



Dissecting the role of eosinophil cationic protein in upper airway disease

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Purpose of review

Eosinophil granulocyte myeloid cells are increased in atopic and nonatopic rhinitis, chronic rhinosinusitis (CRS) and atopic keratoconjunctivitis, diseases of the upper respiratory tract. Eosinophils contain several basic granule proteins, the best known being the eosinophil cationic protein (ECP). ECP is a cytotoxic, pro-fibrotic ribonuclease, which is found deposited in these eosinophil-related diseases and is often used in parallel with blood eosinophilia to monitor those diseases. The contribution of eosinophils and their granule proteins to disease pathogenesis have been debated; recent findings might bring these cells to the center of attention.

Recent findings

Novel mediators of atopic disease, interleukin-17 (IL-17) and IL-33 have been found in the upper respiratory tract. These cytokines stimulate eosinophils to survival and degranulation, IL-17 via granulocyte-macrophage colony-stimulating factor (GM-CSF), and IL-33 directly. Transforming growth factor (TGF)- β has been found in CRS and atopic keratoconjunctivitis mucosa, its production possibly stimulated by ECP. ECP is detected in nasal mucosa of local allergic reactions, entopy, in rhinitis and CRS. ECP might be released from freely circulating eosinophil granules or in association with eosinophil mitochondrial DNA, both means of release for pathogen defence.

Summary

Novel evidence suggests that eosinophils and ECP might have new prominent roles in development of diseases of the upper respiratory tract.

Keywords

eosinophil cationic protein, entopy, IL-17, IL-33, mepolizumab

INTRODUCTION

The upper respiratory tract includes the nasal cavity and trachea, which is the passageway for respired air. During inhalation, incoming air is diverted by several choncha in the nasal cavity to weaken and moisten the airflow. This region is heavily exposed to airborne pathogens and exogenous particles carried by the incoming air. The nasal mucosa and the adjacent sinus cavities are covered by goblet cell rich ciliated pseudostratified columnar mucosal epithelium. The mucosa and lamina propria is rich in lymphocytes and inflammatory cells such as mast cells and eosinophils [1,2^{***}] (Fig. 1). A number of diseases are attributed to the upper respiratory tract. The origin of the diseases is the constant exposure to pathogens, allergens and various particles found in the inhaled air or oral ingestion in combination with genetic factors. Bacteria, fungi and viruses can cause rhinitis, rhinosinusitis and conjunctivitis (Fig. 2). Eosinophil granulocytes are often associated

with diseases of this region, but their purpose there remains uncertain. Recent discoveries of novel inflammatory mediators, the emergence of local allergy and development of pharmacological tools

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Curr Opin Allergy Clin Immunol 2012, 12:000–000

DOI:10.1097/ACI.0b013e32834eccaf

KEY POINTS

- Innate cytokines IL-33 and IL-17 activate eosinophils in diseases of the upper respiratory tract, which via eosinophil cationic protein (ECP) release might contribute to TGF- β -mediated fibrosis in chronic rhinosinusitis (CRS) and atopic keratoconjunctivitis.
- ECP can be used to assess whether a local allergic reaction is cause of rhinitis and CRSwNP, but it is unknown whether ECP contributes to Th2 development.
- ECP can be released from freely circulating granules by LTC₄ stimulation or be released in complex with mitochondrial DNA.
- It would be invaluable to determine whether diseases of upper respiratory tract with eosinophil exacerbations could benefit from usage of biologics to remove eosinophils as was the case for subgroups of asthmatic patients.

have increased our understanding of the incidence of these cells. Eosinophil granulocytes harbor a number of large granules and released granule content is often detectable in biological fluids from the upper respiratory tract, nasal secretions, nasal

lavages and tear fluid. These proteins have been carefully analyzed and recent findings relating to one of the proteins, the eosinophil cationic protein (ECP) [2^{••}] will in this review be correlated with the novel understanding of eosinophils in the upper respiratory tract.

EOSINOPHIL GRANULOCYTES IN HEALTH AND DISEASE

Eosinophil granulocytes are hematopoietic cells of myeloid origin present in low numbers in peripheral blood, mucosa and skin during homeostasis. Whether these cells contribute to biological homeostasis or participate in pathogen fighting remains unanswered. The cell numbers increase dramatically in response to the T helper cell type 2 cytokine interleukin 5 (IL-5) induced by helminth or parasite infection or by overproduction of such cytokines in some forms of hypereosinophilic syndrome [3]. The functions of the cells were thought to be parasite and helminth fighting, but this remains controversial [4]. As the role for the cells remains elusive, the search for functions is ongoing. During the recent year, studies have proposed novel and unexpected roles for eosinophils at new locations, attributing

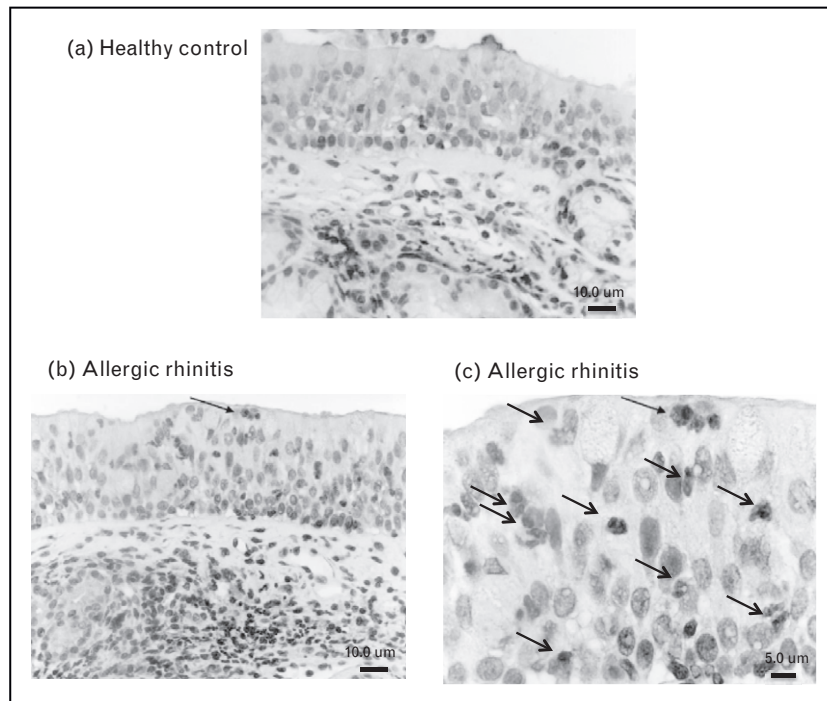


FIGURE 1. Columnar epithelial cells and submucosa of the nasal cavity. Eosinophils are visualized using an antibody (EG2) detecting the granule protein ECP. Eosinophil granulocytes are rarely visible in a healthy control (a). In contrast, eosinophils are abundant between epithelial cells and in submucosa in a rhinitis patient (b) (scale bar 10 μm). (c) Higher magnification highlighting eosinophil granules residing in the epithelium (arrows, scale bar 5 μm). Reproduced from Bystrom *et al.* [2^{••}].

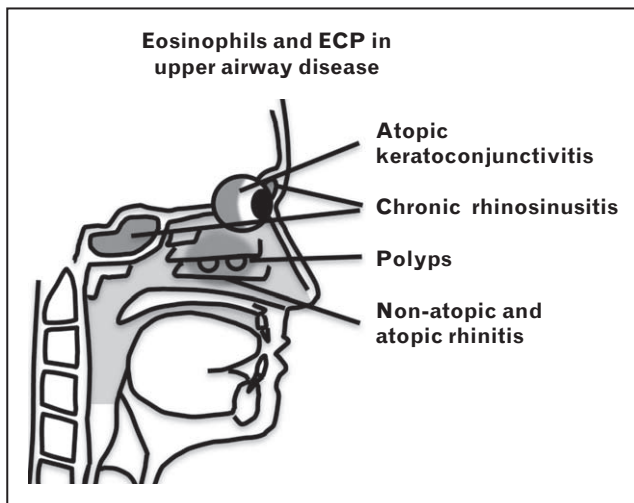


FIGURE 2. Anatomical image showing regions of the upper respiratory tract where eosinophils often are present and ECP released during diseases. For the purpose of assessing eosinophil participation, ECP can be detected in tear fluid, nasal lavage fluid, nasal secretion and sputum [2^{••}].

immune-regulatory and homeostasis-maintaining actions. For example, one study [5[•]] suggests that eosinophils support bone marrow resident antibody-producing plasma cells. Another study [6[•]] located eosinophils to adipose tissue in which they regulated the inflammatory state of macrophages, thereby altering local inflammation and insulin resistance.

Eosinophils are present in mucosa of the entire digestive and respiratory system and have been implicated in pathophysiology of several mucosa-associated diseases such as allergic asthma, eosinophil esophagitis, Crohn's disease and ulcerative colitis. At the beginning of this century, research surprisingly suggested that eosinophils have no role in asthma [7]. However, recent evidence using an antibody directed against IL-5, mepolizumab, showed that an eosinophil-rich form of asthma exists in which exacerbations are reduced when peripheral eosinophils are reduced [8,9]. Similarly, the use of an anti-IgE antibody, omalizumab, for the treatment of house dust mite induced asthma showed best results for eosinophil-rich disease [10]. Another study [11] (without biologics treatment) found interstitial and sputum eosinophilia and sputum ECP associated with accelerated forced expiratory volume decline and change in basement membrane thickness in a group of asthmatic patients.

Eosinophils are often part of the clinical picture of both allergic and nonallergic rhinitis, chronic rhinosinusitis with or without polyps and atopic keratoconjunctivitis. Eosinophils are principally

present in nasal polyps and their granule proteins readily detected there. Nasal polyps are described in asthma, rhinitis and rhinosinusitis, origin in sinuses and prolapse in the nasal cavity. Polyps are generally located above or below the middle concha and have variable sizes (Fig. 2). Although subgroups of asthmatics have benefited from biologics mepolizumab, omalizumab, these or the recently developed anti IL-5 receptor antibody MEDI-563 [12] have not so far been reported in treatment of diseases of the upper respiratory tract.

EOSINOPHIL CATIONIC PROTEIN: RECENT FINDINGS

The eosinophils contain granules filled with basic proteins. Although these proteins, stained red by the dye eosin, are a major focal point of the cells, their actual functions still remain to be fully understood. One of the proteins, ECP, is a basic, fibrosis promoting, cytotoxic ribonuclease and its functions are reviewed extensively in another study [2^{••}]. Analytical techniques for ECP in biological fluids have been developed to assess eosinophil-associated diseases. ECP often correlates with eosinophil numbers in circulation and might better discriminate eosinophil activation [13]. Several studies have concluded that measurement of serum ECP cannot, however, differentiate specific diseases. ECP is detected in allergic and nonallergic asthma, some cases of atopic dermatitis, some cases of eosinophil esophagitis and even cases of sepsis [2^{••},14,15] and endotoxin exposure [16]. However, ECP levels are useful in monitoring disease progression, for example, recently in cases of atopic dermatitis [17] and during immune therapy [18]. Several reports have recently dealt with the mechanism leading to ECP distribution in the surrounding mucosa. It has been proposed that whole granules are released from the eosinophils and subsequently the granule content is expelled, for example, in response to stimulation by cysteinyl leukotrienes [19[•]]. As leukotriene C₄ is prevalent in upper respiratory disease, especially in aspirin-triggered allergic rhinitis, this action could, at least partly, explain efficacy of leukotriene inhibitors such as montelukast. Alternatively, eosinophils have been reported to release mitochondrial DNA coated with cytotoxic granule proteins found attached, to trap and kill pathogens [20]. These mitochondrial DNA/protein complexes have been reported in skin diseases [21[•]] and in human allergic asthmatic airways [22[•]] but not yet in the upper respiratory tract.

This review will discuss the findings from analysis of eosinophils and ECP in the upper respiratory tract in publications during the recent year.

What, if any, is the contribution of the eosinophils and ECP to disease? Is there evidence that eosinophil-dominated disease would benefit from anti-IL5 treatment to relieve symptoms of released granule proteins? Are there additional situations in which ECP could be beneficial?

EOSINOPHIL GRANULOCYTES AND ECP IN NON-ALLERGIC AND ALLERGIC RHINITIS

Rhinitis is inflammation of the nasal mucosa leading to rhinorrhea, nasal obstruction, nasal itching and sneezing. Rhinitis might be induced by repeated inhalation of irritants (occupational), by pathogens or by allergens inducing allergic rhinitis in genetically susceptible individuals. Eosinophils are often present in the nasal mucosa of all forms of rhinitis and can be assessed by ECP levels in nasal lavages [2²²]. Farm workers with rhinitis induced by extended endotoxin exposure presented with elevated levels of ECP in nasal lavage [16]. ECP in nasal lavage was also increased in response to occupational exposures to flour, tea and animal proteins [23]. Eosinophil-rich rhinitis with no known cause is called nonallergic rhinitis with eosinophil syndrome (NARES). This form of rhinitis responds well to corticosteroids and can be monitored by ECP in nasal secretions [24]. Allergen exposure triggers a T helper cell type 2 response with IgE production and concurrent eosinophil accumulation in susceptible individuals. Persistent allergic rhinitis is characterized by a significantly greater eosinophilia and predominantly Th2 cell-mediated nasal inflammatory profile compared with intermittent allergic rhinitis [25]. Nasal polyps were found to be an independent risk factor for bronchial hyperresponsiveness in patients with allergic rhinitis, highlighting the link between allergic disease in the upper and lower respiratory tract [26]. Systemic eosinophils from established allergic asthma or allergic rhinitis patients showed a similar pattern of ECP degranulation [27]. Asthmatic patients' eosinophils, however, were slightly more prone to ECP release than rhinitis patients' [28]. It has recently been proposed that T helper cell type 2 response can occur locally with IgE production detected in the nasal mucosa only but not by skin prick test. This is called entopy [24,29] and NARES can in some cases be explained by such a local allergic reaction. Increased ECP has been detected in nasal lavages of patients with rhinitis and entopy [30²]. ECP can potentially influence many types of cells and could contribute to this local atopy development, for example, by inducing mast cell degranulation, promoting tissue remodeling or influencing lymphocytes [2²²,5²].

Allergic rhinitis might be treated by corticosteroids or immune therapy and even helminthes have been applied with the aim to alter the atopic response. In many cases, ECP levels have been found useful in assessing efficacy of the treatment [15]. Serum ECP was used to monitor efficacy of sublingual immune therapy in patients with allergic rhinitis to house dust mites. Somewhat surprisingly high levels of ECP (and eosinophils) were found to predict efficacy [18]. Blood eosinophils were raised as a consequence of an attempt to resolve allergic rhinitis by oral administration of the helminth *Trichuris suis* to patients [31]. The regimen failed, but gave the byproduct finding that raised eosinophils, during such a period of exposure, do not influence the disease either positively or negatively.

EOSINOPHIL GRANULOCYTES AND ECP IN FUNGAL AND CHRONIC RHINOSINUSITIS WITH OR WITHOUT POLYPS

Allergic fungal rhinosinusitis is caused by inhaled fungi and presents with massive eosinophilia. One such fungus, *Alternaria* activates eosinophils through proteinase-activated receptor-2 leading to granule protein release [32]. Chronic rhinosinusitis (CRS) is diagnosed as lasting more than 3 months, often with Th2-type characteristics and eosinophilia. CRS might present with (CRSwNP) or without (CRSSNP) nasal polyps with the former form being more severe. Patients without polyps have Th1 cells, neutrophils, mast cells, few eosinophils and base membrane thickening. CRSwNP is recurrent with a very high level of reoccurrence of polyps after surgical removal. Polyps of east Asian and Japanese CRSwNP patients often lack eosinophils, but the clinical phenotype eosinophilic chronic rhinosinusitis show many similarities [33]. *Staphylococcus (S.) aureus* infection and its superantigen staphylococcal enterotoxin B (SEB) has been suggested as a cause. Eosinophils are abundant in the polyps. Increased expression of the eosinophil chemokines eotaxin-2 protein, eotaxin-1 and eotaxin-3 mRNA in polyps might explain this eosinophil accumulation [34–36]. Interestingly, children with CRS accumulate fewer eosinophils than adults [37]. About half of the cases are associated with atopy. However, a local allergic reaction (entopy) is taking place in some CRS polyps, just as in cases of rhinitis (see above). SEB have been shown to stimulate B cells and specific IgE antibodies for SEB have been detected. Activated B cells produce IL-5 that activate eosinophils and evoke ECP release. Two studies during the recent year have suggested that polyp-residing

eosinophils and other cells produce IL-17. IL-17 is a cytokine linked to a form of asthma dominated by neutrophils [38]. However, the situation is different in the polyps; one study [39[■]] found nasal polyp macrophages and eosinophils-produced IL-17A in response to SEB stimulation dependent on prostaglandin E₂ and IL-23. IL-17A induced GM-CSF by polyp cells, a cytokine known to prolong survival of eosinophils. The other study [40] found eosinophils and T cells producing IL-17. Increased TGF- β activity has been found in ethmoidal sinus mucosa [41]. Eosinophils have previously been suggested to produce TGF- β . However, released ECP also stimulates TGF- β release from fibroblasts [2[■]]. Eosinophils might, therefore, contribute to remodeling in polyps and sinuses. One alternative speculative explanation why eosinophils are attracted to polyps is for the aim of fighting *S. aureus*. Released ECP associated with mitochondrial DNA [20] could be an innate defense system activated under such circumstances.

EOSINOPHIL GRANULOCYTES AND ECP IN ATOPIC KERATOCONJUNCTIVITIS

Atopic keratoconjunctivitis is a chronic allergic ocular inflammatory condition that leads to corneal scarring. Atopic keratoconjunctivitis invariably involves eosinophils localized to the conjunctival epithelium [42], which can be assayed by ECP in tear fluid [2[■]]. A recent study has identified IL-33 expressed by conjunctival epithelial cells [42]. The innate cytokine IL-33 is associated with atopy and asthma and is suggested to initiate the disease [38]. The receptor for IL-33, ST2 is expressed on eosinophils [43]. IL-33 prolongs survival of eosinophils [44[■]] and cause degranulation [43]. Levels of IL-33 do not, however, discriminate eosinophilic disease as well as ECP do [45[■]]. As tissue scarring is a feature of atopic keratoconjunctivitis, it is not surprising that TGF- β activity is detected. TGF- β was found to stimulate tissue eosinophilia in the disease [46]. Furthermore ECP has previously been shown to induce TGF- β release possibly contributing to tissue scarring [2[■]].

CONCLUSION

Over the recent year, novel insights have been gained into the role of eosinophils and ECP in diseases of the upper respiratory tract. IL-33, a potent activator of eosinophils, was found expressed by epithelial cells in atopic keratoconjunctivitis [42], whereas eosinophils have been shown to be inducers of the inflammatory mediator IL-17 in CRS nasal polyps in response to SEB [39[■],40]. ECP and IgE in nasal lavage from 'nonallergic' rhinitis [30[■]]

and rhinosinusitis patients are useful biomarkers to determine whether entropy is the cause of disease [47]. New research suggests that eosinophil granules are released from the cells for subsequent degranulation. Cysteinyl leukotriene receptors have been found on these granules and when engaged releasing granule content [19[■]]. Additionally, eosinophils can expel mitochondrial DNA associated with granule proteins in skin disease and airways of asthmatic patients [21[■],22[■]]. Anti-eosinophil biologics have been found helpful in subgroups of asthmatic patients with eosinophil-dominated disease. Mepolizumab, omalizumab and recently developed anti-IL5 receptor antibody MEDI-563 might, therefore, be useful also in eosinophil-dominated diseases of the upper respiratory tract.

Acknowledgements

Pfizer paid salary to Jonas Bystrom, although the company is not supporting research related to this review.

Conflicts of interest

None declared.

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