced than seen in HT-HDM-pulsed DCs (Fig. 3A,B). In concordance, LT-HDM-pulsed DCs produced 13-fold less IL-6 and 30-fold less IL-10 compared with HT-HDM DCs (Fig. 3C).

Exposure of HDM-pulsed DCs induced a cellular but not a humoral immune response to HDM

Next, we administered HT-HDM-pulsed BMDC, LT-HDM-pulsed BMDCs or, as a control, unpulsed DCs to the airways of naïve Balb/c mice to induce an immune response to HDM. After 10 days, mice were challenged with a single i.n. exposure with the corresponding HT-HDM or LT-HDM extract.

As shown before by Zhang et al. and Plantinga et al. (22, 23), i.t. administration of HT-HDM-pulsed BMDCs followed by a single i.n. challenge with HT-HDM recruited more eosinophils to the bronchoalveolar compartment compared to mice instilled with unpulsed DCs (Fig. 4A, left panel). In concordance, IL-4, IL-5, IL-13, and IL-17A production in lung-draining lymph node cells was increased (Fig. 4B, left panels) and airway histology analysis showed an increase in mucus production and peribronchial inflammatory infiltrates (Fig. 4C,D, upper panels).

Although i.t. administered DCs pulsed with LT-HDM were less mature and produced less inflammatory cytokines (Fig. 3), a clear eosinophilic recruitment, mucus production, and IL-4, IL-5, IL-13 and IL-17A production was observed after a single i.n. challenge with LT-HDM (Fig. 4A–D).

Although exposure of LT-HDM DCs or HT-HDM DCs both induced a strong IL-4 production, the induction of total IgE, HDM-specific IgE (data not shown), or HDM-specific IgG was completely absent (Fig. 4E), in contrast to mice sensitized through direct airway exposure to HT-HDM extract (Fig. 2E). To investigate whether the absence of soluble unprocessed HDM, needed by B cells to be activated toward antibody production, could explain the lack of an antibody response, we co-administered LT-HDM with LT-HDM-pulsed DCs. Exposure to unprocessed soluble LT-HDM did, however, not result in induction of HDM-specific IgG₁, increase of total IgE (Fig. 4E), or induction of HDM-specific IgE (data not shown). The immunological parameters that

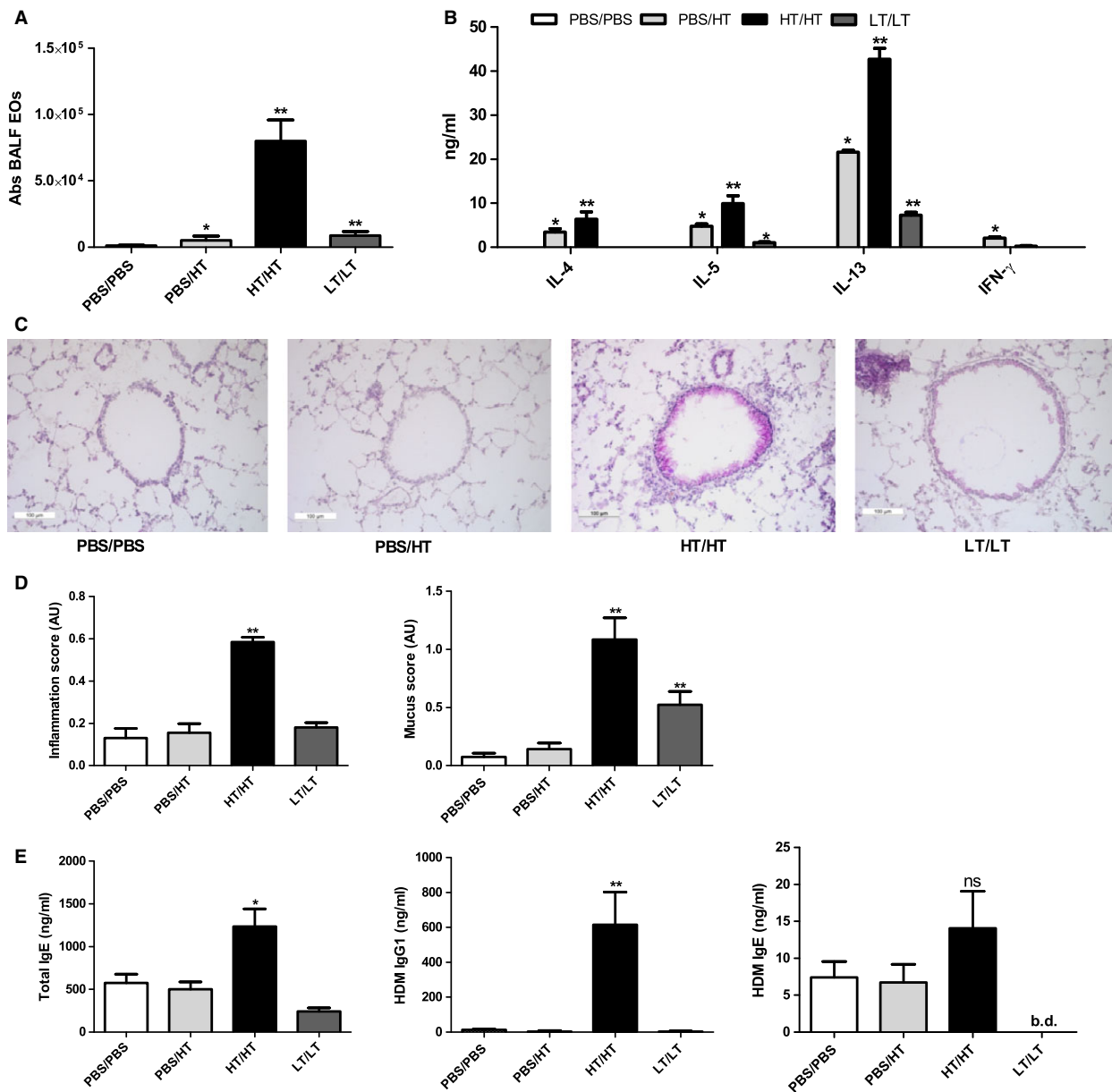


Figure 2 Mice were exposed via the airways to HT-HDM (HT/HT), LT-HDM (LT/LT), or as a control to PBS (PBS/PBS) at day 0 and 10 or as an additional control to a single intranasal exposure to HT-HDM (PBS/HT). At day 13, the recruitment of eosinophils to the BALF compartment was analyzed (A), production of Th2 cytokines IL-4, IL-5, IL-13, and IFN γ was determined in supernatants of ex vivo restimulated lung-draining lymph node cells with HDM (B).

were induced by LT-HDM-pulsed DCs such as eosinophils and Th2 cytokines were not affected by the addition of soluble LT-HDM (Fig. 4A–D).

IL-33 promotes a humoral immune response to HDM

Activation of airway epithelium by HT-HDM has been reported to induce IL-33 (3), a central innate cytokine for the

Lung slides were stained with PAS to determine peribronchial inflammatory infiltrates and mucus production (C). Peribronchial infiltrates (left graph) and mucus production (right graph) were quantified (D). Total IgE, HDM IgG1, and HDM IgE were determined in serum by ELISA (E). Data are presented as means $\pm$ SEM. B.d.: below detection limit, * $P \leq 0.05$, ** $P \leq 0.01$ vs PBS/PBS. HDM, house dust mite.

development of HDM-induced inflammation (23). As airway epithelium is not directly exposed to HDM by instillation of HDM-pulsed DCs, we compared IL-33 release upon exposure to HT-HDM to HT-HDM-pulsed DCs, to LT-HDM or to PBS. Only exposure of airway ECs to soluble HT-HDM enhanced IL-33 mRNA expression and IL-33 protein production in lung tissue. Neither HT-HDM-pulsed DCs nor LT-HDM did affect IL-33 expression (Fig. 5A).

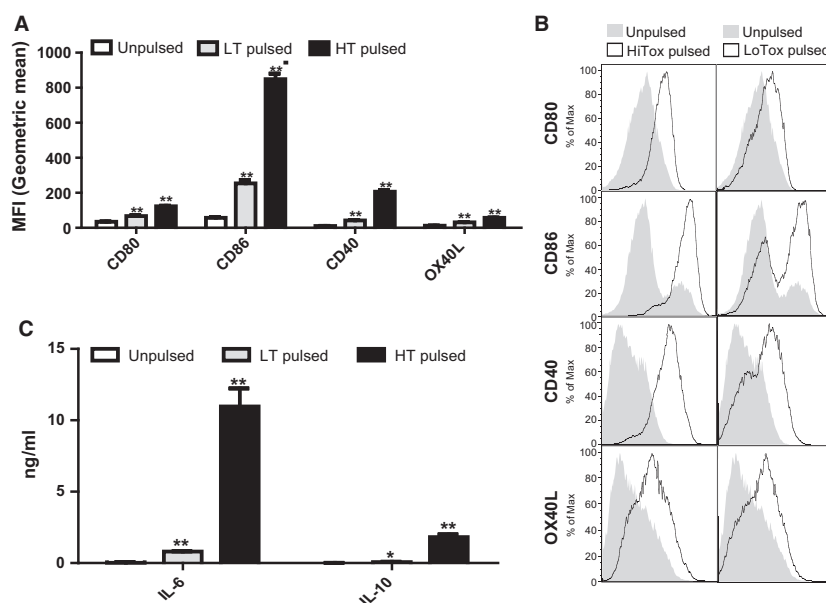


Figure 3 Bone marrow-derived DCs were pulsed overnight at day 9 with two different house dust mite (HDM) extracts, HT-HDM and LT-HDM, and were analyzed on day 10 for the expression of costimulatory molecules CD80, CD86, CD40, and

OX40L (A). The concentration IL-6 and IL-10 was determined in the supernatant by ELISA (B). Data are presented as means $\pm$ SEM. * $P \leq 0.05$, ** $P \leq 0.01$ vs unpulsed DCs and LT-HDM-pulsed DCs.

To investigate whether the lack of an IL-33 response could explain the absence of an antibody response, we investigated whether simultaneous administration of IL-33 together with soluble LT-HDM (LT/IL-33) or with LT-HDM-pulsed DCs plus soluble LT-HDM (LTDC/LT/IL-33) would result in the induction of an antibody response. Exogenously administered IL-33 together with LT-HDM-pulsed DCs (LTDC/LT/IL-33) induced HDM IgG₁ and total IgE (but not HDM IgE, data not shown), which was accompanied by an enhanced IL-4 production, increased recruitment of eosinophils, more severe peribronchial infiltration, and mucus production, in comparison with LTDC/LT-sensitized mice (Fig. 5B–F). Unexpectedly, LTDC/LT-sensitized mice had an increased total IgE compared with PBS control mice (Fig. 5F, left panel), but not in comparison with mice which received unpulsed DCs (Fig. 4E, lower left panel). This indicates that the adaptive transfer of DCs, independent of pulsing with HDM, was capable of inducing total IgE. However, HDM-specific IgG₁ was not induced in LTDC/LT-sensitized mice compared with PBS control mice. Remarkably, simultaneous administration of IL-33 and LT-HDM, without administering LT-HDM-pulsed DCs, was as efficient in inducing HDM IgG₁ and total IgE, although IL-4, IL-5, and IL-13 production and airway inflammation were lower than induced by a LTDC/LT/IL-33 sensitization (Fig. 5C–E).

Finally, we investigated whether the IL-33-facilitated immunoglobulin responses upon exposure to LT-HDM would be mediated via maturation of airway DCs. Mice were exposed i.t. to HT-HDM, LT-HDM, LT-HDM/IL-33, or as a control to PBS. Twenty-four hours later, airway MHCII-

^{hi}CD11c⁺CD3⁺CD19⁺DCs were analyzed for CD80, CD86, CD40, and OX40L. In airways exposed to HT-HDM, all measured costimulatory molecules, except OX40L, were upregulated on airway DCs, while DCs in LT-HDM- and in LT-HDM/IL-33-exposed lungs were not different from PBS-exposed airways (Fig. 6A). However, the percentage of DCs of total lung cells was increased after exposure to LT-HDM/IL-33 in a similar degree as seen after HT-HDM exposure but not in LT-HDM- or PBS-exposed lungs (Fig. 6B).

Discussion

In the present study, we report an important role for IL-33 in the induction of immunoglobulin responses upon inhalation of HDM. An IL-33-driven, but not a HDM DC driven, immune response resulted in increased levels of IgE and HDM-specific IgG₁.

Recently, much progress has been made in understanding the underlying mechanisms for IL-33 in the development of an allergic response (12). Several reports have shown a role for IL-33 via recruitment and activation of DCs (5, 8, 11). It was therefore of interest to investigate whether DCs were sufficient to induce a humoral immune response or whether IL-33 was needed.

Our study revealed that HDM-pulsed DCs were capable of inducing a Th2-mediated airway inflammation, as seen after direct exposure to HDM, but this was not accompanied by HDM-specific IgG₁ and total IgE. Intratracheal administration of HT-HDM-pulsed DCs bypasses the direct interaction of ECs with HT-HDM. In contrast to direct exposure to

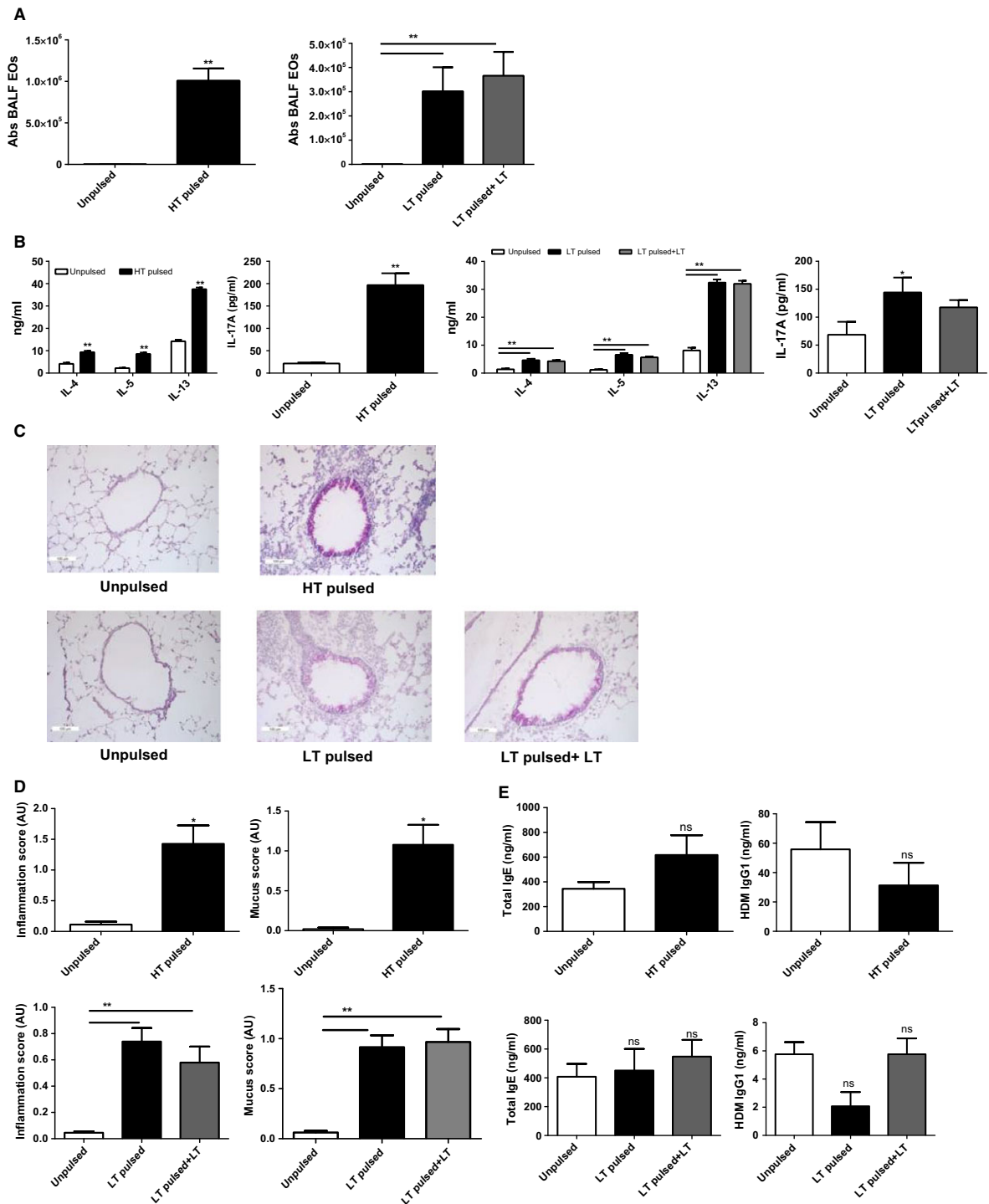


Figure 4 Mice were intratracheally instilled with HT-HDM-pulsed or LT-HDM-pulsed BMDCs. To investigate their potential to induce an immune response to inhaled HDM, mice were challenged with, respectively, HT-HDM or LT-HDM at day 10. The need for free HDM for immunoglobulin induction was investigated by the simultaneous administration of LT-HDM and LT-HDM-pulsed DCs at day 0. Eosinophil recruitment to the airways was examined at day 13 (A). Production of Th2 cytokines IL-4, IL-5, IL-13, and IL-17A was

determined in supernatants of *ex vivo* restimulated lung-draining lymph node cells with HDM (B). Lung slides were stained with PAS to determine peribronchial inflammatory infiltrates and mucus production (C). Peribronchial infiltrates (left graph) and mucus production were quantified (right graph) (D). Total IgE and HDM IgG1 were determined in serum by ELISA (E). Data are presented as means $\pm$ SEM. * $P \leq 0.05$, ** $P \leq 0.01$ vs unpulsed DCs. HDM, house dust mite.

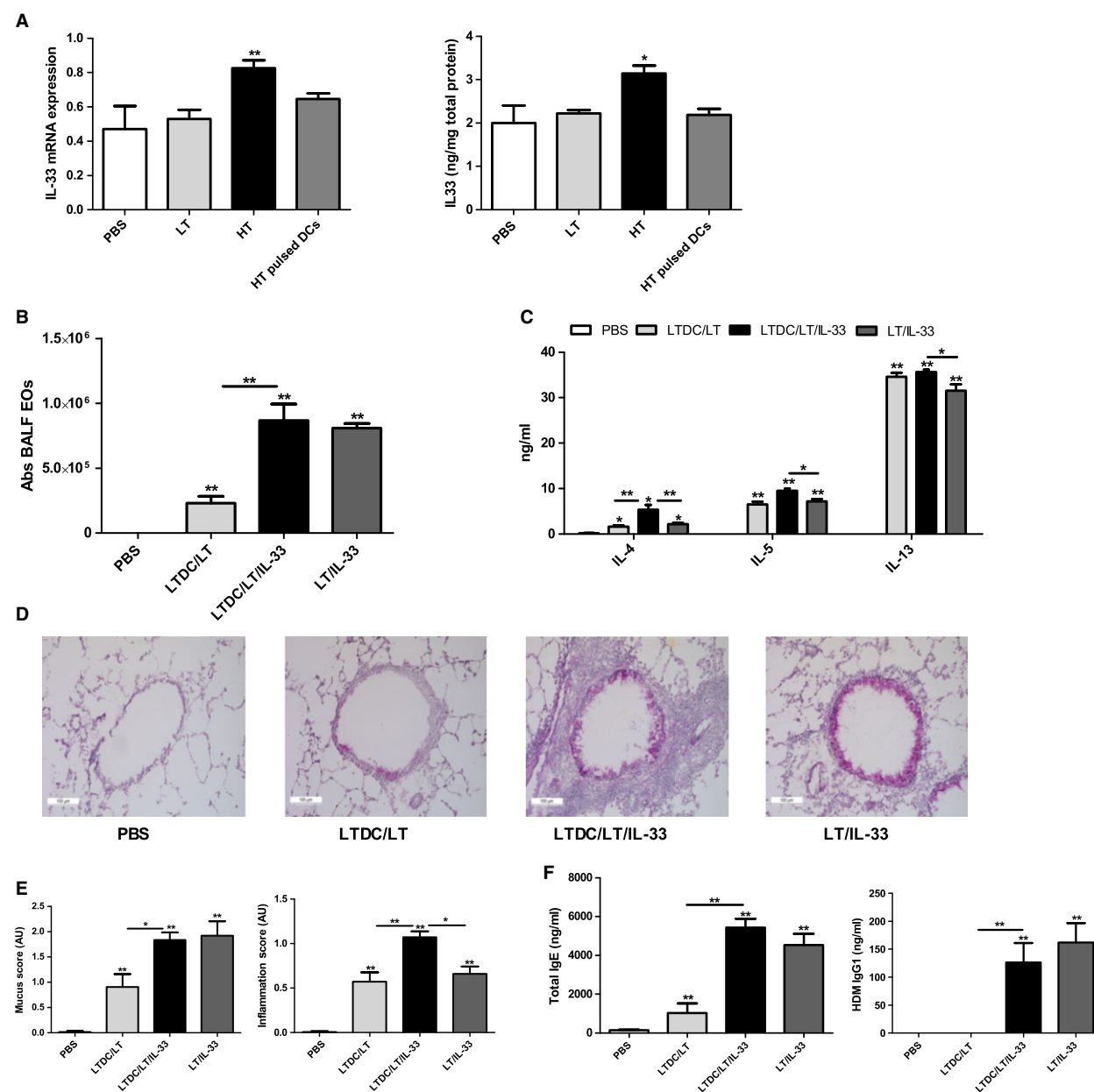


Figure 5 Mice were exposed intratracheally to PBS, LT-HDM, HT-HDM, or HT-HDM-pulsed DCs, and after 24 h, IL-33 mRNA expression and protein concentration were analyzed in the lung tissue by real-time PCR and ELISA, respectively (A). Mice were exposed at day 0 by (1) PBS, (2) LT-HDM-pulsed DCs with additional LT-HDM (LTDC/LT), (3) LT-HDM-pulsed DCs with additional LT-HDM and IL-33 (LTDC/LT/IL-33), or (4) LT-HDM and IL-33 (LT/IL-33). All mice were challenged i.n. at day 10 with LT-HDM. Eosinophil recruitment to the airways was exam-

ined at day 13 (B). Production of Th2 cytokines IL-4, IL-5, and IL-13 was determined in supernatants of *ex vivo* restimulated lung-draining lymph node cells with HDM (C). Lung slides were stained with PAS to determine mucus production and peribronchial inflammatory infiltrates (D). Mucus production was quantified (left graph) and peribronchial infiltrates (right graph) (E). Total IgE and HDM IgG₁ were determined in serum by ELISA (F). Data are presented as means $\pm$ SEM. * $P \leq 0.05$, ** $P \leq 0.01$ vs PBS. HDM, house dust mite.

HT-HDM, no IL-33 was detected in lung tissue after exposure to HT-HDM-pulsed DCs (Fig. 5A). House dust mite-pulsed DCs were also not likely to be a source for IL-33 because IL-33 could not be detected in either LT- or HT-HDM-pulsed DC culture supernatants (data not shown). We demonstrated that simultaneous exposure to IL-33, one of

the innate cytokines secreted by the epithelium after exposure to HT-HDM (2), elevated total IgE and HDM IgG₁ in serum.

Other groups have demonstrated that HDM-pulsed bone marrow or lymph node-derived DCs are able to induce Th2 immunity and in several studies, this was accompanied by a

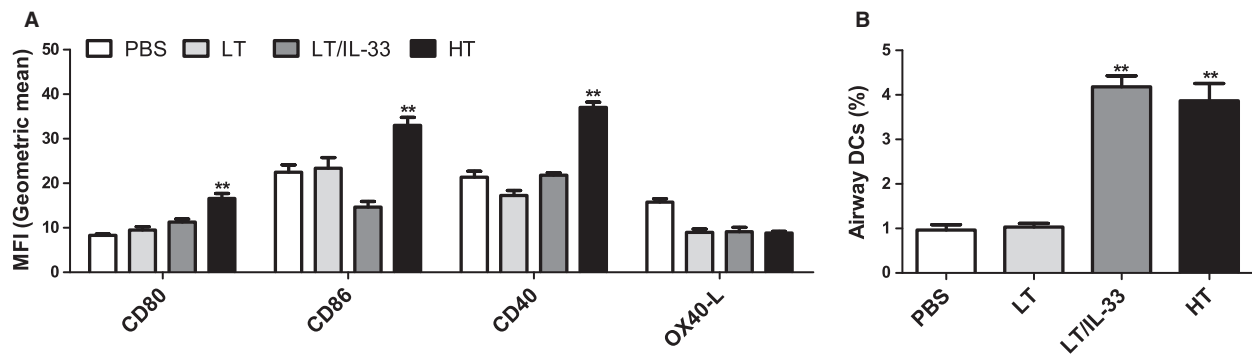


Figure 6 Mice were exposed intratracheally to PBS, LT-HDM (LT), LT-HDM with IL-33 (LT/IL-33), or HT-HDM (HT), and 24 h later, airway DCs were analyzed for expression of costimulatory molecules

CD80, CD86, CD40, and OX40L (A), and the percentage of airway DCs of total lung cells was determined (B). Data are presented as means $\pm$ SEM. ** $P \leq 0.01$ vs PBS. HDM, house dust mite.

humoral immune response. However, in most of these studies, mice were exposed to multiple mucosal challenges (6, 22–27). The repeated stimulation of airway epithelium might have induced secretion of inflammatory cytokines, such as IL-33, which was sufficient to induce an antibody response. However, Zhang et al. (22) showed an increase of total IgE after HDM-pulsed DC sensitization followed by a single HDM challenge, but they did not investigate HDM-specific IgG₁ levels. Interestingly, we observed that transfer of LT-DCs was able to increase the level of total IgE compared with PBS-transferred mice but not in comparison with mice which received unpulsed DCs (Figs 4E and 5F). This indicates that a ‘natural’ (not HDM related) IgE response was boosted by the DC transfer itself. We speculate that recruitment/activation of IL-4-producing innate cells, such as basophils, by release of chemokines by the transferred DCs might have been involved. However, sensitization with HDM-pulsed DCs did not result in a HDM-specific IgG₁ response in comparison with unpulsed DCs or in comparison with PBS control mice.

The absence of an antibody response after DC transfer could not be explained by the lack of free antigen, which is needed to stimulate B cells via their Ig-receptors. Simultaneous administration of LT-HDM and LT-HDM-pulsed DCs did not facilitate a humoral immune response, despite a strong IL-4 production. We chose LT-HDM extract, because by itself it does not induce sensitization nor IL-33 production in the lung upon mucosal exposure. In contrast, HT-HDM is capable of doing both without pulsed DCs, making it impossible to establish whether B cells require free allergen in combination with pulsed DCs for being activated (Fig. 5A).

Several reports are in support of a direct role of IL-33 in antibody production (5, 8, 28–31). Interestingly, blockade of IL-33 signaling in our direct exposure model at the time of sensitization, but not during challenge, did not affect immunoglobulin production (Fig. S1). A difference with our study is that in confirmative reports, weaker stimuli were used, compared with our direct exposure model in which we challenged mice with a high concentration HDM (100 μ g dry

weight HDM extract equivalent to 1 μ g Der p 1). In these studies, either OVA was used, an innocuous protein without proteolytic activity, or a much lower dose (1 μ g) of HDM was administered. Another difference is that in some studies, IL-33 signaling was blocked during sensitization and challenge by anti-IL-33 or that soluble ST2 (IL-33 receptor) was used. However, our data indicate that upon exposure to high concentrations of HDM, the antibody response is not exclusively regulated by IL-33. A high dose of HDM might have activated complementary pathways that compensate for the loss of IL-33 signaling, but it cannot be excluded that our protocol to block IL-33 may have played a role in the observed discrepancy with previous reports.

IL-33 has been described to affect the inflammatory milieu in many ways, such as recruitment and activation of airway DCs potentiating Th2 help for B cells (8, 11). Simultaneous exposure of IL-33 and LT-HDM induced an increased recruitment of DCs to the airways and enhanced IL-4 production compared with exposure to LT-HDM alone. This might have contributed to the induction of the humoral immune response by IL-33. As an adoptive transfer of HDM-pulsed DCs was not sufficient to induce a humoral immune response, despite a strong IL-4 production, IL-33 might have activated an additional pathway. IL-33 has also been described to increase CD40L expression on CD4⁺ T cells which could have contributed to the induction of immunoglobulin production by B cells (32). An alternative pathway for IL-33-enhanced Th2 help for B cells could be via IL-33 that recruited and activated innate cells, such as ILC2s, eosinophils, and mast cells, which can provide a source of IL-4 and in case of ILC2s can interact with B cells via ICOS-ICOSL expression (8, 32–34). The rapid immune response that we observed after a single exposure to HT-HDM suggests that the epithelial release of several chemo attractants (35), such as IL-33, recruited innate inflammatory cells which might have been crucial for the induction of immunoglobulins during the secondary immune response to inhaled HT-HDM. The involvement of innate cells in the immunoglobulin production by B cells will have to be elucidated in future experiments.

In conclusion, our data indicate that a DC-mediated Th2 response was not able to induce a humoral immune response after a single HDM exposure but that IL-33 was sufficient.

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Conflicts of interest

All authors declare that they have no conflicts of interest.

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Author contributions

DC, LU, RvR, and LvR were involved in conception and design of the study. DC, AL, and LvR were involved in the acquisition of data.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Mice were treated at day 0, with 50 μ g anti-IL-33 (or isotype control; both R&D Systems Europe, Abingdon, UK) i.p. 30 min before i.t. administration of 100 μ g HT-HDM together with 10 μ g anti-IL-33 (or isotype control).

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