

REVIEW

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Eosinophilic bioactivities in severe asthma



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Abstract

Asthma is clearly related to airway or blood eosinophilia, and asthmatics with significant eosinophilia are at higher risk for more severe disease. Eosinophils actively contribute to innate and adaptive immune responses and inflammatory cascades through the production and release of diverse chemokines, cytokines, lipid mediators and other growth factors. Eosinophils may persist in the blood and airways despite guidelines-based treatment. This review details eosinophil effector mechanisms, surface markers, and clinical outcomes associated with eosinophilia and asthma severity. There is interest in the potential of eosinophils or their products to predict treatment response with biotherapeutics and their usefulness as biomarkers. This is important as monoclonal antibodies are targeting cytokines and eosinophils in different lung environments for treating severe asthma. Identifying disease state-specific eosinophil biomarkers would help to refine these strategies and choose likely responders to biotherapeutics.

Keywords: Severe asthma, Eosinophils, Eosinophilia, Bioactivities, Biotherapeutics

Background

Eosinophils are terminally differentiated granulocytes that play a role in innate host defense against pathogens, particularly parasites and viruses. Eosinophils damage both pathogenic and host cells through the release of toxic granule proteins and reactive oxygen species. Far from maintaining a bystander role, however, eosinophils actively contribute to innate and adaptive inflammatory cascades through the production and release of diverse chemokines, cytokines, lipid mediators and other growth factors. Through these effector mechanisms, eosinophils can influence tissue specific function. Asthma, a chronic airways disease, is often related to airway or blood eosinophilia. Importantly, asthmatics with significant eosinophilia are at higher risk for more severe disease. Strategies for identifying and treating these individuals will provide much needed progress toward personalized medical care. This review details eosinophil effector mechanisms, surface markers, and clinical outcomes associated with eosinophilia and asthma severity.

Key points

- Eosinophils contain highly charged proteins in their granules, mediating toxicity toward pathogens and

tissues, and produce a variety of inflammatory proteins which further contribute to tissue pathology

- Eosinophil accumulation in the airways in severe asthma correlate with markers of local tissue and extracellular matrix (ECM) remodeling
- Eosinophilia is a marker of severe asthma and those at risk for more frequent exacerbations

Unmet needs

- Understanding phenotypic and functional plasticity of eosinophils in the context of the changing or altered lung microenvironment occurring within different asthma endotypes and severity
- Discovery of how the phenotype and function of eosinophils contribute to asthma exacerbations
- Identification of novel eosinophil-restricted biomarkers, both internal and external, with potential to be targeted for therapeutic intervention with specific biotherapeutics and monoclonal antibodies.

Effector and pro-inflammatory mechanisms of eosinophils

Eosinophils are terminally differentiated granulocytic effector cells that produce and store biologically active molecules, including the cytotoxic proteins major basic protein (MBP), eosinophil peroxidase (EPX), eosinophil

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cationic protein (ECP), and eosinophil derived neurotoxin (EDN), lipid mediators, chemotactic peptides, as well as cytokines (Fig. 1) [1, 2]. They are believed to play an important role in the innate immune system by contributing to host resistance to parasites, particularly helminths, but also antimicrobial activities toward bacterial, viral and fungal pathogens [3]. These pathogens can be killed by granule proteins released from activated eosinophils [4]. However, the eosinophil-derived granule proteins are not only toxic to pathogens but also to other cells within immune responses, causing tissue damage and consequently organ dysfunction (Fig. 2). MBP is expressed as two homologs, MBP-1 and MBP-2; MBP-1 has a markedly basic pH and is directly toxic to host and parasite cells [5]. EDN exerts antiviral effects through RNase activity [6]. Besides granule proteins, eosinophil reactive oxygen species have also been shown to damage cells and tissues. EPX catalyzes the development of reactive oxygen species in the presence of hydrogen peroxide. Not only the cytotoxic activity itself, but also the interactions with other immune cells, enhanced the notion of a pro-inflammatory role for the eosinophil [7]. It could be demonstrated that MBP induces histamine release by both mast cells and basophils [8]. Furthermore, EPX in combination with H₂O₂ and halides was shown to induce mast cell secretion [9].

To release granule proteins and to produce toxic oxygen radicals, eosinophils need to be activated (Fig. 2). Following priming with eosinophil hematopoietins, such as IL-5 and GM-CSF, eosinophils exhibit an increased capacity to respond to other inflammatory mediators [10]. Adhesion molecule engagement can also stimulate eosinophil activation [11]. Activation of the mitogen-activated protein kinase (MAPK) pathway is central for degranulation [12]. Granule proteins can be detected in tissues following eosinophil degranulation, sometimes even in the absence of sustained eosinophil infiltration [13], suggesting not only little degradation but also limited toxicity under such conditions. Extracellular granule proteins can be detected in association with collagen fibers, within DNA traps and extracellular free granules (Fig. 2) [14]. MBP has been shown to occur as an amyloid structure within flame figures of patients with eosinophilic dermatitis [15] and eosinophil DNA traps have been demonstrated in multiple eosinophilic diseases, including eosinophilic asthma [16]. Although the DNA scaffold clearly increases the toxicity of granule proteins against bacteria, it remains unclear whether eosinophil DNA traps mediate or limit immunopathology [17]. Extracellular free granules are believed to be the result of eosinophil cytolysis, a non-apoptotic cell death

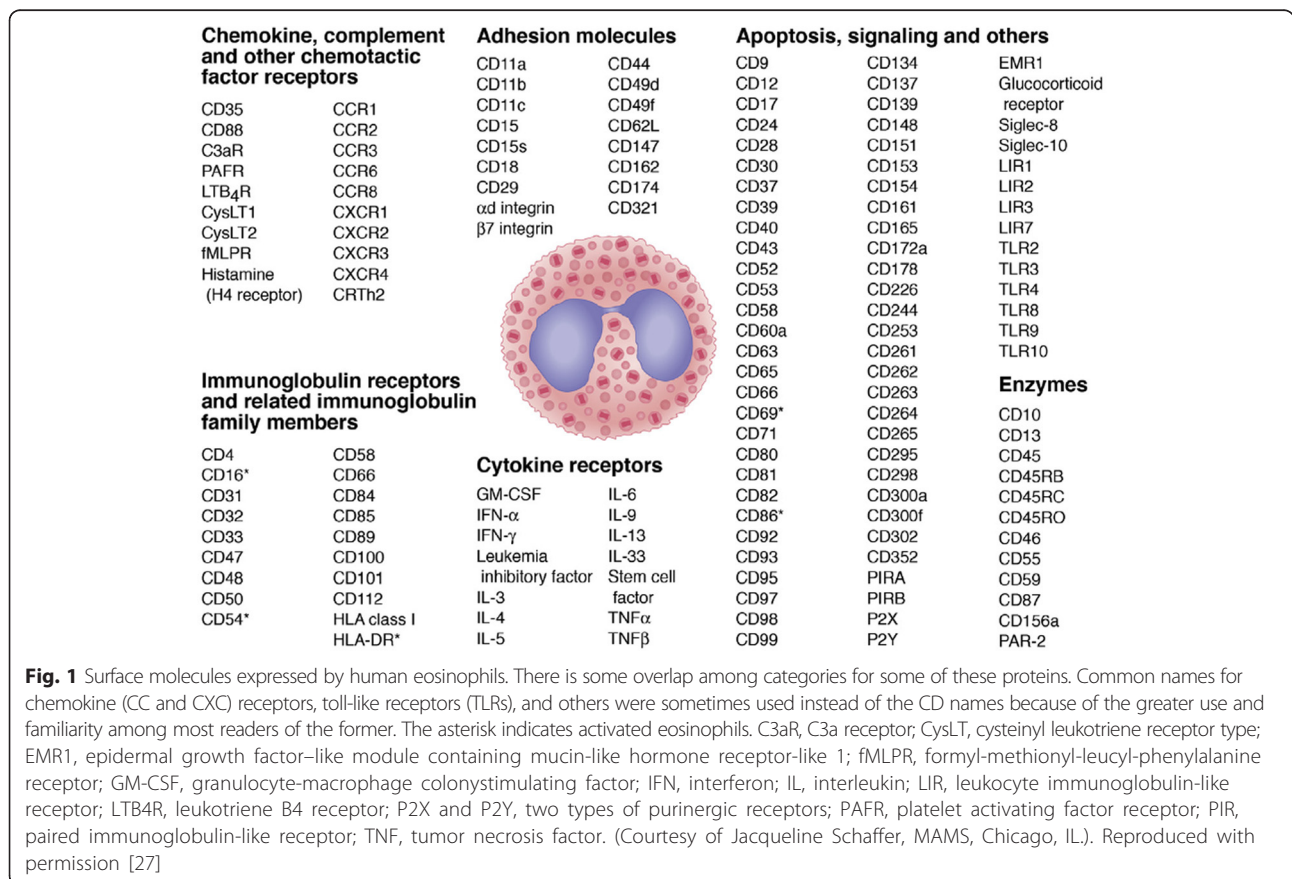
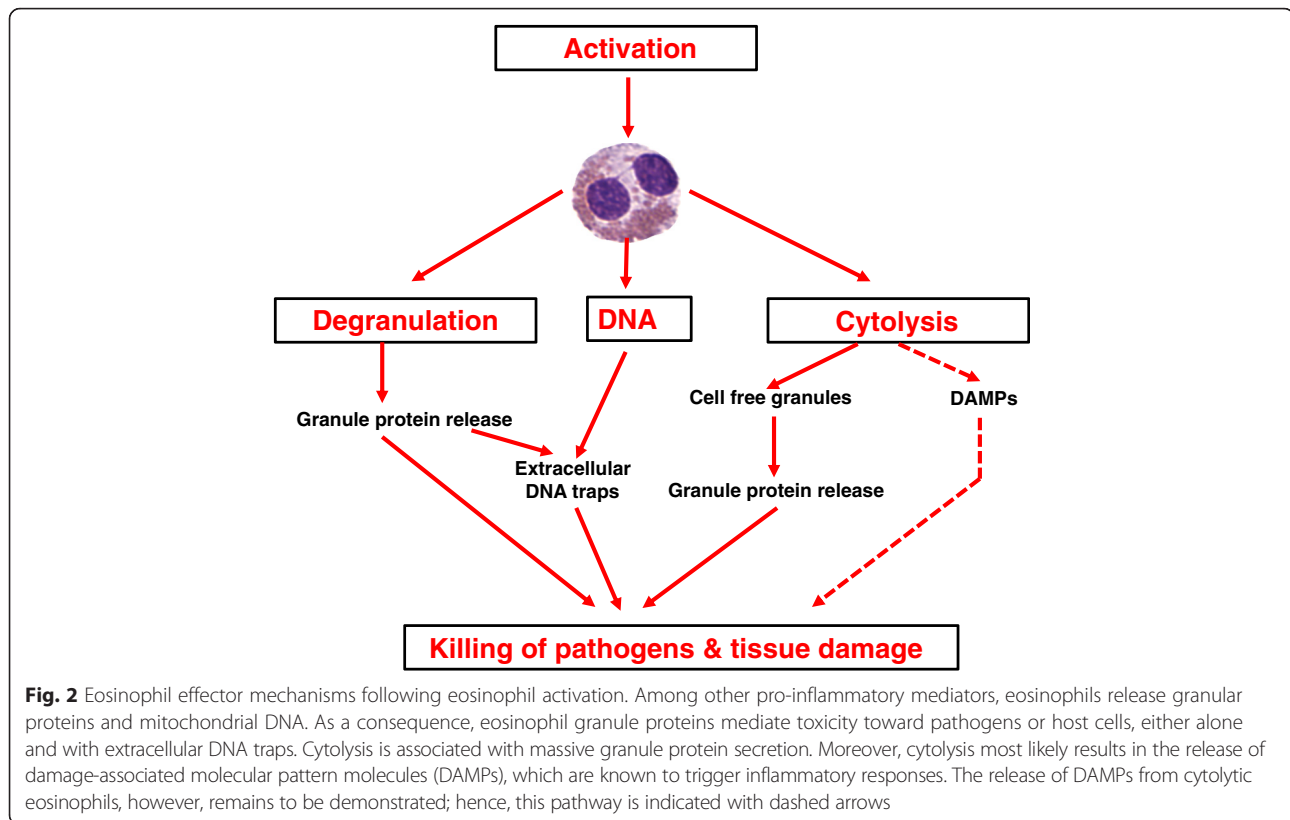


Fig. 1 Surface molecules expressed by human eosinophils. There is some overlap among categories for some of these proteins. Common names for chemokine (CC and CX) receptors, toll-like receptors (TLRs), and others were sometimes used instead of the CD names because of the greater use and familiarity among most readers of the former. The asterisk indicates activated eosinophils. C3aR, C3a receptor; CysLT, cysteinyl leukotriene receptor type; EMR1, epidermal growth factor-like module containing mucin-like hormone receptor-like 1; fMLPR, formyl-methionyl-leucyl-phenylalanine receptor; GM-CSF, granulocyte-macrophage colonystimulating factor; IFN, interferon; IL, interleukin; LIR, leukocyte immunoglobulin-like receptor; LTB₄R, leukotriene B₄ receptor; P2X and P2Y, two types of purinergic receptors; PAFR, platelet activating factor receptor; PIR, paired immunoglobulin-like receptor; TNF, tumor necrosis factor. (Courtesy of Jacqueline Schaffer, MAMS, Chicago, IL). Reproduced with permission [27]



occurring as a consequence of utmost eosinophil activation [18] (Fig. 2). The molecular mechanism leading to eosinophil cytolysis, however, is not known.

The eosinophil's granule proteins exhibit different toxicity when tested *in vitro*. While EDN is only marginally toxic, MBP, EPO and ECP are toxic to a variety of tissues, including heart, brain, skin, and bronchial epithelium [4]. ECP has been shown to be 8–10 times more potent than MBP [19]. Recently, however, it has been demonstrated that the toxicity of MBP is regulated by aggregation [15]. Following degranulation, MBP forms functional amyloid structures exhibiting high toxicity. The continuation of the aggregation process, however, is associated with a loss of toxicity explaining the little damage seen in tissues containing large MBP deposits. MBP can stimulate histamine and leukotriene C4 release from human basophils and can activate mast cells. Interestingly, heparin, a product of mast cells, can rapidly inhibit MBP toxicity [15]. Therefore, MBP toxicity is not stable and depends on polymorphic self-association pathways. Taken together, first insights have been obtained on how the non-selective mechanism of MBP toxicity toward host cells, such as bronchial epithelial cells in asthma, is regulated.

In addition to the direct tissue toxic effect of eosinophil activation and degranulation, eosinophils can

contribute to inflammatory pathways through their capacity to synthesize and secrete a remarkable number of pro-inflammatory cytokines and chemokines [20] (Fig. 1). For example, eosinophils may produce type-2 pathway factors such as interleukin (IL)-4, IL-5, IL-13, and IL-25, and may have been shown to do so in the airways of asthmatics [21]. Chemokines such as CCL5/RANTES, CCL11/eotaxin and CCL3 are secreted by eosinophils and can recruit leukocytes to the site of eosinophilic inflammation. Alternately, following allergen challenge, airway eosinophils have been shown to express GM-CSF and CXCL8/IL-8 [22] thereby inducing neutrophil recruitment. Eosinophils may contribute to airway remodeling in severe asthma through release of transforming growth factor β -1 [23, 24]. Interferon-gamma, a type-1 cytokine secreted by eosinophils, can also potently activate eosinophils [25], and is elevated in the serum of some acute severe asthmatic patients [26], underscoring the importance of these pathways in severe asthma.

The pleiotropic effects of eosinophils therefore include host defense, host tissue damage, regulation and potentiation of inflammatory cascades, each of which may contribute to asthma severity. Therapies that target eosinophils may therefore help control diseases associated with eosinophil-mediated tissue damage and inflammation [7, 27].

Markers on eosinophils

Like all leukocytes, the eosinophil displays a wide range of cell surface proteins, glycans, and lipids (see Fig. 1) [2, 28]. Functions associated with this phenotype include adhesion, migration, proliferation, activation and promotion of survival and death. Some cell surface receptors are G-protein coupled receptors (e.g., chemokine receptors), some are members of the immunoglobulin gene superfamily or have enzymatic activity, while others allow the eosinophil to interact with various soluble mediators released by other immune-related proteins such as cytokines and immunoglobulins. Eosinophils are also equipped with certain intracellular receptors that regulate function (e.g., some toll-like receptors and the glucocorticoid receptor). The only known surface structure that is completely unique to the eosinophil is epidermal growth factor-like module containing mucin-like hormone-like receptor 1 (EMR1) [29, 30]. Other cell surface structures are relatively specific for eosinophils, but are also expressed on mast cells and basophils, such as CCR3 (the receptor for eotaxins), CRTh2 (the receptor for prostaglandin D2), the IL-5 receptor, and Siglec-8. Glycomic analysis of materials derived from cell lysates showed sizable amounts of terminal N-acetylglucosamine containing structures in both eosinophils and basophils, while mast cells have more in the way of sialylated terminal glycans [31].

Markers of eosinophil activation may differ among the atopic diseases, both by site and severity. Identification of markers on tissue resident eosinophils has been limited by the availability of adequate tissue for this analysis, particularly in severe asthma. An extensive review on the role of allergic biomarkers of asthma and other atopic disease was published recently by the World Allergy Organization [32].

Airway eosinophilia occurs in approximately 50 % of severe asthma patients, and is especially prominent in severe asthmatics with Type 2-like late onset disease [33]. The number of eosinophils in sputum can be used as a marker of disease severity, with strong associations between airway eosinophilia and severity of disease symptoms, worsened lung function, and incidence of fatal asthma [33, 34]. Eosinophil numbers in the airways correlate with tissue biomarkers such as periostin, fractional exhaled nitric oxide expression of chemokine CCL26 (eotaxin-3), and thickening of the reticular basement membrane with deposition of extracellular matrix (ECM) proteins, specifically laminin and tenascin [35–37]. Airway eosinophils in asthma exhibit a hyperadhesive phenotype towards various ECM components, and are thought to play an active role in lung tissue remodeling processes and thickening of the reticular basement membrane through their release of TGF- β and proteases [38, 39].

Following recruitment into the airways, there are certain predictable and characteristic changes in the

surface phenotype of the eosinophil reflecting their transendothelial and transepithelial migration and interactions with the tissue and ECM, such as shedding of L-selectin (CD62L), CD31, the IL-5 receptor and CD162, upregulation and/or enhanced function of CD11b, CD11c, CD11d, CD35, CD44 (the receptor for hyaluronic acid), CD66 and CD81, and the de novo appearance of HLA-DR, ICAM-1 and CD69 [40, 41]. Upregulation of an allosterically active form of integrin CD11b is particularly characteristic of the airway eosinophil phenotype in asthma, mediating enhanced adhesion and migration of eosinophils to fibrinogen and diverse ECM ligands [42]. CD11b was also recently established as a primary adhesive and promigratory receptor for periostin [43]. An interesting possibility has been proposed that in patients with severe eosinophilic asthma, in situ maturation of CD34⁺ and CD125⁺ (IL-5R α) eosinophil progenitors rather than recruitment of mature cells could also contribute to persistent airway eosinophilia [44, 45], potentially governed by IL-5 produced by group 2 innate lymphoid cells [46].

By now, it is widely recognized that asthma is a highly heterogeneous disease. Several endotypes potentially exist even in forms of disease characterized by eosinophil presence in the airways, where diverse factors could dictate specific eosinophil phenotypes [47]. Our understanding of eosinophil phenotypic plasticity across different asthma endotypes is currently lacking. Identifying disease state-specific eosinophil biomarkers would not only allow for better understanding of the nature of eosinophil-tissue relationship, but also would help to refine our strategies for targeting these cells in different lung microenvironments.

Eosinophils, severe asthma and allergic inflammation

Eosinophils are present in the blood and some tissues of healthy individuals and are increased in numbers in a variety of disease states. Systemic diseases, such as parasitic infection, drug allergy, vasculitis, or malignancy, can present with blood or organ-specific eosinophilia. Similarly, eosinophilia can be a marker of many diseases of the airway, such as chronic sinusitis, chronic obstructive pulmonary disease, hypersensitivity pneumonitis, acute and chronic eosinophilic pneumonia, and of course, asthma. Indeed, elevated eosinophil levels are seen in approximately half of individuals with asthma [48–51]. Here we discuss the relationship between atopy and eosinophilia as it may pertain to severe asthma.

Atopy is a prominent feature of some asthma phenotypes. Environmental allergens, such as *Dermatophagoides* spp., *Alternaria alternata*, pollens and pet dander have been implicated in the development, or severity, of asthma

in epidemiologic studies [52, 53]. These allergens presumably exert their effects through activation of mast cells and basophils. Mast cells are bone marrow derived cells of the innate immune system which are induced by stem cell factor and IL-3, mature and reside in tissues, and can proliferate in tissues after maturation. Mast cell granules contain pre-formed mediators including histamine, tryptase, and variably other enzymes such as chymase and carboxypeptidase. Allergen-specific IgE antibodies noncovalently bind to the high affinity IgE receptor (FcεRI) on the surface of tissue resident mast cells. Mast cells can be activated by cross-linking of those FcεRI molecules upon exposure of the mast cell to the offending antigen. This event initiates signalling cascades within the mast cell involving protein tyrosine kinases. Three main pathways predominate. The first involves phosphatidylinositol bisphosphate catabolism and activation of protein kinase C, which together facilitate mast cell degranulation and release of the aforementioned preformed mediators. The mast cell activation cascade also activates phospholipase A₂, which induces development of arachadonic acid, and the subsequent production of the lipid mediators prostaglandin D₂ and the cysteinyl-leukotrienes. Finally, activation of the kinase cascades leads to nuclear translocation of transcription factors which stimulate gene expression and protein production of cytokines such as IL-4, IL-5, IL-13 and tumor necrosis factor. The IL-5 released stimulates bone marrow production and release of eosinophils, which are then recruited to tissues via ICAM-1, P-selectin and VCAM-1. Type-2 helper CD4⁺ T lymphocytes are recruited, and chronically contribute proinflammatory mediators which potentiate this cycle.

As discussed earlier in this chapter, eosinophils can cause direct toxic effects on host tissues and promote inflammatory cascades through release of a variety of inflammatory mediators. These effects are reflected in clinical outcomes, particularly severity of asthma and risk of exacerbation. Severe asthma is defined as asthma that requires treatment with high dose inhaled corticosteroids (ICS) plus a second controller for the previous year, and/or systemic corticosteroids for at least half of the previous year, to prevent it from becoming 'uncontrolled' or which remains 'uncontrolled' despite this therapy. Uncontrolled asthma is defined as the presence at least one of the following characteristics: persistently poor symptom control, two or more exacerbations requiring bursts of systemic corticosteroids in the preceding year, at least one serious exacerbation requiring hospitalization in the previous year, or chronic airflow limitation of FEV₁ < 80 % predicted with FEV₁/FVC ratio less than the lower limit of normal [54].

An analysis using the National Health and Nutrition Examination Survey, an annual cross-sectional survey of the US general population, revealed that individuals with

asthma and blood eosinophil count greater than 300 cells per microliter were more likely to report asthma attacks [55]. Similarly, adults with higher blood eosinophil counts seem to have more frequent exacerbations than those with low eosinophil counts [56]. In the National Institutes of Health-sponsored Severe Asthma Research Program (SARP), which enrolled and carefully assessed large cohorts of mild, moderate, and severe asthmatic adults and children, eosinophilic and other cellular markers were assessed in relationship to disease outcomes. Those individuals with significant sputum eosinophilia, often in the presence of sputum neutrophilia, had more severe asthma. Importantly, these groups also had increased medication use, bursts of systemic corticosteroids, and hospitalizations [57, 58]. Reduction of eosinophil levels in blood and sputum is also related to fewer exacerbations and less health care utilization for asthma [59, 60]. However, in some severe asthmatics, high eosinophil levels can persist despite the use of high dose controller medications, including corticosteroids [61].

Importantly, eosinophilia is a marker of beneficial response to corticosteroid therapy [61–64]. Therefore, identification of asthmatics with significant eosinophilic inflammation is an important step towards practicing personalized, or precision, medicine. Eosinophilia can be present in the airway lumen, bronchial walls, and blood, however levels in these compartments do not always correlate. Cell counts and gene expression patterns in the sputum can accurately identify steroid-responders [62, 64]; however, induced sputum collection and measurement is time consuming, labor-intensive, and not available for routine use [65]. Blood eosinophil measurements, while simple and widely available, are a good predictor of steroid response [59, 66]. However, blood eosinophil levels can fluctuate throughout the day, with higher levels in the evening, impacting accuracy of measurements. ECP can be used as a biomarker of eosinophilic inflammation, and can be measured in both blood and airway compartments. In plasma, ECP may be a time sensitive marker of eosinophil activation [67].

Anti-IL-5 monoclonal antibodies, mepolizumab, and reslizumab are available for the treatment of severe eosinophilic asthma. Phase three studies supported its use for reduction of exacerbation frequency and steroid sparing effect [68–70]. Long term effects on airway remodeling remain to be seen.

Conclusions

Eosinophils can affect airway biology as both a source of epithelial damage and airway remodeling in asthma. As such eosinophils contribute to the severity of asthma and may persist despite guidelines-based treatment. Eosinophils also may act as biomarkers for severity of asthma and may also identify the response to treatment

and control for severe asthma. Eosinophils or their products are of great interest for potential usefulness in predicting treatment response and as biomarkers. This is especially important since new and expensive biotherapeutic treatments (monoclonal antibodies) are being directed towards eosinophils in the treatment of severe asthma.

Abbreviations

ECM, extra cellular matrix; ECP, eosinophil cationic protein; EDN, eosinophil derived neurotoxin; EPX/EPO, eosinophil peroxidase; MAPK, mitogen activated protein kinase; MBP, major basic protein

Acknowledgement

The authors would like to acknowledge the editorial support of Sofia Dorsano, World Allergy Organization. The distribution of this paper was made possible by an unrestricted educational grant from Teva Pharmaceuticals.

Funding

Not applicable.

Availability of data and materials

Not applicable.

Authors' contributions

This paper was commissioned as an initiative of the Collaborative on Severe Asthma (COSA), World Allergy Organization. TFC and LJR contributed the section on eosinophils, severe asthma and allergic inflammation. TFC and HUS contributed the section on effector and pro-inflammatory mechanisms of eosinophils. SB and BSB contributed the section on markers on eosinophils. All authors reviewed, edited and approved the final paper.

Competing interests

BSB has current or recent consulting or scientific advisory board arrangements with, or has received honoraria from, Sanofi-Aventis, Pfizer, Biogen Idec, TEVA, AstraZeneca and Allakos and owns stock in Allakos and Glycomimetics. He receives publication-related royalty payments from Elsevier and UpToDate™, and is a co-inventor on existing Siglec-8-related patents and thus is entitled to a share of royalties received by Johns Hopkins University on the potential sales of such products. BSB is also a co-founder of Allakos, which makes him subject to certain restrictions under University policy. The terms of this arrangement are being managed by the Johns Hopkins University and Northwestern University in accordance with their conflict of interest policies. The other authors have no conflicts of interest to declare regarding this paper.

Consent for publication

Not applicable.

Ethics approval and consent to participate

Not applicable.

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Received: 29 March 2016 Accepted: 15 June 2016

Published online: 27 June 2016

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