Clinical characterization of allergic sensitization patterns and the role of mucosal dendritic cells
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Chapter 5

General discussion and conclusions
General discussion and conclusions

Allergic rhinitis is a symptomatic disorder of the nose induced by an IgE-mediated inflammation of the nasal mucosa after allergen exposure. To improve treatment for allergic disease it is important to understand the underlying mechanisms, leading to these symptoms. Antigen-presenting cells, such as dendritic cells, in the nasal mucosa start the immune response by binding of the allergen, processing the captured allergen, and presenting it to T cells. Allergic inflammation in the airway mucosa is regulated by allergen-specific T helper 2 cells, which produce key cytokines like IL-4, IL-5 and IL-13 that regulate the isotype switching and synthesis of allergen-specific IgE from B cells which is a critical step in the allergic cascade. DC subtypes differ in their functional potential, and the expression of function is flexible and regulated by environmental factors. Dendritic cell subtypes and their function have been studied in many different organs and diseases, however the multitude of DC subtypes and their opposing function makes interpretation of their role in allergic inflammation difficult.

In this thesis the following questions/themes were addressed.
Can observed differences in clinical response, such as response to nasal allergen challenge and response to treatment, be explained by differences in local regulation by dendritic cells?
What is the role of myeloid, plasmacytoid, and Langerhans type dendritic cells of the nasal and oral mucosa in allergic rhinitis?
What is the role of dendritic cell subtypes in several models of immunological dysfunction?

Clinical characterization of allergic sensitization patterns

What is the difference in clinical response to nasal allergen challenge between subjects with different sensitizations?
An interesting observation in two of our studies was the difference in response to grass pollen nasal allergen challenge between subjects with GP mono-sensitization and subjects with persistent AR and poly-sensitization. Subjects with GP mono-sensitization had a significantly higher total nasal symptom score and significantly lower peak nasal inspiratory flow values than the subjects with poly-sensitization. It is not clear yet what causes this difference in response.
By definition, allergens are proteins with the ability to elicit powerful Th2 responses, leading to production of IgE antibodies. Some specific proteins cause aberrant immune responses, which may be caused by several molecular paths that affect allergenicity of specific proteins, depending on intrinsic biological activity of the allergens. Recent data have suggested that the cysteine protease activity of house dust mite allergen Der p 1 may play a significant role in its ability to elicit IgE antibody responses, mainly through
cleavage of membrane CD23 on B cells and increased IL-4 synthesis and secretion. Der p 1 also cleaves the α subunit of the IL-2 receptor from the surface of human peripheral blood T cells. Given that the IL-2 receptor is pivotal for the propagation of Th1 cells, its cleavage by Der p 1 may consequently bias the immune response towards Th2 cells, thereby creating an allergic microenvironment (1). The proteolytic activity of Der p 1 also leads to increased production of IL-8 in human airway epithelial cells, and decreased production of interleukin-12 (2;3). Beside proteolytic activity also lipid binding has been proposed as an adjuvant for allergenicity, and is found for more than 50% of the major allergens. Structural biology of allergens may enhance allergenicity of proteins via Toll-like receptor (TLR) pathways. Recently it has become apparent that lipopolysaccharide (LPS)-binding may contribute to the allergenicity of proteins. Main house dust mite allergen Der p 2 was identified to exhibit structural and functional homology with MD-2, an important co-factor for TLR-4 signalling (4;5). Non-intrinsic properties, such as cross-reactivity (6) and solubility of airborne particles (1), may also play a role in the allergenicity of proteins. Furthermore, there is growing evidence that epithelial cells do not only have a passive barrier function, but also play an active role in allergen-induced immune responses. The respiratory epithelium is likely to be involved in the process of sensitization, and contributes to the immunological response by generating a microenvironment that regulates the attraction and activation of immune competent cells (8-11). In vitro it has been shown that the response of airway epithelial cells to grass pollen allergen and house dust mite allergen is different on gene expression level (12-14). Downstream this leads to differences in levels of mediators involved in allergic inflammation. Further studies are necessary to investigate whether this is responsible for the observed difference in clinical response.

*Does the difference in clinical response to nasal allergen challenge between subjects with different sensitizations have consequences for therapy?*

In a placebo controlled treatment study with an antihistamine (desloratadine) we also observed the clinical difference in response to nasal allergen challenge between allergic rhinitis subjects with grass pollen monosensitization and subjects with polysensitization. Besides the less pronounced clinical response to grass pollen allergen challenge in the polysensitized group, we observed an improvement in symptoms after desloratadine treatment in the polysensitized subjects, especially reflected in less blockage and nasal itching, lower total nasal symptom score and better peak nasal inspiratory flow (unpublished data). Whereas the GP monosensitized subjects had higher symptom scores after nasal allergen challenge and no significant improvement in response to nasal allergen challenge after one-week desloratadine treatment. Whether this difference in response also occurs during natural allergen exposure and/or with other medication needs to be further studied.

The difference in treatment effect between pollen allergic subjects and house dust mite subjects is well known in allergen specific immunotherapy. Immunotherapy is more effective
in pollen allergic subjects than in house dust mite allergic rhinitis patients. However the underlying mechanism for this difference is not clear, and is probably multifactorial. That house dust mite allergy seems to be more difficult to control was also shown in a german study population, where the majority of patients with grass pollen allergy were treated by their general practitioners, while those with house dust mite allergy were treated by a specialist (dermatologists, pulmonologists, and otorhinolaryngologists) with a background in allergic diseases (2).

**How can the difference in clinical response to allergen challenge and difference in response to antihistamine treatment be explained?**

The most obvious explanation would be in differences in histamine release after allergen induced specific IgE-cross linking. Therefore, we measured serum total IgE, allergen-specific IgE, and allergen-specific IgG4. Also, biological activity of IgE was determined by basophil histamine release in vitro. Although the median ratio of Phleum pratense (Phl p)-specific IgE to total IgE was significantly higher in the grass pollen monosensitized subjects than the polysensitized subjects, this did not affect basophil histamine release.

Contrary to IgE antibodies, IgG4 represents a noninflammatory and blocking antibody isotype, and is induced in human high-dose antigen tolerance models. IgG4 antibodies are thought to antagonize or block the allergic inflammation cascade resulting from allergen recognition by IgE (3). Therefore we hypothesized that high levels of IgG4 could represent a less pro-inflammatory environment. However, in our study there was a trend for the levels of specific IgG4 to be higher in mono-sensitized subjects than in poly-sensitized subjects. A protective role or IgG4 as an explanation for the observed difference between mono- and poly-sensitized patients is therefore highly unlikely.

Since dendritic cells play a pivotal role in several distinct phases of the allergic response, not only in inducing the primary immune response to inhaled antigen leading to allergic sensitization, but also in the maintenance of eosinophilic airway inflammation (17;18), we hypothesized that dendritic cells in the nasal mucosa may differ between subjects with monosensitization and subjects with polysensitization. We hypothesized that this difference may be reflected either in the number of dendritic cell subtypes, or in the ratio of functionally opposing dendritic cell subtypes in the nasal mucosa.

**Mucosal dendritic cells in allergic disease**

**What is the role of dendritic cell subtypes in the respiratory mucosa?**

Mouse models and *in vitro* studies show that DCs play a central immunoregulatory role, and suggest a differential role for myeloid DCs and plasmacytoid DCs in the pathogenesis of allergic airway disease (4-7). We showed the presence in human nasal mucosa of mDCs, characterized by BDCA-1 and BDCA-3, and pDCs, characterized by BDCA-2 and BDCA-4, previously identified on circulating blood dendritic cells (8). We found these 4 subtypes of
dendritic cells, both in the epithelium and in the lamina propria of the nasal mucosa, with comparable number of each subtype in allergic and healthy individuals. We did not find significant changes in numbers of DC subtypes after nasal allergen challenge. Previous studies, using different immunohistochemical staining protocols and other DC markers for mDCs and pDCs, have reported elevated numbers of DCs in the nasal mucosa of allergic rhinitis subjects, as well as an increase in DCs after nasal allergen provocation (24-28). Immunohistochemical staining protocols have several limitations in assessing DCs. The markers we have used to identify DCs in the nose may overlap within distinct DC subset so that the different stainings do not indicate more than the three distinct DC subtypes (pDC, mDC, and LDC). Potential overlap between dendritic cell markers has been shown before. For instance, in peripheral blood most of the BDCA-2 positive cells are also positive for BDCA-4, while in lung mucosa the majority of CD1a+ DCs also express BDCA-1 (23;29). This is further complicated by the fact that there are major differences in cell surface markers, activation markers, and TLR distribution between murine and human dendritic cells (9-13). Multiple DC subtypes with multiple expression patterns and functions exist, with myeloid DCs thought to enhance and plasmacytoid DCs to inhibit immune responses. Given the opposing functions of mDCs and pDCs we assessed the immune status through the median ratio of BDCA-1 positive (mDC) over BDCA-2 positive (pDC) cells. The ratio of mDCs to pDCs is often used to describe the overall immunogenic status (14-17). Although the extent of DC marker overlap and specificity cannot be fully established with our immunohistochemical staining protocols, the main conclusions on the presence of the different DC subtypes and the mDC/pDC ratio should not be affected. We found that this ratio dropped in the epithelium of healthy subjects after nasal allergen provocation, and was significantly different from allergic subjects where the ratio did not change after allergen challenge. This suggests a lack of immunosuppression in the allergic subjects.

Are there differences in dendritic cell subsets in the nasal mucosa of allergic rhinitis subjects with different allergic sensitizations?

We found that numbers of mDCs, pDCs and Langerhans cells differ between subjects with different allergic sensitizations. Many factors could be of influence causing these differences, such as the type of allergen, the number of allergen sensitizations, persistent or intermittent allergy, seasonal fluctuations (in pollen allergy), and the interaction between effector and regulatory inflammatory cells. High baseline levels of mDCs in mono-pollen allergic subjects could reflect ongoing minimal persistent inflammation outside of the pollen season (18;19), showing that pollen allergic individuals are not normal even when free of symptoms. Whether mDCs are responsible for maintaining allergic inflammation outside of the pollen season or for the increased response to nasal allergen provocation remains to be explored. In pulmonary mucosa a shift in the myeloid dendritic cell population towards a Langerhans phenotype is found in COPD patients, with a further increase in langerin+ DCs in relation to the severity of disease and no difference between current
smoking and ex-smoking COPD patients, supporting the concept of ongoing inflammation in COPD, despite smoking cessation. This shows that a shift in DC subset distribution and/or function can lead to persistent disease, even when the initial cause has been eliminated. Shifts in DC distribution are also reflected in the ratio of myeloid DCs to plasmacytoid DCs. In the nasal mucosa the median ratio of BDCA-1 positive (mDC) to BDCA-2 positive (pDC) cells differed among monosensitized and polysensitized subjects. In healthy controls the mDC/pDC ratio dropped by half after nasal allergen provocation, in contrast the ratio increased in mono-sensitized subjects, whereas in poly-sensitized subjects this ratio decreased slightly. This may reflect the molecular mechanism of the less severe clinical response to grass pollen in polysensitized subjects.

As we have not determined the activation state of the different subclasses of dendritic cells by evaluating the expression levels of activation markers (e.g. CD80, CD83, CD86) or inhibitory markers (e.g. CTLA4) on the surface of the DCs we are solely depending on changes in cell numbers to evaluate the status of the immune system. Despite these limitations this approach has been successful in characterizing the immune status in the lower respiratory tract. Further research in human in vivo models is necessary to assess the function and relevance of present DCs.

Do oral mucosal DCs play a role in allergic disease?
Our analysis of the oral mucosa of allergic subjects and healthy controls revealed abundant numbers of CD207 (langerin) and CD1a positive Langerhans cells, in accordance with other reports of oral mucosal DCs (20-23). Furthermore, we found CD141 (BDCA-3) positive DCs in a remarkably different distribution than in the nasal mucosa, and with significantly more CD141 positive mDCs in epithelium of allergic subjects compared to healthy controls. For the first time, we demonstrated the presence of plasmacytoid DCs, characterized by CD303 (BDCA-2), in the human oral mucosa in very low numbers. Thus the ratio of mDCs to pDCs, which is often used to describe the overall immunogenic status, is very high in human oral mucosa. In other tissues a high mDC/pDC reflects a pro-inflammatory state. We found this in the nasal mucosa, where the mDC to pDC ratio would describe the clinical observations seen in the response to nasal allergen provocation. However, this ratio seems not adequate to describe the overall tolerogenic environment of the human oral mucosa.

How can we explain the tolerogenic function of oral DCs?
The oral mucosa is an immune-privileged site. Despite the exposure of the oral mucosa to millions of bacteria and many antigens, severe inflammatory and acute allergic reactions rarely occur. Oral DCs overall have a tolerogenic function in absence of danger signals. We found very low levels of plasmacytoid DCs in the oral mucosa, which suggests that pDCs overall do not play a significant role in oral tolerance, in contrast to nasal and bronchial mucosae. The differences between the nasal and oral mucosa with respect to the ability of
DCs to induce tolerance is of specific clinical interest with regard to possible immunologic mechanisms that play a role in sublingual immunotherapy. A better understanding of these mechanisms could lead to optimization of sublingual immunotherapy efficacy. Previously the Langerhans cells were reported as the predominating DC population in human oral mucosa, and these are thought to contribute to the effectiveness of SLIT (20,22). We found elevated levels of CD141+ DCs in the oral mucosa of allergic subjects, which suggests immunostimulatory properties, since CD141+ DCs lead to a Th2-polarized cytokine response by allergen-specific T-cells (41). On the other hand, CD141+ DCs express a more immature phenotype and secrete more anti-inflammatory cytokine IL-10 (24). This may represent the tolerogenic function of the oral mucosa, based on the increase of number and activity of regulatory T cells. This has been suggested as a mechanism that plays a role in immunotherapy (25). Sublingual immunotherapy reduces allergen-induced inflammation and changes polarization of allergen-specific CD4+ T-cell responses, leading to clinical tolerance (26). However, understanding of the interaction of allergen and oral mucosal DCs during SLIT is limited. Further studies are necessary to investigate whether CD141 can be a target to improve SLIT efficacy.

Mucosal dendritic cells in models of immunological dysfunction

The mucosa of each organ seems to have a unique composition of dendritic cell subsets, probably based on the specific function(s) of the organ. In healthy state there is a balance in these DC subsets leading to a balanced immune response. Both genetic and environmental influences can lead to changes in the amount of DCs and/or ratio between DCs subsets. These shifts in mucosal DC subset composition may result in disease, and furthermore this may commence a vicious circle of DC subset disbalance leading to persistent disease even after the initial stimulus or provocation has been taken away.

In chapters 3.1, 3.2, 3.3, and 3.4 the distribution of several DC subsets is described in some models of immunological dysfunction. The mucosa of several organs was studied to assess whether there are common changes in DC subset shifts, with special emphasis on the ratio between myeloid and plasmacytoid DCs. The nasal mucosa of IgA Nephropathy patients was studied, because these patients are known to exhibit a defective immune response when challenged intranasally, leading to a deficient mucosal IgA immune response after primary mucosal immunization (27). We investigated whether this IgA hyporesponse in IgAN patients could be explained by reduced numbers or altered subset distribution of dendritic cells in the nasal mucosa.
Furthermore, we studied the numbers and subset distribution of DCs in pulmonary mucosa of COPD patients, and colon mucosa in Crohn’s disease.

**Nasal DCs in IgA Nephropathy**

We have shown the presence of mDCs, pDCs, and Langerhans type DCs in the nasal mucosa. In patients with IgA Nephropathy, we observed higher numbers of intra-epithelial
CD1a+ cells and DC-SIGN+ cells in the lamina propria compared to healthy controls. It is not clear yet whether this change in the composition of DCs is responsible for this hyporesponsiveness. In vitro it was found that DCs from IgAN patients are less effective in inducing IgA production in naïve B cells. This suggests that DC surface molecules are responsible for the difference in IgA production that was found between IgAN patients and controls (28). It would be interesting to study functional activities of mucosal DCs of IgAN patients. However, techniques and availability of tissue are presently not sufficient to perform proper functional analysis. An alternative way to get more information on DC function could be further phenotypic analysis of tissue DCs, preferably during antigen challenge.

**Pulmonary DCs in COPD**

In pulmonary mucosa langerin+ DCs are mainly present in the epithelium, whereas DC-SIGN+ DCs are mainly localized in the lamina propria and adventitia. This is in partial agreement with our observations in the nasal mucosa where langerin+ DCs are the predominant cell type in the epithelium and the lamina propria. Previously mDCs were shown to be more abundant than pDCs in pulmonary mucosa (29,30). DCs infiltrate the airways in COPD (31). In the pulmonary mucosa of COPD patients we found a shift of the myeloid dendritic cell population towards a Langerhans phenotype with higher numbers of langerin+ DCs in COPD patients compared to never smokers and ex-smokers without COPD. Furthermore, the number of langerin+ DCs further increased with the severity of the disease. Thus the Langerhans type DC subset seems to have an important role in the initiation of airway inflammation in susceptible smokers and perpetuation of this destructive process in COPD, even after smoking cessation.

Quantification of BDCA-1 positive DCs revealed a completely different result with a significantly lower number of these DCs in COPD patients compared to never smokers, especially in the lamina propria. Moreover, the number of BDCA-1 positive DCs further decreased with the severity of the disease. Plasmacytoid DCs were identified in small airways of human lungs, and in lymphoid follicles. There was a significant accumulation of pDCs in lymphoid follicles of patients with mild to moderate COPD. As pDCs are known for their tolerogenic properties, one could speculate that this increase in pDCs is a compensatory mechanism to balance the exaggerated immune response. An imbalance between mDCs and pDCs in the lungs could cause a derailment in the control of inflammation. Indeed, in COPD GOLD stage I, we observed a significant positive correlation between the number of langerin-positive mDCs and the number of pDCs in the total airway wall, which supports our hypothesis of the importance of mDC/pDC ratio for a coordinated immune response. This positive correlation was completely lost in COPD GOLD stage III–IV. However, the reduced number of pDCs could be explained by the use of inhaled and systemic corticosteroids by patients with severe and very severe COPD, as these drugs are known to induce apoptosis in circulating and tissue-resident pDCs (32-35). The effect of
corticosteroids on pDCs could not be evaluated in the other disease models we studied. The nasal mucosa biopsies were taken from allergic rhinitis patients and IgA Nephropathy patients, who were not allowed to use any systemic corticosteroids during the study, and only a low stable dose of inhaled corticosteroids. In the patients with Crohn’s disease, 66% of the study subjects used corticosteroids, however due to the very low number of plasmacytoid DCs in the lamina propria and complete absence of pDCs in the epithelium both in Crohn’s disease patients and healthy controls, it was not possible to assess any effect of corticosteroid use.

The DC maturation process in pulmonary mucosa, evaluated by the number of CD83 positive DCs, was not significantly altered in smokers and COPD patients. We conclude that DC differentiation, but not maturation, is altered in small airways of current smokers and COPD patients.

Colon DCs in Crohn’s disease
Three different subtypes of myeloid DCs populate the human colon mucosa and mesenteric lymph nodes; immature DCs expressing DC-SIGN, mature DCs that express S-100 or CD83, and mature DCs that express BDCA-3. BDCA-3 positive DCs were mostly found in the LP, suggesting involvement in antigen-capturing processes. In contrast, CD1a, BDCA-1, BDCA-2, and BDCA-4 positive DCs are virtually absent in colon and mesenteric lymph nodes. BDCA4 expression was only found around blood vessels, suggesting expression on endothelial cells instead of DCs. This expression pattern of BDCA-4 around blood vessels was also found in the lamina propria of oral and nasal mucosal biopsies.

Increasing evidence indicates dendritic cells play an important role in the immunological pathogenesis of inflammatory bowel disease (36-39). Crohn’s disease is a chronic inflammatory bowel disease. DCs of Crohn’s disease patients seem to have an intrinsic abnormal responsiveness to antigens from the lumen of the gut (40-43). The mDC populations may play an important role in the pathogenesis of Crohn’s disease. S-100+ DCs were only found in subcapsular sinuses of mesenteric lymph nodes of Crohn’s disease patients and not in healthy controls, indicating that there is an increased influx of mature DCs into the T-cell areas in Crohn’s disease patients. There was no difference in BDCA-2+ DCs between Crohn’s disease and healthy subjects. Therefore plasmacytoid DCs, which seem to be mainly involved in antigen-capturing processes in the colon, are not likely contributing to mucosal inflammation in Crohn’s disease.

Concluding remarks
Allergic rhinitis is probably not one disease, but a collective noun for several immunological dysfunctions leading to a common pathway of IgE-mediated inflammation of the nasal mucosa after allergen exposure. Each step in the immunological process leading to sensitization and allergen-specific IgE production is subject to intrinsic and environmental
influences. It is well known that the clinical symptoms of allergic rhinitis due to grass pollen differ from those caused by house dust mite allergen.

In this thesis we have shown that different allergic sensitizations lead to different clinical phenotypes. A concomitant house dust allergy beside a grass pollen allergy influences the response to a nasal allergen challenge with grass pollen. Contrary to common belief that patients with multiple allergies have more severe disease, we found that subjects with grass pollen allergy and a concomitant house dust mite allergy had a less severe response to nasal allergen provocation. Moreover these subjects benefited more from a one-week treatment with an antihistamine (desloratadine 5 mg od) prior to nasal allergen challenge. A multitude of factors can be of influence on the severity of allergic rhinitis symptoms; the number and type of sensitization, the extend of exposure to this allergen, and the intrinsic biological activity of allergens, which is caused by several molecular patterns that affect allergenicity of specific proteins. These mechanisms include protease activity, lipid binding, and Toll-like receptor signaling. Moreover, there is evidence that epithelial cells play an active role in allergen-induced immune responses, both in the process of sensitization and in the attraction and activation of immune competent cells. In vitro it has been shown that the response of airway epithelial cells to grass pollen allergen and house dust mite allergen is different on gene expression level. Airway epithelial factors have an important modulating role in DC function, differentiation, and maturation. On the other hand, DCs can bind the epithelial cells through the expression of adhesion molecules and tight junction molecules, which allow them to sample the surface without disrupting the mucosal integrity. The activation of airway epithelial cells with different allergens not only induces the expression of chemokines resulting in the recruitment of DCs, but also creates an immunomodulatory microenvironment in which DCs exert their function.

Dendritic cells in the nasal mucosa, specifically the mDC/pDC ratio, seem to play a role in the difference in clinical response to nasal allergen exposure. This is in accordance with the pulmonary mucosa where the balance between mDCs and pDCs determines whether there will be a pro-inflammatory or a tolerogenic immune response. In the gastrointestinal tract the plasmacytoid DCs, characterized by BDCA-2 and BDCA-4, are virtually absent and do not seem to play a role in the overall tolerogenic function. We suggest that myeloid DCs with an immature phenotype in the gastro-intestinal tract are important for maintaining a tolerogenic immune status. Shifts in distribution patterns of DCs and/or shifts in phenotype (maturation) lead to immunological dysfunction and may result in several very distinct diseases. Additionally, this may start a vicious circle of DC subset disbalance leading to persistent disease even after the initial stimulus or provocation has ceased.
Directions for future research

It is clear that DC subpopulations play an important immunoregulatory role in healthy state and that disbalance in dendritic cell composition may lead to allergic and inflammatory diseases. However, further studies are necessary to characterize dendritic cell subtypes in other mucosae in healthy state and in other inflammatory diseases. Furthermore, investigations are warranted to elucidate the functional differences between subsets of DCs in order to better understand the role of the different DC subsets in the initiation and perpetuation of inflammation. Function of the different DC subsets has been studied in vitro and in murine models. In a mouse model of asthma, depletion of CD11c+ DCs abolished the characteristic features of asthma. After adoptive transfer of CD11c+ DCs in CD11c-depleted mice, eosinophilic inflammation and Th2 cytokine secretion were restored. Thus in mice these DCs are identified as key proinflammatory cells that are necessary and sufficient for Th2 cell stimulation during ongoing airway inflammation (51). However, the complex interactions at the level of the airway epithelium, the importance of environmental factors, and the large differences in expression profiles on DCs between mice and men make interpretation of these data and extrapolation to the human in vivo situation very difficult. Further studies are necessary to investigate which DC subsets in humans are responsible for allergic disease and how they influence clinical symptoms.

It would be interesting to obtain mucosal biopsies at different time points and isolate dendritic cells from the sections with laser capture technique to investigate dynamics and function of different DC subsets in vivo. Also the interaction between DCs and epithelial cells deserves further attention, with special emphasis on gene expression levels and the production of cytokines and chemokines by the epithelium. Primary tissue culture systems can be used to mimic the in vivo situation.

More studies in healthy subjects are necessary to investigate the ‘normal’ immune response. We suggest further unraveling of the tolerogenic function of the oral mucosa. Based on our findings in allergic rhinitis and healthy individuals, we speculate that CD141+ myeloid dendritic cells could be a target to improve allergen-specific sublingual immunotherapy.
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