Ann Allergy Asthma Immunol xxx (2015) 1–9

Contents lists available at ScienceDirect

Immune profile modulation of blood and mucosal eosinophils in nasal polyposis with concomitant asthma

Geoffrey Mortuaire, MD [*,†,‡,§]; Isabelle Gengler, MD [*,‡]; Claire Vandenhende-Szymanski, MD [*,‡]; Marie Delbeke [*]; Solène Gatault, PharmD [*]; Dominique Chevalier, MD [‡]; Lionel Prin, MD, PhD [†,§]; and Monique Capron, PharmD, PhD [*]

[*] *INSERM U995, Université de Lille, Lille, France*
[†] *EA 2686, Université de Lille, Lille, France*
[‡] *Department of Otorhinolaryngology—Head and Neck Surgery, University Hospital, Lille, France*
[§] *French Eosinophil Network, University Hospital, Lille, France*

ARTICLE INFO

Article history:
Received for publication October 22, 2014.
Received in revised form December 1, 2014.
Accepted for publication January 23, 2015.

ABSTRACT

Background: Chronic rhinosinusitis with nasal polyps (CRSwNP) is frequently associated with asthma. Mucosal eosinophil (EO) infiltrate has been found to correlate with asthma and disease severity but not necessarily in every patient. Other multifactorial immune processes are required to determine disease endotypes and response to treatment.

Objective: To evaluate EO immunomodulation for migration and survival in accordance with inflammatory protein profiles and asthmatic status in CRSwNP.

Methods: Ninety-three patients (47 with asthma) with CRSwNP were included. Each patient was staged clinically according to symptom severity and polyp size. Nasal secretions were collected to establish a cytokine profile. The EOs were purified from blood samples and nasal polyps to delineate specific immunophenotypes by flow cytometry and determine in vitro EO survival in relation to asthmatic status.

Results: The CRSwNP in patients with asthma was characterized by eosinophilia and a high level of interleukin (IL)-5 in nasal secretions. Although EOs exhibited activation profiles after mucosal migration, there was relative down-expression of IL-5 receptor-α (IL-5Rα) on nasal EOs in patients with asthma. The EO culture with IL-5 and IL-9 showed an antiapoptotic effect in patients with asthma through IL-5Rα modulation.

Conclusion: Mucosal eosinophilia seems to be induced by EO nasal trapping through modulation of adhesion receptors. In patients with asthma, EO involvement is enhanced by the antiapoptotic synergistic action of T-helper cell type 2 cytokines on IL-5Rα expression. This study shows for the first time that IL-9 is involved in EO homeostasis in CRSwNP and could explain the low benefit of anti—IL-5 therapy for some patients with asthma and nasal polyposis.

Introduction

Chronic rhinosinusitis with nasal polyps (CRSwNP) is a clinical syndrome characterized by persistent symptomatic inflammation of the mucosa of the nose and paranasal sinuses. It affects 1% to 4.3% of the general population.[1–3] The impact of the disease on quality of life is considerable, with chronic nasal obstruction and dysosmia.[4] Functional endoscopic sinus surgery is required when symptoms persist despite optimal medical treatment, which is still based on topical and systemic corticosteroid.[3,5] Medical failure is observed mainly in concomitant asthma or acetylsalicylic acid intolerance, which represents 42% or 15% of patients, respectively.[6–10] Similarly, asthma associated with CRSwNP frequently accounts for patients with substantial residual disease despite contemporary airway treatment approaches.[3]

The pathogenesis of CRSwNP is still poorly understood, given that this syndrome encompasses major variants or endotypes. The impact of allergy as an excessive inflammation driven by aberrant T-helper cell type 2 (T_H2) immunity is controversial because allergy incidences in patients with CRSwNP and the general population are equal.[3] Environmental factors such as fungal (*Alternaria* species) or

Reprints: Geoffrey Mortuaire, MD, Service d'ORL de chirurgie cervico-faciale, Hôpital Huriez, CHRU Lille, 59000 France; E-mail: g-mortuaire@chru-lille.fr.
Drs Mortuaire and Gengler contributed equally to this work.
Disclosure: Authors have nothing to disclose.
Funding: This work was supported by grants from the Otology Research Association (Lille, France) and the French National Institutes for Health and Medical Research (INSERM).

http://dx.doi.org/10.1016/j.anai.2015.01.012
1081-1206/

bacterial (*Staphylococcus aureus*) agents could act as triggers of nasal mucosa disruption and stromal inflammation through innate and adaptive immune responses.[11–13] Whatever the hypothesis, the characteristic of CRSwNP inflammation in the white population is substantial eosinophil (EO) mucosal infiltrate.[14–18]

Nasal infiltrate requires active blood EO recruitment and EO survival within the mucosa after transendothelial migration. Adhesion agents are described as pivotal in the interaction of EOs with nasal vessels and mucosal extracellular matrix components.[19,20] Many of them, such as CD44, lymphocyte function-associated antigen-1 (LFA-1 or CD11a/CD18) β_2-integrin, or very late antigen-4 (VLA-4 or CD49b/CD29) β_1-integrin, have been studied in airway inflammation.[21–23] The expression modulation of these interacting and homing receptors and their role in patients with CRSwNP with and without asthma require further investigations.

Concerning EO priming within the mucosa, many studies have emphasized the major role of proinflammatory cytokines by the epithelial thymic stromal lymphopoietin–dependent signaling pathway and innate lymphoid cell activation.[24–26] Interleukin-5 (IL-5), mainly produced by activated T cells, mast cells, and EOs, is associated with the clinical severity of asthma.[27] In CRSwNP, high levels of IL-5 in nasal polyps have been proved to be associated with comorbid asthma.[28] Although anti–IL-5 therapies with mepolizumab or reslizumab are effective to improve quality-of-life scores and decrease exacerbation rates and blood and sputum EO counts in asthma,[29] only short-term efficacy has been observed on symptoms and polyp eosinophilia in patients with severe nasal polyposis.[30–33] Among other cytokines produced by T cells and EOs, interleukin 9 (IL-9) has been studied in lower airway inflammation and has been shown to be involved in blood EO maturation and survival by enhancing IL-5 receptor-α (IL-5Rα) membrane expression. Its interaction with IL-5 in CRSwNP has not yet been clearly evaluated.[34]

To investigate the relation between asthma and CRSwNP further for EO involvement and disease variants, patients were compared for eosinophilia and levels of proinflammatory markers in blood samples and nasal secretions. Subsequently, patients' EO profiles were examined for adhesion and interleukin receptor expression. In patients with CRSwNP and asthma, a specific inflammatory endotype was delineated and potential additive effects of IL-5 and IL-9 on EO survival were found.

Methods

Patients

Ninety-three patients older than 18 years with CRSwNP (according to guidelines of the European Position Paper on Rhinosinusitis and Nasal Polyps[3]) were included. All patients provided written informed consent before participation. The study on EO sample collection was approved by local ethics review board (registration number 2009-A00314-53). The patients were initially referred to the authors' department for persistent symptoms despite optimal medical treatment (≥3 courses of 7-day oral corticosteroid and maximum dose of topical nasal corticosteroid spray). Concomitant asthma (diagnosed by spirometry), allergy (confirmed with skin prick testing), and history of acetylsalicylic acid intolerance were assessed. Patients were enrolled after maximum medical management failure and functional endoscopic sinus surgical indication. Patients with immunodeficiency, autoimmune disease, and/or cystic fibrosis were excluded.

The burden of sinonasal symptoms was measured by a visual analog scale (0 = no symptom). Polyp size was measured endoscopically using a 30° rigid nasal fiberoptic scope according to the grading system of Lildholdt et al[35] (maximum total score 3). A computed tomographic (CT) scan was performed to evaluate sinus opacifications by the scoring system of Lund and Mackay[36] (maximum total score 24).

Patients with asthma (n = 47) were systematically compared with patients without asthma (n = 46; Table 1). These 2 groups were comparable in age, sex, duration of CRSwNP before surgery in the authors' institution, and tobacco use. For severity, mean visual analog scale score, polyp score, and CT scan opacification staging did not differ between these 2 groups of patients. However, prior surgery (P = .036) and allergy (P = .027) were more frequent in patients with asthma.

Samples Collection

Samples collected from the 93 patients were used for cytokine measurement, EO purification, and/or histopathologic analysis.

To improve the homogeneity of the population, any kind of medical therapy (oral and topical corticosteroid) except nasal douching with saline solution was stopped at least 1 month before surgery. Blood samples for EO count and EO purification were obtained 3 weeks before surgery in Vacutainer tubes containing ethylenediaminetetraacetic acid (BD Medical, Franklin Lakes, New Jersey). Nasal secretions were collected during the surgical procedure. Two surgical patties measuring 1.27 × 2.54 cm (Codman, Raynham, Massachusetts) were placed in each nasal fossa for 5 minutes. Patties were put in 3 mL of phosphate buffered saline (PBS) and stored for 1 hour at 4°C. Nasal fluid was centrifuged at 1,400 rpm for 8 minutes to separate the cellular components. Then, supernatants were portioned according to the Bradford assay for protein concentration and stored at −70°C until cytokine determination. Nasal polyps were obtained at the beginning of the surgical procedure. Through an endoscope, 1 polyp was gently removed from each patient and kept for a maximum of 1 hour at room temperature (RT) in sterile saline before further procedures.

EO Purification from Blood Sample

The EOs were isolated as previously described[37] on Percoll gradient (Pharmacia Biotech, Uppsala, Sweden) followed by a negative immunomagnetic selection using anti–CD16-, anti–CD2- and anti–CD14-coated microbeads on a Cs column (Miltenyi Biotec, Bergisch Gladbach, Germany) for 30 minutes at 4°C. Purity was determined on cytospin preparations after RAL-555 staining and was greater 98%.

EO Purification from Nasal Polyp

The protocol was adapted from a previously published procedure.[38] Piecemeal tissue was subjected to a first mechanical dissociation with a GentleMacs Dissociator (Miltenyi Biotec). The fragments were subsequently digested by incubation for 1 hour at

Table 1
Demographic and clinical description of study population

	With asthma	Without asthma	*P* value
Population (n = 93)	47	46	NS
Age (y), mean	49.5	46.6	NS
Men/women	31/16	36/10	NS
Duration of CRSwNP (y)	12.4	10.4	NS
Prior surgery, %	53.2	30.4	.036[a]
Allergy, %	44.7	21.7	.027[a]
ASA intolerance, n	14	—	—
Tobacco use (n = 9)	2	7	NS
Polyp size score (1–3), mean	2.47	2.38	NS
CT scan score (1–24), mean	19.09	19.15	NS
VAS score (1–10), mean	7.3	7.1	NS

Abbreviations: ASA, acetylsalicylic acid; CRSwNP, chronic rhinosinusitis with nasal polyps; CT, computed tomographic; NS, nonsignificant; VAS, visual analog scale.
[a]By χ^2 test.