Tnfaip3 expression in pulmonary conventional type 1 Langerin-expressing dendritic cells regulates T helper 2-mediated airway inflammation in mice

Heleen Vroman1,2 | Denise van Uden1 | Ingrid M. Bergen1 | Jennifer A. C. van Hulst1 | Melanie Lukkes1 | Geert van Loo2,3 | Björn E. Clausen4 | Louis Boon5 | Bart N. Lambrecht1,2,6 | Hamida Hammad2,7 | Rudi W. Hendriks1 | Mirjam Kool1

1Department of Pulmonary Medicine, Erasmus MC, Rotterdam, The Netherlands
2VIB Center for Inflammation Research, Ghent, Belgium
3Department of Biomedical Molecular Biology, Ghent University, Ghent, Belgium
4Institute for Molecular Medicine, University Medical Center of the Johannes Gutenberg-University Mainz, Mainz, Germany
5BioceroS, Utrecht, The Netherlands
6Department of Respiratory Medicine, Ghent University, Ghent, Belgium
7Department of Internal Medicine, Ghent University, Ghent, Belgium

Correspondence
Mirjam Kool, Department of Pulmonary Medicine, Erasmus MC, Rotterdam, The Netherlands.
Email: m.kool@erasmusmc.nl

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Abstract
Background: Conventional type 1 dendritic cells (cDC1s) control anti-viral and anti-tumor immunity by inducing antigen-specific cytotoxic CD8+ T-cell responses. Controversy exists whether cDC1s also control CD4+ T helper 2 (Th2) cell responses, since suppressive and activating roles have been reported. DC activation status, controlled by the transcription factor NF-κB, might determine the precise outcome of Th-cell differentiation upon encounter with cDC1s. To investigate the role of activated cDC1s in Th2-driven immune responses, pulmonary cDC1s were activated by targeted deletion of A20/Tnfaip3, a negative regulator of NF-κB signaling.

Methods: To target pulmonary cDC1s, Cd207 (Langerin)-mediated excision of Tnfaip3 was used, generating Tnfaip3fl/flxCd207+/cre (Tnfaip3Lg-KO) mice. Mice were exposed to house dust mite (HDM) to provoke Th2-mediated immune responses.

Results: Mice harboring Tnfaip3-deficient cDC1s did not develop Th2-driven eosinophilic airway inflammation upon HDM exposure, but rather showed elevated numbers of IFNγ-expressing CD8+ T cells. In addition, Tnfaip3KO mice harbored increased numbers of IL-12-expressing cDC1s and elevated PD-L1 expression in all pulmonary DC subsets. Blocking either IL-12 or IFNγ in Tnfaip3KO mice restored Th2 responses, whereas administration of recombinant IFNγ during HDM sensitization in C57Bl/6 mice blocked Th2 development.

Conclusions: These findings indicate that the activation status of cDC1s, shown by their specific expression of co-inhibitory molecules and cytokines, critically contributes to the development of Th2 cell-mediated disorders, most likely by influencing IFNγ production in CD8+ T cells.
INTRODUCTION

T helper (Th) 2-mediated diseases such as allergic asthma affect people all over the world. Th2 cytokines facilitate the classical allergic response, such as IgE class switching by B cells (IL-4), eosinophilic inflammation (IL-5), and goblet cell hyperplasia (IL-13). Dendritic cells (DCs) are potent antigen-presenting cells that induce the differentiation of naïve Th cells into Th2 cells or various other T helper cell subsets. Based on surface markers and transcription factor expression, two main conventional DC (cDC) subsets can be identified: type 1 cDCs (cDC1s) and cDC2s. During inflammation, a third population of monocyte-derived DCs (moDCs) arises. IRF-4–dependent cDC2s are considered to be efficient at priming CD4+ T cells through MHC class II-restricted antigen (Ag) presentation. Upon allergen inhalation, cDC2s drive Th2 differentiation, whereas moDCs maintain Th2 inflammation through local secretion of chemokines. cDC1s have superior Ag-cross-presenting capabilities and play pivotal roles in antiviral and antitumor immunity through the induction of antigen-specific cytotoxic CD8+ T-cell responses. Conflicting data exist concerning cDC1 function in Th2-mediated diseases, whereby cDC1s were reported to either suppress allergic airway inflammation, be redundant, or essential for Th2 immune responses. During experimental helminth infection, cDC1s limit the amplitude of Th2 immune responses through their IL-12 production.

DC activation is needed for proper Th-cell differentiation into Th1, Th2, and Th17 cells. Although there is evidence that pattern recognition receptors (PRRs) on lung epithelial cells contribute to allergic airway inflammation, activation of DCs in Th2-mediated diseases also often occurs through allergen-mediated triggering of PRRs on DCs. PRR triggering on DCs activates the transcription factor NF-κB, which initiates transcription of pro-inflammatory cytokines. NF-κB activation is negatively regulated by TNFAIP3 (TNFα-induced protein 3, also known as A20), a ubiquitin-modifying enzyme that can (de)ubiquitinate several NF-κB signaling molecules to terminate NF-κB activation. Several models of DC-specific deletion of TNFAIP3 in CD11c+ cells have shown that this molecule limits DC activation, and in the absence of TNFAIP3, DCs produce higher levels of cytokines, express higher co-stimulatory molecules, resist to cell death, and activate auto-reactive T- and B-cell responses. TNFAIP3 is also implicated in allergic disorders in humans, as genetic polymorphisms in the TNFAIP3 and TNFAIP3 interacting protein (TNIP) loci have been associated with asthma and allergies. Also, reduced TNFAIP3 expression in peripheral blood mononuclear cells was observed in asthmatic children compared healthy controls. Recently, we found that increasing the activation status of DCs by ablation of the Tnfaip3 gene in myeloid cells induced a neutrophilic inflammation in a house dust mite (HDM)-mediated murine asthma model, which was accompanied by elevated numbers of Th17-cells.
However, whether TNFAIP3 levels in cDC1s affect the development of asthma is currently unknown. Therefore, in this study, we investigated whether TNFAIP3 depletion in cDC1s would affect their activation status and thereby affect asthma development. To this end, we crossed Tnfaip3 floxed mice\textsuperscript{22} with a transgenic mouse line that expresses Cre under the control of the Langerin (Cd207) promoter, which targets Langerin-expressing cDC1s,\textsuperscript{28} and performed HDM-driven allergic airway inflammation experiments. Our data indicate that TNFAIP3 depletion in Langerin-expressing cDC1s inhibited the Th2-mediated immune responses. This was associated with an increase in the production of the Th2-suppressive cytokine IL-12 by cDC1s, leading to increased IFN$\gamma$ production by CD8$^+$ T cells. Increased production of IFN$\gamma$ subsequently increased the expression of the co-inhibitory molecule PD-L1 on all pulmonary DC subsets. Strikingly, administration of IFN$\gamma$ only during HDM sensitization was already sufficient to abrogate Th2 development and eosinophilic inflammation.

2 | MATERIALS AND METHODS

2.1 | Mice

Tnfaip3$^{fl/fl}$ mice\textsuperscript{22} were crossed to Cd207\textsuperscript{CRE/+} (Langerin-CRE) mice\textsuperscript{28} to generate Tnfaip3$^{−/−}$ mice. Tnfaip3$^{−/−}$ mice were crossed to Rosa26-stop-EYFP reporter (ROSA26$^{EYFP}$) mice. Mice were backcrossed to the C57BL/6 genetic background for at least six generations. Mice were housed and bred under SPF conditions at the Erasmus MC and analyzed at 6-12 weeks of age. All experiments were performed with approval by the animal ethics committee of the Erasmus MC.

2.2 | HDM-induced allergic airway inflammation

During HDM exposures, mice were anesthetized using isoflurane. Mice were sensitized intranasally (i.n.) with 1 μg/40 μL HDM (Greer) or 40 μL PBS as a control on day 1 (GIBCO Life Technologies), and challenged i.n. on days 7-11 with 10 μg/40 μL HDM.\textsuperscript{7} Mice were killed on day 15. Bronchoalveolar lavage (BAL) was obtained by flushing the lungs three times with 1 mL PBS containing 0.5 mM EDTA (Sigma-Aldrich). Lungs were either inflated with PBS/OCT (1:1) solution and placed in 4% PFA and embedded in paraffin or single-cell suspensions were prepared.

2.3 | HDM-induced acute immune responses

For the induction of an HDM-mediated acute innate response, mice were treated with 100 μg/80 μL of HDM extract intra-tracheally (i.t.). Twenty-four hours later, single-cell suspensions were obtained from lungs by digesting the lungs using DNase (Sigma) and Liberase TM (Roche) for 30 minutes at 37°C.\textsuperscript{7} After digestion, the lungs were homogenized through a 100 μm cell strainer (BD Biosciences). Red blood cells were lysed using osmotic lysis buffer (8.3% NH$_4$Cl, 1% KHCO$_3$, and 0.04% NA$_2$EDTA in Milli-Q). Cell suspensions were prepared and used for flow cytometry procedures.

2.4 | Antibody treatment of mice during HDM-induced allergic airway inflammation

To study the effect of IL-12, IFN$\gamma$, and PD-L1 on development of HDM-induced allergic airway inflammation in Tnfaip3$^{−/−}$ mice, mice were treated i.p. with 500 μg anti–IL-12p40 (clone C17/8), 500 μg anti–IFN$\gamma$ (clone XMG1.2) antibodies, 250 μg anti–PD-L1 (clone MIH5) antibodies or with monoclonal antibody β-galactosidase (GL113) as isotype control, 11, 7, 4, and day prior to 1 μg/40 μL HDM sensitization on day 0, and at days 0, 3, 7, and 10 during the HDM-induced allergic airway inflammation model. Fifty ng recombinant IFN$\gamma$ (R&D systems) was administered together with 1 μg/40 μL HDM sensitization on day 0. Mice were killed on day 15 after HDM sensitization. For the HDM-induced acute immune response, mice were treated with monoclonal antibodies or isotype control 10, 7, 4, and 1 day prior to 100 μg/80 μL HDM administration on day 0. Mice were killed on day 1.

2.5 | Flow cytometry

Single-cell suspensions were prepared from bronchoalveolar lavage (BAL) and MLN using standard procedures. MLNs were homogenized through a 100 μm cell strainer (BD Biosciences). Flow cytometry surface and intracellular staining procedures have been described previously.\textsuperscript{23} Monoclonal antibodies used for mouse flow cytometric analyses are listed in Table S1. In all experiments, dead cells were excluded using Fixable viability dye (eBioscience). To measure cytokine production by T cells, cells were stimulated at 37°C using 10 ng/mL PMA (Sigma-Aldrich), 250 ng/mL ionomycin (Sigma-Aldrich) and GolgiStop (BD Bioscience), for 4 hours. To measure cytokine production by DCs, cells were incubated at 37°C in the presence of GolgiPlug (BD Biosciences) for 4 hours. Data were acquired using an LSR II flow cytometer (Beckton Dickinson) and FACS software (Beckton Dickinson) and analyzed by FlowJo version 9 (Tree Star Inc software).

2.6 | Lung histology

Five-um-thick paraffin-embedded lung sections were stained with periodic acid and Schiff’s reagents to visualize mucus-producing cells.

2.7 | ELISA

Total IgE and HDM-specific IgG1 were measured in serum (Opteia, BD Biosciences).
FIGURE 1  Depletion of TNFAIP3 in Langerin⁺ cDC1s increased PD-L1 expression and pulmonary DC numbers. (A) Flow cytometry histograms of lung DC subsets in C57BL/6 mice showing CD207 expression in cDC1s, cDC2s, moDCs, and pDCs. (B) Quantification of the percentage CD207 expression in different DC subsets. (C) Flow cytometric histograms of EYFP expression in lung DC subsets of Langerin-cre x Rosa26-stop⁺ EYFP mice. (D) Quantification of the percentage EYFP expression in different DC subsets by flow cytometry in BAL, lung, and MLN. (E) EYFP expression in different DC subsets in the lung of Tnfaip3⁺ WT Rosa26-stop⁺ EYFP and Tnfaip3⁺ KO Rosa26-stop⁺ EYFP mice. (F) Quantification of mean fluorescent intensity of MHCII, CD86, and PD-L1 in lung cDC1s of Tnfaip3⁺ WT mice (n = 6) and cDC1s of Tnfaip3⁺ KO (n = 4) by flow cytometry. (G) Quantification of PD-L1 in EYFP⁺ and EYFP⁻ cDC1s in the lung of Tnfaip3⁺ WT x Rosa26-stop⁺ EYFP (n = 6) and Tnfaip3⁺ KO x Rosa26-stop⁺ EYFP mice (n = 4). Results are presented as mean ± SEM of n = 2-7 per group and representative of two independent experiments.
2.8 | Statistical analysis

Mann-Whitney U tests were used for comparison between two relevant groups, which either differ only in genotype of the mice, or in the protocol/treatment received, and a P-value of <.05 was considered statistically significant. Analysis was determined using Prism (GraphPad Software).

3 | RESULTS

3.1 | Depletion of TNFAIP3 in Langerin+ cDC1s increases PD-L1 expressing lung DCs

Langerin (CD207) is expressed in Langerhans cells in the skin and in a proportion of pulmonary cDC1s. Consistent with other
FIGURE 3  TNFAIP3 deletion in cDC1s increases PD-L1 and IL-12 expression in all pulmonary DC subsets, together with increased IFNγ expression by CD8+ T cells. (A) Flow cytometric histogram showing PD-L1 expression in pulmonary DCs in Tnfaip3−/− mice upon a single provocation of PBS or 100 μg HDM. (B) Quantification of MFI of PD-L1 in cDC1s, cDC2s, moDCs, and pDCs of PBS or HDM-treated Tnfaip3−/− (n = 6) or Tnfaip3−/− n = 4) mice by flow cytometry. (C) Flow cytometric histogram of MHCII expression in pulmonary DCs upon PBS or HDM treatment. (D) Quantification of MFI of MHCII in cDC1s, cDC2s, moDCs, and pDCs of PBS or HDM-treated Tnfaip3−/− (n = 6) or Tnfaip3−/− KO (n = 4) mice by flow cytometry. (E) Flow cytometric gating of pulmonary IL-12-secreting cDC1s. (F) Percentage of IL-12 secreting cDC1s in lungs of PBS or HDM-treated Tnfaip3−/− (n = 6) or Tnfaip3−/− (n = 6) mice by flow cytometry. (G) Proportion of IFNγ+ CD4+ and CD8+ T cells in lungs of Tnfaip3−/− mice 24 h after single PBS or HDM provocation by flow cytometry. (H) Quantification of the number of IFNγ+ CD4+ and CD8+ T cells in BAL of Tnfaip3−/− (n = 6) and Tnfaip3−/− KO (n = 4) mice after HDM sensitization and challenge. (I) Quantification of the total numbers of IFNγ-producing cells in BAL of HDM-sensitized and HDM-challenged Tnfaip3−/− WT mice (n = 6) and Tnfaip3−/− KO mice (n = 6). (J) Distribution of IFNγ-expressing cells in the BAL of HDM-sensitized and HDM-challenged Tnfaip3−/− WT mice (n = 6) and Tnfaip3−/− KO mice (n = 6). Results are presented as mean ± SEM of n = 2-6 mice per group and representative of two or more independent experiments. *P < .05, **P < .01
publications, we also found that Langerin was expressed by 15% of cDC1s in the lung, whereas in other pulmonary DC subsets, Langerin expression was not detected (Figure 1A,B). To
determine whether Langerin-Cre-mediated targeting of cDC1s reflects CD207 expression, we crossed Langerin-Cre mice to Rosa26-stop\textsuperscript{IEYFP} mice (Langerin-Cre x Rosa26-stop\textsuperscript{IEYFP} mice).
DC subsets were examined according to the gating strategy as shown in Figure S1. In the lungs and lung-draining mediastinal lymph node (MLN) of naive mice at the age of 6–8 weeks, approximately 10% of the cDC1s expressed EYFP, whereas in the bronchoalveolar lavage (BAL), 35% of cDC1s expressed EYFP (Figure 1C,D). Expression of EYFP in other DC subsets in BAL, lung, or MLN was below 3%. TNFAIP3 deletion did not affect EYFP expression as the proportions of EYFP-expressing pulmonary DCs were not different between Tnfaip3−/− mice by i.p. injections with anti–IL-12p40 and anti–IFNγ on days −10, −7, −4, and −1. Tnfaip3−/− mice were treated i.t. with PBS or 100 μg HDM on day 0. Analysis was performed on day 1. (B–C) Quantification of MFI of PD-L1 expression in pulmonary total DCs (B) and DC subsets (C) by flow cytometry. Results are presented as mean ± SEM of n = 3–4 mice per group and representative of two independent experiments. *P < .05, **P < .01.

3.2 | Th2-mediated HDM-induced airway inflammation is reduced in Tnfaip3Le−/− mice

To investigate the effects of cDC1-specific TNFAIP3 deficiency on Th2-cell differentiation, we exposed Tnfaip3Le−/− mice to inhaled HDM to induce allergic airway inflammation (Figure 2A). As previously reported,31 HDM sensitization followed by repetitive HDM challenge increased eosinophils, B cells, and T cells in the BAL as compared with PBS sensitization in WT mice (Figure 2B,C). Strikingly, HDM-sensitized and HDM-challenged Tnfaip3Le−/− mice had reduced eosinophil and T-cell numbers in the BAL compared to Tnfaip3Le−/− mice, whereas the numbers of neutrophils, macrophages, and B cells were unchanged (Figure 2B,C). Th2 cytokine-secreting CD4+ T cells were increased in HDM-sensitized Tnfaip3Le−/− mice, whereas IL-5+ and IL-13+ T cells in HDM-sensitized Tnfaip3Le−/− were not
elevated as compared with PBS-sensitized controls. In this model, IL-17 was not induced in Th cells (Figure 2E). Serum IgE increased upon HDM sensitization and challenge in Tnfaip3<sup>3<sup>le-KO</sup> mice, but not in Tnfaip3<sup>3<sup>le-WT</sup> mice, compared to PBS-sensitized control mice (Figure 2F). Accordingly, upon immunohistochemistry analysis of the lungs, HDM-sensitized Tnfaip3<sup>3<sup>le-KO</sup> mice showed no lung inflammation and mucus production, which were readily observed in Tnfaip3<sup>3<sup>le-WT</sup> mice (Figure 2G). As Th2-mediated airway inflammation was hampered in HDM-sensitized Tnfaip3<sup>3<sup>le-KO</sup> mice, we wondered whether anti-inflammatory cells would be increased in HDM-treated Tnfaip3<sup>3<sup>le-KO</sup> mice. MLNs of both PBS- and HDM-sensitized Tnfaip3<sup>3<sup>le-KO</sup> mice had increased proportions of Foxp3<sup>+</sup>CD25<sup>+</sup> Tregs compared to PBS- and HDM-sensitized Tnfaip3<sup>3<sup>le-WT</sup> mice, respectively (Figure S5). However, this increase in Tregs in Tnfaip3<sup>3<sup>le-KO</sup> mice was not responsible for the reduced Th2-driven immune responses, as Tnfaip3<sup>3<sup>le-KO</sup> mice depleted of Tregs by an anti-CD25-depleting antibody (PC61), still failed to develop Th2-mediated inflammation (Figure S6).

Taken together, these findings indicate that TNFAIP3 deletion in cDC1s hampers the induction of eosinophilia, Th2 cytokine-producing T cells, and increased serum IgE upon HDM exposure. Furthermore, Tnfaip3<sup>3<sup>le-KO</sup> mice had higher numbers of anti-inflammatory Tregs following HDM exposure. Depleting Tregs in Tnfaip3<sup>3<sup>le-KO</sup> mice did not restore Th2-mediated inflammation, indicating that Tregs are not essential for the suppression of Th2-mediated inflammation by TNFAIP3-deficient cDC1s.

### 3.4 IFNγ expression is increased by T cells of Tnfaip3<sup>3<sup>le-KO</sup> mice</p>

As IL-12 expression by cDC1s and PD-L1 in all DC subsets was increased in PBS-treated and HDM-treated Tnfaip3<sup>3<sup>le-KO</sup> mice, we wondered whether this was accompanied by induction of IFNγ expression in T cells upon HDM exposure, as PD-L1 is a type I interferon-inducible protein. We first investigated whether IFNγ expression was already induced upon a single HDM exposure. Both the proportion of IFNγ-producing CD4<sup>+</sup> and CD8<sup>+</sup> T cells were already augmented in lungs of PBS-treated Tnfaip3<sup>3<sup>le-WT</sup> mice as compared to control mice (Figure 3G). HDM exposure specifically induced IFNγ-producing CD8<sup>+</sup> T cells in both Tnfaip3<sup>3<sup>le-WT</sup> and Tnfaip3<sup>3<sup>le-KO</sup> mice (Figure 3G). In contrast, in the acute HDM-driven allergic inflammation model IFNγ expression in CD4<sup>+</sup> T cells was not induced upon HDM sensitization, whereas the number of IFNγ<sup>+</sup> CD8<sup>+</sup> T cells was specifically increased in BAL of HDM-sensitized Tnfaip3<sup>3<sup>le-KO</sup> mice, whereas this was not observed in Tnfaip3<sup>3<sup>le-WT</sup> mice (Figure 3G,H).

Next, we evaluated whether the increase in IFNγ expression was specific for CD8<sup>+</sup> T cells, and not by other cells, by gating first all IFNγ expressing cells (Figure S9). The total amount of IFNγ expressing cells in the BAL was not different between HDM-sensitized Tnfaip3<sup>3<sup>le-WT</sup> and Tnfaip3<sup>3<sup>le-KO</sup> mice (Figure S3). Also, of all IFNγ expressing cells, the majority is expressed by CD4<sup>+</sup> T cells, but only the expression of IFNγ in CD8<sup>+</sup> T cells was increased in HDM-sensitized and HDM-challenged Tnfaip3<sup>3<sup>le-KO</sup> mice (Figure S3). Also, IFNγ expression by other cells, that are not T cells, which could include...
ILCs or macrophages was decreased in BAL of HDM-sensitized and HDM-challenged Tnfaip3-/-KO mice (Figure 3J).

In conclusion, these data indicate that mice harboring TNFAIP3-deficient Langerin+ cDC1s especially displayed increased numbers of IFNg-producing CD8+ T cells upon either a single HDM exposure or repetitive exposure with HDM.

3.5 | Blockade of IFNg restores eosinophilic airway inflammation in Tnfaip33/-KO mice

Both the numbers of IL-12-expressing cDC1s and IFNg-producing CD8+ T cells were enhanced in Tnfaip33/-KO mice. Previous work demonstrated that both of these populations can efficiently suppress Th2 immune responses. Accordingly, we found that when we exposed mice to recombinant IFNg during HDM sensitization, eosinophilia was significantly reduced (data not shown). To determine whether IL-12 or IFNg were crucial in the suppression of Th2-mediated eosinophilic airway inflammation in Tnfaip33/-KO mice, we used antibodies to block IL-12p40 or IFNg starting 10 days before antigen sensitization in the HDM-induced allergic airway inflammation model (Figure 4A). Blocking IFNg completely restored eosinophilic, neutrophilic, B-cell, and T-cell infiltration in BAL of HDM-treated Tnfaip33/-KO mice to levels found in HDM-treated Tnfaip33/-WT mice (Figure 4B,C). Blockade of IL-12 partially (eosinophils, neutrophils) or completely (macrophages, B cells, and T cells) restored infiltration in BAL of HDM-exposed Tnfaip33/-KO mice, as compared to HDM-treated Tnfaip33/-WT mice (Figure 4B,C). Blocking either IL-12 or IFNg did not alter Th or CD8+ T-cell numbers, but increased IL-5 expression specifically in CD8+ T cells as compared to isotype-treated Tnfaip33/-KO mice (Figure 4D,E). Furthermore, anti-IFNg antibody treatment strongly reduced the number of IFNg+ Th cells and CD8+ T cells and IL-10+ Th cells and CD8+ T cells in Tnfaip33/-KO mice as compared to isotype-treated Tnfaip33/-KO mice to similar numbers as observed in Tnfaip33/-WT mice (Figure 4F,G).

These data imply that blocking IFNg restores development of Th2-mediated allergic airway inflammation in Tnfaip33/-KO mice and IL-12 is partially or indirectly involved.

3.6 | IL-12 and IFNg are essential for the enhanced PD-L1 expression on DC subsets in Tnfaip33/-KO mice

IL-12 is essential for the induction of IFNg secretion by CD8+ T cells, and IFNg can subsequently induce PD-L1 expression. To determine whether IL-12, IFNg, or both are responsible for the increased PD-L1 expression as observed in pulmonary DCs of Tnfaip33/-KO mice, we treated mice with anti-IL-12p40 or anti-IFNg antibodies for 10 days prior to a single HDM exposure (Figure 5A). Strikingly, blocking IL-12 completely prevented PD-L1 upregulation after HDM exposure in all pulmonary DC subsets of Tnfaip33/-KO mice. Anti-IFNg antibodies also inhibited the upregulation of PD-L1 in DCs of Tnfaip33/-KO mice, although less vigorously than anti-IL-12 treatment (Figure 5B).

We therefore conclude that IL-12p40 or IFNg blockade can separately reduce the increased expression of PD-L1 on DC subsets in Tnfaip33/-KO mice, and could therefore be essential for the increase in PD-L1 expression on all pulmonary DC subsets after HDM exposure in Tnfaip33/-KO mice.

4 | DISCUSSION

DCs are critically involved in the pathogenesis of Th2-mediated disorders; however, the exact function of cDC1s in allergic asthma is still debated, as it has been shown that cDC1s can either suppress or induce Th2-mediated inflammation.11,13,14,16 Our results indicate that TNFAIP3 deletion in Langerin+ cDC1s elevated IL-12 expression specifically in cDC1s. Increased IL-12 expression by Tnfaip3-deficient cDC1s could provoke an augmented IFNg production by CD8+ T cells. In turn, IFNg was responsible for the enhanced PD-L1 expression on all pulmonary DC subsets. IFNg during sensitization controlled the development of Th2-mediated allergic inflammation upon HDM.

Our observation that mice harboring TNFAIP3-deficient Langerin+ cDC1s do not develop Th2-mediated inflammation upon exposure to HDM agrees with other reports that cDC1s suppress Th2-mediated pulmonary inflammation upon helminth infection, ovalbumin sensitization, and chronic HDM exposure. Additionally, Helicobacter pylori infection suppresses allergic airway inflammation through activation of cDC1s. In contrast, others have reported that cDC1s are essential for the induction of Th2-mediated differentiation in response to HDM, and that deleting cDC1s attenuates Th2-mediated eosinophilic inflammation. These conflicting findings may be due to the type of allergen used, timing of administration, or the use of different mouse models, such as Basic Leucine Zipper ATF-Like Transcription Factor 3 (BATF3) KO mice, BH2x mice7 that harbor a spontaneous point mutation (R294C) of IRF8, Langerin-DTR mice,7 CD103+KO mice,41 or XCR1+KO mice.42 Furthermore, the lung is unique, as pulmonary cDC1s contain a mixture of Langerin+ and Langerin- cDC1s. Differences in ontogeny and function between Langerin+ and Langerin- cDC1s are currently unknown. Specific deletion of lung Langerin+ cDC1s using Langerin-DTR mice did not affect eosinophilic inflammation,4 which could indicate that activation of these Langerin+ cDC1s provides an additional trigger enhancing their Th2-suppressive character.

Various reports have established cDC1s as major IL-12 producers that drive protective Th1 immunity against several pathogens. IL-12 production by cDC1s is also known to inhibit Th2 immune responses during chronic Schistosoma mansoni infection. Additionally, IL-12-producing DCs, established through retroviral overexpression, are also unable to prime mice for pulmonary Th2-mediated eosinophilic inflammation. There are case reports describing that blocking IL-12 with ustekinumab (monoclonal antibody to the p40 subunit of IL-12 and IL-23) in psoriasis patients exacerbated their atopic dermatitis, indicating a possible role for IL-12 in regulating ongoing Th2 responses, next to controlling the induction of Th2 immunity. Somewhat unexpected considering the increased IL-12 expression,
we did not observe enhanced Th1-cell differentiation, but rather an increase in IFNγ-production by CD8+ T cells, which is also dependent on IL-12.33 The specific increase in IFNγ-producing CD8+ T cells and not IFNγ-producing Th cells may be related to the unique cross-presenting capacities of cDC1s, which allow cDC1s to load exogenous peptides on MHC-I molecules, making them superior inducers of CD8+ T-cell activation.8,9 Increased numbers of IFNγ-expressing T cells were already found in the lungs of naïve Tnfaip3−/− KO mice. These are likely induced by TNFAIP3-deficient cDC1s in the lungs; however, we cannot exclude that the IFNγ-expressing T cells in the lung were partly induced by TNFAIP3-deficient Langerhans cells in the skin, because skin DCs have been reported to mediate inflammation in the Airways through the skin-lung axis.5

Furthermore, the presence of CD8+ T cells, and specifically allergen-specific CD8+ T cells, hampers the development of allergic diseases.37,46,47 The inhibitory effect of CD8+ T cells is likely caused by increased IFNγ production and was most pronounced during the sensitization phase, as blocking IFNγ during the challenge phase had no effect on Th2-mediated inflammation.44 This was confirmed by our findings that administration of IFNγ during sensitization reduced Th2-mediated HDM-triggered inflammation. Strikingly, CD8+ T cells also contribute to Th2-mediated inflammation by secretion of type-2 cytokines.46 We also found that blocking IL-12 or IFNγ promoted IL-5 expression and reduced IFNγ expression in CD8+ T cells, which is in line with previous reports that IL-12 is implicated in the induction of IFNγ.48 In conclusion, this indicates that the environment is a crucial determinant of cytokine expression by CD8+ T cells.

In our experiments, the IL-12p40 subunit was blocked, which is used by both IL-12 and IL-23. However, previous reports showed that Tnfaip3-deficient cDC1s do not express IL-23,23 and identified cDC1s as key producers of IL-12.16,43,49 making it most likely that the observed effects were mediated by blocking IL-12.

In conclusion, our data establish that mice harboring Tnfaip3-deficient Langerin+ cDC1s develop a strongly reduced Th2-mediated inflammation in response to HDM which is accompanied by the induction of IFNγ-producing CD8+ T cells and increased PD-L1 expression on pulmonary DCs. This indicates that the activation status of pulmonary cDC1s critically controls development of Th2-mediated allergic disorders.

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CONFLICT OF INTEREST

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AUTHOR CONTRIBUTIONS

HV, BNL, HH, RWH, and MK designed the experiments. HV, IB, JvH, ML, DvU, and IT performed experiments and analyzed data. BNL, GvL, and BEC provided transgenic mouse strains used for the experiments. LB provided therapeutic blocking antibodies. HV, RWH, and MK wrote the manuscript. All authors read and approved the final manuscript.

ORCID

Mirjam Kool https://orcid.org/0000-0003-1436-3876

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