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The role of dendritic cells and monocytes in the maintenance and loss of respiratory tolerance

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Abstract

Promoting tolerance to inhaled antigens is an active area of study with the potential to benefit the millions of Americans currently suffering from respiratory allergies and asthma. Interestingly, not all individuals with atopy are symptomatic, arguing that sensitization alone does not lead to an allergic clinical phenotype. Respiratory dendritic cells (rDCs), classically associated with inducing inflammatory responses, can actively promote tolerance. Tolerance can be broken when inflammatory stimuli, including viral infections and other environmental exposures, inhibit rDC-mediated tolerance by allowing innocuous antigen to be presented to initiate type-2 immunity. Importantly, rDCs are composed of multiple subsets, each with a unique response to inhaled antigen that can lead to either tolerance or inflammation. In this review we will discuss how rDC subsets actively maintain tolerance or, alternatively, break tolerance in response to environmental cues.

Introduction

Allergic sensitization is a complex immune phenotype that results from the induction of type-2 immunity against normally harmless antigens. For decades, researchers have examined how type-2 immunity develops and the resulting phenotype characterized by antigen-specific IgE and eosinophilia. With over half of Americans now suffering from at least one type of allergic disease (1), the more pertinent question has become: how do some individuals remain tolerant of exogenous inhaled allergens? Epidemiological studies have established environmental exposures that can increase or decrease a child's risk of developing asthma and/or atopy. Factors that decrease risk include growing up on farms, consumption of raw milk, and exposure to microbial products early in life (2–6). One recent epidemiological study of over 8000 children found exposure to specific types of farming, including exposure to hay, pigs, and poultry, provided protection from asthma (7). In

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contrast, environmental exposures that lead to an increased risk for childhood asthma include respiratory infections, exposure to molds, and living in an urban environment (8–12). These studies suggest that some exposures are protective while others promote the dysregulation of tolerance through the induction of inflammatory responses.

Clinical markers of allergic tolerance

Atopic individuals are characterized by increased circulating Th2 cells, Th2-associated cytokines, eosinophils, and IgE. Recent studies have elucidated markers of tolerance by comparing individuals with and without allergic lung diseases. Increased circulating regulatory T cells (Tregs) are correlated with lower levels of atopy and asthma (13, 14). Tregs are a key cell type regulating tolerance by inhibiting inflammatory Th2 responses in the lungs (15). The increase in Treg numbers in healthy individuals is associated with an increase in anti-inflammatory mediators in serum. For example, non-atopic individuals have more circulating Indoleamine 2,3-dioxygenase (IDO) (16). IDO can stabilize Tregs by preventing their conversion to effector Th17 cells and preventing the differentiation of Th1 and Th17 cells (17, 18). The contribution of IDO in allergic airways disease in humans is an active area of study. In one study, IDO activity was significantly decreased in the BAL of patients with allergic asthma, providing a link between low IDO and airway hyperreactivity (19).

Another marker of tolerance is circulating vitamin D levels. Peruvian children without asthma had higher circulating 25-hydroxy vitamin D3 compared to children with asthma, and low vitamin D was also associated with asthma exacerbations in a Puerto Rican cohort (20, 21). However, no association between vitamin D and atopy or asthma was found in two other studies examining Danish adults or Taiwanese children (22, 23). The complex nature of allergic disease may explain these discrepancies. In a study comparing symptomatic and nonsymptomatic patients with atopy, IDO and IL-10 was significantly higher in the asymptomatic group, despite both groups having aeroallergen-specific IgE (24). This result would argue that sensitization alone is not sufficient break tolerance. Instead, loss of tolerance required both sensitization to antigen plus a reduction in IDO, IL-10, and other anti-inflammatory mediators. The mechanism by which these two events synergize is unclear. As DCs drive sensitization, but can also produce IDO and anti-inflammatory mediators, they are likely the cell type controlling inhaled allergen tolerance.

Environmental exposures that promote lung tolerance

Epidemiological studies have found that reduced risk of asthma and allergies correlates to environmental exposures, leading to the suggestion that this protection is through pattern recognition receptors (PRRs). In murine models, high-dose exposures to PRRs, particularly TLR3 and TLR4 agonists, have been shown to augment allergic responses (25, 26). However, low-dose exposure to TLR agonists can promote tolerance. For example, high-dose lipopolysaccharide (LPS) delivered intranasally during sensitization induces sensitization, but a low-dose inhibited sensitization (26). To mimic physiologic conditions, allergen has also been administered in combination with sterilized house dust extract (HDE) isolated from bedroom carpets (27, 28). Daily HDE reduced lung-infiltrating eosinophils and

eotaxin after challenge. Interestingly, this effect also occurred in TLR4-KO mice, arguing that the ability of HDE to promote tolerance may involve other PRRs. These studies suggest that exposures to microbial products, including endotoxin, can be beneficial in that it reduces Th2 polarization and prevents airway hyperreactivity.

While dust studies reveal the microbial components that promote tolerance, specific commensal bacteria have also been described that can directly promote tolerance. Dog ownership is linked to lower rates of childhood allergies (29); however, a recent study indicated that dog exposure alters the microbiome by increasing *Lactobacillus johnsonii*, a bacteria residing in the gut which can inhibit respiratory Th2 responses. In a similar manner, two species of bacteria isolated from farm cowsheds, *Acinetobacter lwoffii* F78 and *Lactococcus lactis* G121, were sufficient to protect mice from allergy development (30). Taken together, these data indicate that both microbial products and live bacteria have the potential to inhibit allergies by regulating the pulmonary immune response.

Maintenance of tolerance by respiratory DCs

Inhaled antigens and microbial products have a profound ability to regulate antigen presenting cells (APCs). APCs in the lungs express a wide variety of PRRs including toll-like receptors (TLRs), C-type lectin receptors (CLRs), RIG-I like receptors (RLRs), and Nod-like receptors (NLRs) that are capable of recognizing inhaled particulates (31). Particularly important are respiratory DCs (rDCs) that express PRRs and also have the capacity to initiate adaptive immune responses to inhaled allergens. While it has been well studied that the downstream effects of atopic asthma are dependent on type 2 CD4⁺ helper T cells (Th2) and more recently type 2 innate lymphoid cells (ILC2s), the mechanisms that induce rDCs to break respiratory tolerance and initiate this cascade remains unclear. A critical role for rDCs in initiating Th2 responses in the lungs was first demonstrated with the CD11c-DTR mice that fail to develop eosinophilia, goblet cell hyperplasia, or bronchial hyperreactivity when CD11c⁺ cells were depleted (32). In mice, the integrin CD11c is found at high levels on rDCs and macrophages and at intermediate levels on monocytes (33). Since CD11c is present on multiple cell types and subsets, recent investigations have assessed which subpopulation is the primary driver of allergic airway inflammation. Each rDC subset responds to specific environmental stimuli that can either enhance or inhibit sensitization.

One mechanism by which rDCs maintain airway tolerance is through the induction and maintenance of Tregs (Figure 1A). Depleting Tregs prevents mice from becoming tolerant to an inhaled allergen (15). Recent studies have elucidated the specific types of DCs that can induce Tregs and enhance Treg function. CD103⁺ cDCs, while classically associated with presentation of viral antigens, also play a critical role in inducing tolerogenic responses in the gut, ocular mucosa, and lung (34–36). In murine models, CD103⁺ cDCs have been shown to induce airway tolerance rather than sensitization as mice lacking this subset are unable to induce Tregs in the lungs (36). The primary mechanism of this regulation was dependent on retinoic acid and TGFβ, which promoted Treg differentiation. Interestingly, when cholera toxin was used as an adjuvant, CD103⁺ cDCs had a diminished capability to induce Tregs. This indicates that pro-inflammatory signals in the lungs during allergen exposure may prevent the tolerogenic functions of CD103⁺ cDCs. In contrast to the above

studies, mice lacking CD103⁺ cDCs had attenuated responses in vivo after allergen challenge, and CD103⁺ cDCs were able to induce Th2 differentiation in vitro (37). In humans, CD141 is a marker of DCs with similar function to murine CD103⁺ DCs (38). CD141⁺ DCs in skin have been described as potent inducers of Tregs although it is unknown if they play a similar role in the lung (39). Vitamin D has a direct effect on human DCs in vitro by upregulating the inhibitory receptor ILT3 (40). These ILT3-expressing DCs produce IL-10 and are potent inducers of Tregs.

Although traditionally associated with anti-viral immunity, plasmacytoid (pDCs) also play an important role in lung tolerance. Depleting pDCs in mice prior to antigen challenge was sufficient to drive sensitization, indicating that pDCs can prevent allergic airway responses (41). More recently, a subset of pDCs characterized by the expression of CD8 α was found to induce Tregs in the lungs and reduce airway reactivity in a murine model. Induction of Tregs was dependent on retinoic acid acting directly on the pDCs (42). The ability of pDCs to regulate immune responses is likely through constitutive expression of the immunosuppressive enzyme IDO (43, 44). These studies suggest that low IDO in atopic individuals may be driven by dysfunctional pDCs, which fail to produce IDO in the tissues to regulate normal immune homeostasis.

Loss of inhaled tolerance

Breaking respiratory tolerance has been associated with viral infections or other stimuli that promote airway inflammation (Figure 1B). There is also a role for non-pulmonary atopic disorders leading to respiratory allergies, as atopic dermatitis is a risk factor for the development of respiratory atopy and asthma (45). Other factors include colonization of specific microbes in the nasal passages, such as *Staphylococcus aureus* (46). The presence of allergen-specific IgE in asymptomatic individuals suggests that subclinical sensitization to allergen may be a common phenomenon (1, 47). These risk factors may act on rDCs to prime Th2 differentiation and inhibit regulatory responses.

Viral infections rapidly activate lung pDCs, which may prevent them from regulating allergic sensitization and may explain the link between viral infection and asthma exacerbations. Activation with nucleic acids through TLR7 and TLR9 provides a signal for pDCs to switch from regulatory to pro-inflammatory (48). The pDCs stop producing IDO, start producing IFN α , IFN β , and IL-6, and up-regulate antigen presentation machinery (49). The loss of IDO from regulatory pDCs may be sufficient to break tolerance by reducing Tregs and allowing the induction of effector T cells by cDCs.

When anti-inflammatory mediators are no longer present in sufficient quantities in the lung, the opportunity arises for the loss of tolerance to innocuous antigen. Of all rDC subsets, the CD11b⁺ cDCs in mice and CD1c⁺ DCs in humans have been most significantly associated with asthma pathogenesis (50, 51). Using a house dust mite (HDM)-mediated model of asthma in mice, it was shown that CD11b⁺ cDCs were sufficient for development of Th2 responses in the lungs. In particular, they were responsible for migrating to the draining lymph node and initiating Th2 differentiation. Interestingly, two-photon microscopy of the lung has demonstrated that after antigen challenge there is significant accumulation of

CD11b⁺ cDCs around the airways, which can take up antigen and traffic to the draining lymph node (52). Migration of CD11b⁺ cDCs to the draining lymph node has been found to be dependent on CCR7, a chemokine receptor, and CD47, an integrin associated protein, that are upregulated by DCs after antigen uptake (53–55). Once in the draining lymph node, CD11b⁺ cDCs preferentially activate CD4⁺ T cells (32, 56). Whether CD1c⁺ DCs fulfill a similar role in humans is not currently known. Clinical studies have demonstrated that rDCs in induced sputum from patients with asthma express elevated levels of CD1c compared to healthy controls (57). CD1c⁺ DCs are also found to be specifically increased in the airways of asthmatic patients, implicating a role for these cells in allergic respiratory responses (58). These studies identify CD11b⁺ cDCs/CD1c⁺ DCs as a critical participant in the development of allergic airway inflammation.

Recent studies have elucidated mechanisms by which CD11b⁺ DCs can promote allergen sensitization. Studies from our lab using two stimuli, HDM and IgG-immune complexes (ICs), have shown that they signal through FcR γ -associated receptors on CD11b⁺ DCs and lead to induction of Th2 responses (59–61). FcR γ is a common signaling component that associates with several receptors including Dectin-2 and CD16 (Fc γ RIII), which bind HDM and ICs respectively (62, 63). HDM-mediated Th2 responses were shown to be dependent on cysteinyl leukotriene production following Dectin-2 signaling on CD11b⁺ DCs (64) while IC-mediated Th2 responses were dependent on IL-33 production after activation of CD11b⁺ DCs through CD16 (60). We have demonstrated that both models are dependent on FcR γ , suggesting a common pathway through which allergens are able to promote Th2-mediated airway inflammation (65). Furthermore, our lab and other labs have identified interferon regulatory factor 4 (IRF4) as a key transcription factor that mediates the ability of DCs to promote Th2 differentiation (59, 66). IRF4 upregulation in CD11b⁺ bone marrow derived DCs (BMDCs) was found to regulate IL-10 and IL-33 production to promote Th2 differentiation (59). These studies identify a mechanism through which Th2 stimuli ligate FcR γ -associated receptors on CD11b⁺ DCs to upregulate production of Th2 associated transcription factors and cytokines. Whether this particular pathway is also aberrantly activated in humans is an area of ongoing research.

A similar activation pathway may occur in monocytes and monocyte-derived dendritic cells in the lung. Changes in circulating monocyte populations have been reported at 24hr post-allergen challenge in some patients, indicating that inhaled allergen directly effects monocytes in the periphery (67). Monocytes express multiple Fc receptors, and ligation of these receptors could promote an FcR γ signaling cascade promoting allergen sensitization. A greater percentage of CD14⁺CD16⁺ monocytes was found in severe asthmatics compared to less severe asthmatics (68). This may indicate a functional role for CD16 on human monocytes or moDCs in augmenting allergic disease. Under inflammatory conditions, monocytes enter the lungs where they become DC-like in phenotype and function. In many studies analysis of these moDCs was inseparable from cDCs because of the similarities in surface molecule expression in the lungs. However, these cell types are derived from two different progenitor cells, the flt3-dependent common DC progenitor and the GM-CSF-dependent macrophage and DC progenitor (69). Recently moDCs have been found to have specialized functions which are distinct from cDCs. For example, CD11b⁺ moDCs, and not CD11b⁺ cDCs, were the primary producer of chemokines in the lungs following allergen

challenge (51). While moDCs were capable of reaching the draining lymph nodes to present antigen, this only occurred when very high levels of antigen were inoculated. Whether moDCs traffic and present inhaled allergens under physiological conditions is not clear. Instead, this data would suggest that moDCs contribute to allergen sensitization by recruiting other inflammatory cells to the lungs and only have a minor role in antigen presentation.

CD1c⁺ DCs constitutively express the IgE receptor FcεR1a (58). Conflicting data shows that ligation of FcεR1a on DCs in vitro can lead to inflammatory cytokine release through NFκB induction or a tolerogenic state characterized by the production of IL-10 and Indoleamine 2,3-dioxygenase (IDO) (reviewed in (70)). As FcεR1a signals through FcγR, binding of IgE-IC to FcεR1a on human DCs may augment Th2 responses similarly to IgG-IC signaling through FcγRIII on murine DCs. This also implies an important role for secondary signals in promoting allergen-specific immune responses, as IC-mediated Th2 polarization required a TLR4 agonist as a second signal. Genome-wide association studies have linked TLR4 polymorphisms and other innate receptors to asthma and atopy (71, 72). A recent study found that a TLR4 agonist, acting through MyD88, was sufficient to break allergen-specific tolerance *ex vivo* in tonsils and peripheral blood cells (73). Overall, these studies implicate CD11b⁺ cDCs as the key cell type driving Th2 polarization in the lungs following exposure to allergen in the presence of an appropriate secondary signal.

Longitudinal studies have indicated that individuals with atopic dermatitis are at increased risk for developing respiratory allergies, indicating a positive feedback loop for sensitization (74). Recently, DCs have been implicated in this process. In humans, it was found that CD1c⁺ DCs isolated from asthmatic airways express more thymic stromal lymphopoietin receptor (TSLPR) compared to healthy controls. TSLPR ligation has been found to upregulate OX40L on DCs and promote Th2 differentiation (75). Thus, DCs from asthmatic patients are primed to respond to TSLP signaling by polarizing naïve T cells to produce IL-5, IL-13, and IL-9 (76). The upregulation of TSLP signaling globally in the host can explain how sensitization to one allergen leads to subsequent sensitizations.

Summary

Respiratory tolerance is the critical process by which inhaled antigen is deemed harmless to the host and fails to promote an inflammatory response. The mechanism by which rDCs maintain or break respiratory tolerance is an active area of investigation. Recently it has been appreciated that different rDC subsets have specialized functions in the lungs after antigen exposure. CD11b⁺ cDCs have a primary role in allergic sensitization by promoting Th2 differentiation in the draining lymph nodes. CD11b⁺ moDCs produce chemokines in the lung to recruit inflammatory cells to the tissue. CD103⁺ cDCs and pDCs have a critical role in maintaining tolerance through the production of anti-inflammatory mediators including Indoleamine 2,3-dioxygenase (IDO). Loss of respiratory tolerance occurs when antigen enters the lungs in the presence of secondary signals that prevent the tolerogenic function of CD103⁺ cDCs and pDCs while enhancing the ability of CD11b⁺ cDCs to promote Th2 responses.

Viral nucleic acids are potent drivers of pDC maturation and loss of IDO, which in turn may enhance the function of CD11b⁺ cDCs and moDCs. CD103⁺ cDCs have a critical pro-inflammatory role during viral infections through cross-presentation of viral antigens to activate CD8 T cells (77). The loss of inhibitory mediators by pDCs and CD103⁺ cDCs may account for the strong clinical correlations between viral infections and asthma exacerbations. Thus, understanding how rDCs interact in response to a wide variety of environmental exposures is key to preventing asthma and other allergic airways diseases.

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A. Maintenance of Respiratory Tolerance

B. Loss of Respiratory Tolerance

Figure 1. The maintenance and loss of respiratory tolerance

(A). pDCs (1) promote tolerance by producing IDO that maintains Treg function. moDCs (2) and CD11b cDCs (3) do not actively promote Th2 differentiation or inflammation. CD103 cDCs (4) maintain Treg function in a retinoic acid and TGFβ-dependent manner. (B) IDO production is lost when pDCs (1) are activated by viral nucleic acids or PRR stimulation. Additionally, CD103 cDCs can directly prime Th2 responses in some models. moDCs (3) are specialized for chemokine production after allergen challenge and promote immune cell infiltration to the airways. CD11b cDCs (4) are the primary drivers of allergic

sensitization. The ability of cDCs to promote Th2 differentiation is dependent on signaling through both TLRs and an FcR γ -associated receptor. Once these signals are received, CD11b cDCs traffic to the draining lymph node where they polarize naïve T cells to become Th2 cells. Bacterial toxin or other stimuli acts on CD103 cDCs (4) to prevent inhibitory functions.

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