Plasmacytoid dendritic cells drive acute exacerbations of asthma

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ABSTRACT

Background: Although acute exacerbations, mostly triggered by viruses, account for the majority of hospitalizations in asthma, there is still very little known about the pathophysiological mechanisms involved. Plasmacytoid DCs (pDCs), prominent cells of antiviral immunity, exhibit ~~potent~~ either pro-inflammatory ~~and~~or tolerogenic functions depending on the context, yet their involvement in asthma exacerbations remains unexplored.

Objectives: We sought to investigate the role of pDCs in allergic ~~airway~~ inflammation and acute exacerbations of asthma.

Methods: Animal models of allergic airway disease (AAD) and virus-induced AAD exacerbations were employed to dissect pDC function *in vivo* and unwind potential mechanisms involved. Sputum from asthma patients with stable disease or acute exacerbations was further studied to determine pDC presence and correlation with inflammation~~explore disease-relevant associations~~.

Results: pDCs ~~are~~ were key mediators of the immuno-inflammatory cascade that drives asthma exacerbations. In animal models of AAD and RV-induced AAD exacerbations, pDCs ~~are~~ were recruited to the lung during inflammation and migrated to the draining lymph nodes to boost Th2-mediated effector responses. Accordingly, pDC depletion post-allergen challenge or during RV infection ~~abrogates~~ abrogated exacerbation of inflammation and disease. Central to this process ~~is~~ was IL-25, induced by allergen challenge or RV infection that ~~conditions~~ conditioned pDCs for pro-inflammatory function. Consistently, in asthma patients pDCs ~~are~~ were markedly increased during exacerbations, and correlated with the severity of inflammation and the risk for asthmatic attacks.

Conclusions: Our studies uncover a previously unsuspected role of pDCs in asthma exacerbations with important potential diagnostic, prognostic and therapeutic implications. They also propose the therapeutic targeting of pDCs for the treatment of acute asthma.

KEY MESSAGES

- Plasmacytoid dendritic cells (pDCs) are increased in patients' sputum during asthma exacerbations and correlate with disease severity.
- pDC depletion in experimental animal models suppresses established allergic airway disease (AAD) and virus-induced AAD exacerbations
- Monitoring sputum pDCs has important diagnostic and/or prognostic potential, while pDC targeting constitutes a new therapeutic strategy.

CAPSULE SUMMARY

This study establishes plasmacytoid dendritic cells as central mediators of the immuno-inflammatory response that drives asthma exacerbations, and proposes novel diagnostic, prognostic and therapeutic approaches of high translational potential.

KEYWORDS

Plasmacytoid dendritic cells, asthma, animal models of allergic airway disease, rhinovirus, asthma exacerbations

INTRODUCTION

Asthma is the most common noncommunicable respiratory disease, affecting ~300 million people globally[1]. It is characterized by chronic inflammation of the airways, usually of allergic nature, leading to recurrent episodes of wheezing, coughing and shortness of breath. Despite significant advances in asthma management, acute exacerbations continue to occur and still account for the majority of morbidity, mortality and costs of asthma[2]. These are mostly triggered by respiratory viral infections, especially rhinoviruses (RV), which act synergistically with allergen exposure to aggravate disease symptoms[3-5]. Therapeutic options for asthma exacerbations are limited to increasing doses of corticosteroids and short-acting β2 agonists, while there is lack of targeted therapies directed against the immunopathogenic mechanisms that drive the disease process[6, 7].

Extensive research over the years has established the central role of type 2 immunity in asthma. Allergen-specific T helper 2 (Th2) cell and innate lymphoid cell 2 (ILC2) responses direct the immuno-inflammatory process that mediates asthma[6, 8-10], and various type 2-associated gene polymorphisms have been linked to the disease process[11-13]. However, the involvement of type 2 immunity in asthma exacerbations has been more complicated. Traditionally, viral infections induce Th1 responses and IFNγ-secreting $CD8^+$ cytotoxic T cells that cross-regulate type 2 inflammation. The observation therefore that Th2 responses are heightened during exacerbations and correlate with the severity of asthmatic attacks[4, 14, 15] has been puzzling. Although epithelial-derived cytokines such as IL-25 and IL-33, triggered by viral infections, can boost Th2 responses during exacerbations[15-17], the underlying mechanisms involved remain poorly defined.

Plasmacytoid dendritic cells (pDCs) are rare but functionally important bone marrow (BM)-derived cells that circulate in the blood and home to secondary lymphoid organs and sites of inflammation. Although they share functional properties with conventional DCs (cDCs), major drivers of Th2 cell responses in the respiratory track[18], including MHC class II and costimulatory molecule expression, migratory capacity and antigen-presenting function, they constitute a DC subclass on their own because of their 'plasmacytoid' appearance, relatively low antigen-processing capacity and unique ability to secrete type I IFNs[19, 20]. pDCs are prominent in antiviral immunity[21, 22]. They can also stimulate $CD4^+$ and $CD8^+$ T cell activation, and even engage in naive T cell priming and initiation of adaptive immunity[23]. Conversely, pDCs can mediate peripheral and central tolerance by inducing $FoxP3^+$ regulatory T cells, preventing allograft rejection and autoimmunity[24, 25]. This has raised ~~controversy~~ discussions over their 'immunogenic' versus 'tolerogenic' functions which is now attributed

to their notable plasticity in different microenvironments. Although immature pDCs appear to drive 'by default' tolerogenic/immunoregulatory processes, in inflammatory contexts pDCs can promote pro-inflammatory responses.

In asthma, pDCs have not been sufficiently explored. Although they are present in the sputum and bronchoalveolar lavage (BAL) of patients with stable disease, and can be increased upon allergen provocation[26-28], their functional relevance to the disease process has not been addressed. Moreover, although they have been shown to prevent the induction of allergic sensitization and consequently the development of subsequent inflammation in animal models of allergic airway disease (AAD)[29-31], as their depletion under homeostasis results in increased T cell priming, this is not informative about their role in the context of established disease. Thus, it is not known how pDCs function in the allergic inflammatory environment that characterizes human asthma. Most importantly, it is not known how pDCs function during disease exacerbations, in either human or animal model settings, leaving a large gap of knowledge about their function in asthma pathophysiology.

Here, we have used experimental animal models to investigate the role of pDCs in established AAD and RV-induced exacerbations of AAD~~,~~ We have characterized their presence and functional importance in the persistence and exacerbations of Th2 responses, and we have dissected the cellular and molecular mechanisms involved. We have further studied asthma patients, during stable disease or acute exacerbations, to determine pDC presence in their sputum and identify disease-relevant associations. Our findings ~~reveal~~ propose a central disease-exacerbating role of pDCs in AAD and asthma, and challenge the prevailing concept in the field that pDCs are broadly protective.

RESULTS

pDCs are increased in the lung and mediastinal lymph nodes of mice with allergic airway disease

We first employed an established mouse model of AAD based on systemic ovalbumin (OVA) sensitization and subsequent OVA aerosol challenge (Fig 1A) to characterize the spatiotemporal presence of pDCs in the lung and lung-draining mediastinal lymph nodes (MLNs) during the development of inflammation~~ and get clues about their potential function~~. Inflammation in this model peaks as day 1 post-challenge and declines thereafter (Fig E1, A-~~F~~E) as previously reported[32]. Using anti-PDCA-1 staining of lung sections and immunofluorescence microscopy, we observed that pDCs were scarce during homeostasis

and mainly located in the parenchyma (Fig 1B). However, at day 1 post-challenge, the peak of the inflammatory response, pDC numbers markedly increased, especially in peribronchial and perivascular infiltrates around the airways, and clusters in the parenchyma (Fig 1C). We also used flow cytometry to quantify pDC presence in the lung and MLNs of naïve, OVA-sensitized and OVA-challenged mice over a period of 11 days post-challenge. We found that increased pDC numbers, identified as $CD45^+CD19^-B220^+Siglec\text{-}H^+$ cells (Fig E1F), infiltrated the lung at day 1 post-challenge and gradually decreased thereafter (Fig 1D). Increased pDC numbers also infiltrated MLNs, peaking at day 4 post-challenge (Fig 1E). These data indicate that pDCs infiltrate the lung and migrate to the MLNs during the development of allergic airway inflammation, and may therefore be involved in the disease process.

pDCs promote allergic inflammatory responses in the airways during established experimental asthma

To examine whether pDCs can functionally affect established allergic airway disease, and modulate inflammation severity or persistence, we used 120G8, a widely used pDC-depleting antibody binding to PDCA-1[33] (Fig E2, A-B). We observed that repeated intraperitoneal (i.p.) injections of 120G8 at days 1, 3 and 5 post-challenge ~~reduced~~ depleted pDC numbers in the lung by 65% at day 4, and 50% by day 7 and 11 compared to vehicle (PBS), while isotype control (I.C.) antibody had no effect (Fig 2, A-C). 120G8 effectively depleted pDCs in MLNs as well (Fig E2C). Notably, pDC depletion profoundly impacted disease. It rapidly reduced total leukocyte numbers in BAL (Fig 2D), especially eosinophils (Fig 2E) and T cells (Fig E2, D-F). It also decreased peribronchial and perivascular inflammatory cell infiltrates in the lung and OVA-specific Th2 responses in MLNs (Fig 2, F-G) without affecting IL-10 or IL-17 while IFNγ was up-regulated (Fig 2H and Fig E2G). Airway hyper-reactivity measured as metacholine-induced increases in total lung resistance was also decreased in 120G8-treated mice (Fig 2I). On the contrary, administration of 120G8 before OVA challenge had the opposite effect. It increased total leukocyte and eosinophil counts in the BAL, inflammatory cell infiltration in the lung, and Th2 cell responses in MLNs, aggravating overall inflammation and pathology (Fig E3, A-F). In this setting, type I IFNs were not induced (Fig 3EG). This was in accordance to previous studies[30, 31], indicating profound functional differences of pDCs found in a 'homeostatic' versus 'inflammatory' environment. pDCs therefore promote allergic inflammatory responses in the lung once disease has been established.

Activated pDCs carrying allergen reach the MLNs and boost Th2-mediated allergic responses

To understand how pDCs exert their pro-inflammatory effects in AAD, we challenged mice intranasally (i.n) with labeled OVA (OVA-FITC) and examined pDC migration and function in MLNs (Fig 3A). We observed that up to 20% of MLN pDCs at day 1 post-challenge were OVA-FITC^{+}, and among them, a fifth expressed the chemokine receptor CCR7 which is key for cell trafficking to MLNs (Fig 3B). We also observed that pDCs from OVA-challenged mice expressed higher levels of CD40 and OX40L than PBS-challenged mice (Fig 3C), while CD80 and CD86 were not altered (Fig E4A). As CD40 and OX40L are key activation markers involved in T cell priming, these data indicated that activated pDCs carrying antigen reach the MLNs. We subsequently investigated whether pDCs in MLNs could directly affect effector Th2 responses to OVA. We used MLN cultures of OVA-challenged mice depleted from pDCs using cell sorting (Fig E4B) or magnetic beads (Fig E4, C-D), and found that MLNs that lacked pDCs had a profoundly impaired ability to produce IL-5, IL-10 and IL-13 in response to OVA (Fig 3D) demonstrating that pDCs promote Th2 responses and contribute to airway pathology by becoming activated and migrating to the MLNs.

pDCs infiltrate the lung of mice during RV-induced exacerbations of experimental asthma and drive disease

We next investigated whether pDCs are also important during viral exacerbations. We employed a mouse model of RV-induced exacerbations of AAD (Fig 4A) that leads to rapid increases in inflammatory cells including eosinophils and neutrophils in BAL, and OVA-specific Th2 responses in MLNs within 24h of infection (Fig E5, A-C)[34]. In this setting, we observed that pDC numbers were further increased, beyond the levels of OVA-challenged mice, by 50-55% in both the lung and MLNs at day 1 post-infection (Fig 4B, Fig E5D). We therefore studied whether pDCs could also play a functional role in RV-induced exacerbations of AAD by administering 120G8 one day before and during RV challenge to ensure that pDCs are diminished at the time of viral infection (Fig. E5, E-F). We found that pDC depletion completely abrogated RV-induced exacerbation of experimental asthma. It suppressed inflammatory cell infiltration in the BAL and diminished eosinophil, neutrophil and lymphocyte numbers (Fig 4, C-D). It also decreased leukocyte infiltration in the lung (Fig 4E) and abrogated RV-induced airway hyper-reactivity (Fig 4F). Notably, the production of IL-5, IL-10 and IL-13 in MLNs was reduced while IFNγ was up-regulated (Fig 4G). In the BAL, neither IFNα nor IFNβ were detectable (Fig. E3G). Interestingly, pDC depletion down-regulated inflammation in OVA-challenged control mice that received saline (Fig. 4, C-E), demonstrating in a different setting and genetic background of established AAD that these cells exhibit a pro-inflammatory

function. These data therefore highlight the central detrimental role of pDCs in promoting RV-mediated exacerbation of AAD.

IL-25 acts on pDCs to trigger their accumulation and pro-inflammatory activation in the lung

To characterize the molecular link(s) between allergen challenge, viral infections and pDC-triggered inflammation, we hypothesized that epithelial-derived cytokines such as IL-25, IL-33 and/or TSLP could be critically involved. We first studied their presence in the BAL and observed that although IL-33 and TSLP were barely detectable during either established AAD or RV-induced exacerbation models, IL-25 was robustly induced (Fig 5A-B). We also examined the expression of their receptors and found that IL17RA and IL17RB, the two components of the IL-25 receptor, were expressed at the mRNA level in both bone marrow-derived pDCs (Fig 5C) and MLN-sorted pDCs (Fig E4B and E6A) as was IL7RA, TSLP receptor's second chain. In contrast, IL1RL/ST2, the receptor for IL-33, and CRLF2, the unique chain of TSLP receptor, were virtually absent~~,~~ This suggest~~sing~~ that pDCs preferentially respond to IL-25, although it does not exclude a role for TSLP or possibly IL-33 in this process, as CRLF2 can be up-regulated upon TLR activation as well[35] ~~but not IL-33 or SLP~~.

We therefore investigated whether IL-25 could affect pDC function. We noticed that although recombinant IL-25 (rIL-25) by itself did not exert profound effects in pDCs in culture, and did not induce their proliferation, it synergized with ODN (TLR9) and R-848 (TLR7) stimulation to up-regulate pro-inflammatory cytokines and costimulatory molecules such as IL-6, TNF, IFNβ and CD40 (Fig 5D). As IL-6 plays a major role in the development of Th2 over Th1 responses[36, 37], and as CD40 is a critical determinant of optimal T cell activation[38], these data suggest a mechanism by which IL-25 can promote pDC-mediated allergic responses in the airways. Accordingly, we observed that single or repeated i.n. administration of rIL-25 to experimental animals post-challenge (Fig E6B) doubled pDC numbers to the lung and MLNs (Fig 5E and Fig E6C). At the same time, rIL-25 treatment augmented total leukocytes and eosinophils in the BAL (Fig 5, F-G), and inflammatory cell infiltrates in the lung by >50% at day 4 post-challenge (Fig 5H), and increased IL-13 production (Fig E6D). These findings demonstrate that IL-25 directly controls pDC activation, and suggest a mechanism by which allergens and viruses can modulate pDC-mediated pro-inflammatory function.

IL-25 neutralization reduces pDC accumulation in the lung and suppresses AAI

As our findings pointed to a key role of IL-25 in pDC-mediated aggravation of allergic airway inflammation, we investigated whether targeting endogenous IL-25 can prevent pDC

accumulation and pro-inflammatory function in AAD. We found that repeated i.p. administration of DDG91, a potent anti-IL-25 neutralizing antibody[39], post-challenge (Fig 6A) decreased pDC presence in the lung and MLNs (Fig 6B) and reduced total leukocyte and eosinophil numbers in the BAL(Fig 6, C-D) without affecting macrophage and neutrophil presence (Fig E7, A-C), Moreover, anti-IL-25 treatment diminished inflammatory cell infiltration in peribronchial and perivascular areas of the lung (Fig 6E) and suppressed OVA-specific IL-5, IL-10 and IL-13 production in MLNs (Fig 6F). These observations support a central role of endogenous IL-25 in the activation and migration of pDCs to the lung, and the induction of AAD.

IL-25 neutralization reduces pDC infiltration in the lung and suppresses RV-induced exacerbation of experimental asthma

We subsequently examined the effect of IL-25 targeting in the migration of pDCs to the lung and the exacerbation of AAD upon RV infection. We found that administration of DDG91 at the time of virus infection (Fig 7A) prevented pDC accumulation in the lung and MLNs (Fig 7B), and suppressed RV-induced exacerbation of allergic airway inflammation by reducing leukocyte infiltration in the BAL and lung (Fig 7, C-E and Fig E7, D-F), and production of Th2 cytokines in the MLNs (Fig 7F). Interestingly, the production of IFNγ was not affected (Fig E7G), indicating the existence of an IL-25-independent pathway in its regulation. Administration of DDG91 also suppressed airway hyper-reactivity measured as metacholine-induced increases in total lung resistance in (Fig 7G). Taken together, these data favor a causal effect of IL-25-regulated pDC function and exacerbation of allergic airway responses following RV infection.

pDCs are increased in the sputum of patients during asthma exacerbations and correlate with the severity of inflammation and disease

Finally, we sought to investigate whether pDCs are also important in acute exacerbations of asthma in humans. Initially, we assessed pDC presence in the sputum from 6 healthy individuals, 13 asthma patients with stable disease and 13 asthma patients within 48h of first symptoms of exacerbation and prior to any new treatment (Table E1). Multicolor flow cytometry was used to monitor pDCs, identified as $CD45^+HLA\text{-}DR^+CD11c^-CD4^+CD123^+BDCA\text{-}2^+$ cells, as well as various other immune cell populations (Fig 8A). We found that although pDCs were present in all asthma patients, they were markedly up-regulated (up to 4-fold) in patients during acute exacerbations (Fig 8~~, A~~ B). By comparison, pDCs were detectable only in two out of the six healthy individuals examined, and still at very low numbers. These significant

differences in pDC numbers between the groups were not paralleled by similar increases in total leukocyte presence, as higher counts during acute exacerbations were not statistically significant (Fig 8C)~~.~~. Notably, in the same patient sputum pDC numbers were markedly increased during exacerbation compared to stable disease (Fig. 8D), indicating that pDC monitoring constitutes a better approach for distinguishing among these different situations. pDCs from asthma patients exhibited an activated phenotype, expressing higher levels of CD80, CD86 and PDL1 in both groups compared to pDCs from the two healthy individuals (Fig E8A). ~~Notably, i~~Increased pDC numbers were associated with higher total $CD45^+$ leukocyte, eosinophil, neutrophil and T lymphocyte counts during exacerbations but not stable disease (Fig 8D and Fig E8, B-C), and higher levels of inflammatory mediators such as IL-5, IL-6, IL-8, IFNγ, GM-CSF, CCL-2 (MCP-1) CCL3 (MIP-1α) and CCL4 (MIP-1β) (Fig 8E and Fig E8D). Moreover, increased pDC numbers were found in frequent exacerbators, i.e. patients with $\geq$2 exacerbations per year, compared to patients that exhibit exacerbations less often (Fig 8F). These data demonstrate that high pDC numbers are related to a higher risk for exacerbations during stable disease, and more pronounced inflammatory responses during disease exacerbations.

DISCUSSION

Our study uncovers a new role of pDCs in asthma exacerbations. It reveals that pDCs are highly up-regulated in the sputum of patients with acute exacerbations, and correlate with the intensity of asthmatic inflammation and the frequency of asthmatic attacks. Moreover, it demonstrates that pDCs are critically involved in driving allergic airway responses in animal models of established AAD and RV-induced AAD exacerbations. Our study thus positions pDCs at the center of the immunopathogenic process that mediates asthmatic inflammation, sheds light into previously unsuspected aspects of their biology in allergic responses and proposes new potential ways for the diagnosis, prognosis and treatment of acute exacerbations of asthma.

Central to the pro-asthmatic effects of pDCs is their role in type 2 inflammation. pDCs are well established inducers of Th1 and cytotoxic T cell differentiation, especially in the context of viral infections and autoimmunity. However, despite early seminal studies using human cells describing the ability of pDCs to promote Th2 differentiation in culture[40-42] , their involvement in Th2 responses *in vivo* , especially in the context of allergic disease, has ~~remains~~ remained largely unexplored~~, despite early studies describing their ability to promote Th2~~

differentiation in culture[40-42]. Our work thus fills-in this gap by providing ample evidence that pDCs are potent inducers of Th2-mediated allergic responses in the lung driving the persistence of allergic airway disease and its exacerbations. In patients, higher pDC numbers are directly linked to increased numbers of eosinophils, neutrophils, T lymphocytes and pro-inflammatory cytokine networks in their sputum during exacerbations but not during stable disease. These findings highlight the key role of pDCs in Th2-driven allergic responses in the airways with important therapeutic implications.

Our study provides insight into the mechanism of action of pDCs. It shows that in the context of established disease pDCs are activated, take up inhaled allergen and migrate to the MLNs to support the activation of effector Th2 cell responses upon allergen or RV challenge. Although it is not clear how exactly pDCs mediate T cell activation, two possibilities are most likely. First, pDCs may act as classical antigen-presenting cells to boost effector Th2 cell responses and Th2-mediated inflammation. In support of that, pDCs up-regulate CD40 and OX40L, two key costimulatory molecules involved in pDC activation and/or ability to drive Th2 cell differentiation[19 43]. This is independent of their ability to secrete high levels of type I IFNs as these cytokines strongly suppress Th2 responses in this context[44]. Alternatively, pDCs may not directly present antigen but support through their presence conventional DC-T cell interactions. For example, pDCs may affect ILC function and although it has been shown that TLR7/8-activated pDC before the development of AAD suppress ILC2 function through type I IFNs[45], it is possible that in the context of established DC this is different. Further studies to dissect these possibilities will be needed.

The fact that pDCs are activated during established disease and exacerbations may explain the apparent discrepancy between our work and previous studies on the role of pDCs in allergic airway disease in experimental animals. These have shown that depletion of pDCs before allergen challenge (i.e. before the development of inflammation) aggravates disease but pDCs in this context are not activated and have thus the propensity to promote tolerogenic and/or immunoregulatory effects. In contrast, in our model systems pDCs are examined when disease is established and cells are thus activated. In terms of therapy, studying the role of pDCs at the 'chronic' phase of the disease is more relevant as patients that seek medical attention and are in need of treatment, either to control persistent symptoms or exacerbations, suffer from already established asthma.

A question that arises is what controls the pDC activation state and ensures pDC migration to the MLNs to induce effector Th2 responses. Although several factors have been proposed to enhance pDC activation and Th1 cell priming capability including CD40L, TLR

stimulation and viral infection[40, 46, 47], only IL-3 has been shown to effectively promote Th2 development at least in culture[40, 41]. Our study now points to an important role of IL-25 in this process. IL-25 is induced in high levels in response to allergen exposure or viral infection and acts directly on pDCs to condition them towards pro-inflammatory activation. This is not limited to mouse cells as IL-25 acts in synergy with TLR9 to activate human pDCs as well[28]. Accordingly, exogenous IL-25 administration triggers pDC migration to the lung and MLNs in experimental animals and exacerbates AAD, while endogenous IL-25 neutralization exerts the opposite effects. Although these findings do not unequivocally establish the causal effect of IL-25 in these processes, as pDC-independent effects of IL-25 may also be important, they suggest that IL-25 is a key moderator of the pro-inflammatory "switch" of pDCs and their migration to the MLNs. They also underscore the importance of the airway epithelium in the 'education' of pDC function in the respiratory mucosa.

Previous studies have pointed to a potential role of lung pDCs in human asthma. They have shown that pDCs are present in the sputum and BAL of stable asthma patients and can be increased further upon to allergen provocation[26-28]. Our study now greatly expands this earlier work by revealing the major role of pDCs in asthma exacerbations, and shedding light into their functional importance. The observation that pDCs are linked to higher inflammatory burden in the lung during acute exacerbations, and the fact that pDC depletion abrogates exacerbations in experimental animal models, provides the rationale for interfering with pDC function for therapy. Moreover, the demonstration that pDCs are increased in the same patient during an exacerbation, and that higher pDC numbers are present in stable asthma patients with frequent exacerbations supports the tracking of sputum pDC levels for diagnostic and prognostic purposes.

Interestingly, previous studies have also indicated opposing roles of pDCs in the respiratory tract. In the nasal mucosa of chronic rhinosinusitis patients, pDCs have been shown to be activated and capable of promoting both Th1 and/or Th2 cell differentiation depending on the disease phenotype[48]. In contrast, in human tonsils of allergic patients, pDCs have been demonstrated to prevent allergic responses by inducing allergen-specific FoxP3$^+$ regulatory T cell responses[49,50]. The ability therefore of pDCs to induce Th2 versus Th1 or regulatory T cell responses in the respiratory track appears to be context-specific, amenable by inflammatory, environmental and tissue-intrinsic cues, and should be taken into account when therapeutic applications are considered.

In summary, our study reveals a new role of pDCs in AAD and supports the targeting pDCs or their upstream activator IL-25 for the treatment of difficult to control asthma,

especially asthma exacerbations. It also advocates for the monitoring of pDCs in patients' sputum as a potential novel way for diagnosing the severity of asthma and predicting the risk of exacerbations, and has therefore the potential to significantly impact human health.

MATERIALS AND METHODS

Mouse models, treatments and readouts

AAD was induced in C57BL/6 mice that were sensitized and challenged with OVA as previously described[51]. RV exacerbation of AAI was induced in BALB/c mice sensitized and challenged with OVA and exposed i.n. to $1x10^7$ $TCID_{50}$ of RV-1B (RV) or saline as reported[34]. ~~This later model is not functional in the C57BL/6 background[34].~~ For pDC depletion, mice were treated i.p. with 250 µg/mouse of 120G8 anti-mouse antibody (Dendritics) or controls as indicated in the experimental protocol. For IL-25 treatment, mice were administered i.n. with 1 µg/mouse of recombinant IL-25 (Biolegend) or PBS as specified. For IL-25 neutralization, mice were treated i.p. with 500 µg/mouse of anti-mouse IL-25 (clone DDG91; Janssen Pharmaceuticals) or isotype control. Mice were sacrificed at various timepoints for endpoint analyses ~~including inflammatory cell infiltration in the BAL and tissues, immunofluorescence and histopathological analysis, airway hyper-reactivity (AHR) and OVA-specific mediastinal lymph node (MLN) responses~~ as detailed in the online supplement.

Human subjects, sputum induction and analysis

Study subjects were recruited from an open cohort of patients who were followed up for at least 2 years. In total, thirty-four participants with an age range of 34-79 years were enrolled: six healthy individuals, thirteen patients with moderate-to-severe asthma in stable condition and thirteen patients on exacerbation within 48h of manifestation of symptoms. Sputum was induced by inhalation of hypertonic saline aerosol and processed using standard methodologies[52]. Leukocyte fractions were subjected to immediate flow cytometric analysis whereas supernatants were kept at −70 °C for subsequent measurements as detailed in the online supplement.

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