Rapid recruitment of CD14$^+$ monocytes in experimentally induced allergic rhinitis in human subjects

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Background: Activated T_H2 cells and eosinophils are hallmarks of the allergic inflammation seen in patients with allergic rhinitis (AR). However, which cells activate and attract T cells and eosinophils to the inflammatory lesion has not been determined.

Objective: We wanted to assess the role of mucosal mononuclear phagocytes, consisting of monocytes, macrophages, and dendritic cells, in the local allergic inflammatory reaction.

Methods: Patients with AR and nonatopic control subjects were challenged with pollen extract, and nasal symptoms were recorded. Mucosal biopsy specimens obtained at different time points before and after challenge were used for immunostaining *in situ* and flow cytometric cell sorting. Sorted mononuclear phagocytes were subjected to RNA extraction and gene expression profiling.

Results: In an *in vivo* model of AR, we found that CD14$^+$ monocytes were recruited to the nasal mucosa within hours after local allergen challenge, whereas conventional dendritic cells accumulated after several days of continued provocation. Transcriptomic profiling of mucosal mononuclear phagocytes sorted after 1 week of continued allergen challenge showed an activated phenotype at least partially driven by IL-4 signaling, IL-13 signaling, or both. Importantly, gene expression of several T_H2-related chemokines was significantly upregulated by the mononuclear phagocyte population concomitant with an increased recruitment of CD4$^+$ T cells and eosinophils.

Conclusion: Our findings suggest that the mononuclear phagocyte population is directly involved in the production of proinflammatory chemokines that attract other immune cells. Rapid recruitment of CD14$^+$ monocytes to the challenged site indicates that these proinflammatory mononuclear phagocytes have a central role in orchestrating local allergic inflammation.

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Key words: *CD14$^+$ monocytes, resident macrophages, dendritic cells, human airway allergy, nasal mucosa, T_H2-associated chemokines*

Allergic diseases have become a public health concern of pandemic proportions. Allergic rhinitis (AR) alone affects 1 in 5 Europeans and is often a lifelong debilitating disorder with a major effect on quality of life. Moreover, AR and asthma are closely related diseases, and the majority of patients with allergic asthma have concomitant rhinitis.[1,2]

In patients with AR, the inflammatory reaction is characterized by activated T_H2 cells, eosinophils, and mast cells. In addition, the human upper airway mucosa harbors a dense network of antigen-presenting cells (APCs) within and below the surface epithelium.[3-5]

These APCs can be assigned to the mononuclear phagocyte system (MPS). The MPS consists of monocytes, macrophages, and dendritic cells (DCs),[6] which all exhibit multiple functions during an immune response. Cells of the MPS express MHC class II, thus being capable of presenting antigens to T cells. Furthermore, they produce cytokines and chemokines that activate and attract leukocytes.[7] The APC network during homeostasis in the upper airway mucosa consists of spatially closely related macrophages and DCs.[5] Mucosal DCs can be further divided into CD1c$^+$ signal-regulatory protein α (SIRPα)$^+$CD103$^+$ and CD141$^+$CD103$^+$SIRPα$^-$ conventional dendritic cells (cDCs), CD103$^-$SIRPα$^+$ DCs, and CD123$^+$ plasmacytoid dendritic cells (pDCs).[8] cDCs and pDCs originate from a common DC precursor in human subjects,[9] whereas CD103$^-$SIRPα$^+$ DCs are thought to be monocyte derived.[8] Mucosal macrophages are stationary cells with important immunoregulatory functions. Although several studies in mice have shown that macrophages in various tissues are embryonically derived,[10] mucosal macrophages appear to be constantly replenished by circulating monocytes,[11] and in human subjects it is suggested that incoming CD14$^+$ monocytes can differentiate into 2 distinct populations: macrophages that maintain the expression of CD14 and CD103$^-$SIRPα$^+$ DCs that become CD14$^-$.[8]

T_H2 cells are central to the inflammatory reaction in patients with AR. However, local activation of T_H2 cells depends on the presentation of allergenic peptides in the context of MHC class II. The MPS consists of professional APCs, and these cells are thus good candidates for activating T cells locally. Therefore detailed knowledge about the function of these upstream mediators of T-cell activation is important to understand the underlying immunopathology in patients with AR. Applying an *in vivo*

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Abbreviations used
APC: Antigen-presenting cell
AR: Allergic rhinitis
cDC: Conventional dendritic cell
DC: Dendritic cell
DC-SIGN: Dendritic cell–specific intercellular adhesion molecule 3–grabbing nonintegrin
moDC: Monocyte-derived dendritic cell
MPS: Mononuclear phagocyte system
pDC: Plasmacytoid dendritic cell
PNAd: Vascular peripheral lymph node addressin
SIRPα: Signal-regulatory protein α
SPT: Skin prick test

human model of AR, we have shown that both $CD1c^+$ cDCs and pDCs accumulate in the nasal mucosa after several days of repeated allergen challenge accompanied by typical allergic symptoms and eosinophilia.[12,13] Recently, we have demonstrated that $CD1c^+$ cDCs in the nasal mucosa express the receptor for thymic stromal lymphopoietin and that this proallergic cytokine increases their capacity to activate T_H2 cells.[13] cDCs and pDCs have also been shown to play important roles in airway allergy in experimental mice; however, it was shown that monocyte-derived dendritic cells (moDCs) accumulate at high numbers during the effector phase of allergic inflammation and exhibit a strong capacity to recruit and activate T_H2 cells locally.[14] In a rat model of allergic asthma, we have found that MHC class II^+ cells (possibly monocyte derived) were rapidly recruited to the challenged tracheal mucosa and presented allergen to memory T cells.[15] Examining severe asthma exacerbations in children, peripheral blood monocytes were expanded and showed signs of activation, including upregulation of CCR2, a receptor central for monocyte emigration.[16] Together, these reports strongly suggested that recruitment of proinflammatory monocytes might be central players in allergic airway inflammation. Whether monocyte-derived cells play a similar role in patients with AR has not been investigated.

Functional characterization of specific cell types in human diseases is often restricted to *in situ* analysis of a few selected markers, which provides limited information. To obtain a more comprehensive study regarding the role of the MPS in patients with AR, we examined the composition of mononuclear phagocytes that accumulated in the nasal mucosa during experimentally induced AR and performed global transcriptomic profiling of the MPS isolated from the lesion.

METHODS

Ethics statement

All participants in the study provided informed written consent. The study was approved by the Regional Committees for Medical and Health Research Ethics in Norway.

Patients

The size of nasal biopsy specimens obtained after topical anesthesia is limited, impeding the possibility to perform cell sorting and *in situ* immune staining in the same tissue specimen. We avoided taking more than 2 biopsy specimens from the same nostril to reduce the risk of incoercible epistaxis. Therefore 4 cohorts of adult patients (age >20 years) were necessary to complete this study.

In cohort I 8 patients with grass pollen allergy were recruited. The patients were allergen challenged according to the protocol shown in Fig E1, *A*, in this article's Online Repository at www.jacionline.org. In cohort II 9 patients with grass pollen allergy and 5 healthy nonatopic control subjects were included and challenged (see Fig E1, *B*). Cohort III comprised 6 patients with birch pollen allergy whose tissue specimens were processed for cell sorting before and after the allergen provocation protocol (see Fig E1, *C*). In cohort IV 8 patients with birch pollen allergy were recruited and challenged (see Fig E1, *D*). Nasal biopsy specimens obtained for *in situ* staining were either snap-frozen bedside (cohort I and II) or formalin fixed and paraffin embedded (cohort IV).

All patients were challenged according to the same procedure, as described below. Applying the same *in vivo* provocation model, we previously showed that both pollen allergens induce similar symptoms and blood and tissue eosinophilia in challenged allergic patients.[12] All patients fulfilled the Allergic Rhinitis and its Impact on Asthma criteria[17] for persistent moderate-to-severe AR and had been symptomatic for at least the last 3 pollen seasons.

In cohorts I and II all allergic patients had positive skin prick test (SPT) responses to grass pollen. Four subjects in cohort I and 5 subjects in cohort II also had positive SPT responses to birch, house dust mites, and/or cat, dog, or horse dander. All control subjects (cohort II) had negative responses to all tested aeroallergens. Patients in cohorts III and IV had positive SPT responses to birch pollen. Four subjects in cohort III and 5 subjects in cohort IV were also sensitized to grass, mugwort, ragweed, house dust mites, and/or cat, dog, or horse dander.

Any other types of nasal disorder were excluded based on both the patient's history and clinical examination, including nasal endoscopy. Nasal provocation was performed out of the pollen season. No patients experienced nasal allergic symptoms in the months before provocation or received any medication during the challenge period. Control subjects did not have any allergic or nasal disease, and the patients and control subjects were otherwise healthy. None of the participants were smokers.

Nasal allergen challenge

The nasal challenge procedure has been described in detail elsewhere.[12] A solution of pollen extract was delivered once a day into one nostril with a hand-driven pump spray, producing a defined volume of 50 μL (100,000 SQ/mL, either of Aquagen grass or Aquagen birch; ALK-Abelló, Hørsholm, Denmark).

Nasal symptoms

Patients were instructed to score each nasal symptom (sneezing, rhinorrhea, and nasal blockage). For patients in cohorts I and II, this was recorded daily on a 4-point scale (range, 0-3). The subjects in cohorts III and IV used a 10-cm visual analog scale ranging from "not bothered at all" to "extremely bothered."

Nasal biopsy specimens

Before nasal challenge (day 0), a mucosal biopsy specimen was obtained after achievement of topical anesthesia (tetracaine/adrenaline) from the lower turbinate of one nostril. Thereafter, allergen challenge was performed on the other nasal cavity according to the corresponding protocol (see Fig E1). The following nasal biopsy specimens were always taken after achievement of topical anesthesia from the lower turbinate of the challenged nostril. For cohorts I, III, and IV, the last biopsy specimen was obtained 24 hours after the final allergen dose. No biopsy-related complications occurred.

Tissue preparation for cell sorting and gene expression profiling

The biopsy specimens were immediately digested for 60 minutes at 37°C in Dulbecco modified Eagle medium (DMEM; Lonza, Basel, Switzerland) containing 2 mg/mL Liberase TL (Roche Diagnostics, Basel, Switzerland) to release single cells. Before flow cytometric sorting, cells were stained with the antibodies listed in Table E1 in this article's Online Repository at