

## Current Review



# Mechanisms of eosinophilic inflammation

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## ABSTRACT

Eosinophils play roles in the pathogenesis of various diseases. In order to accumulate within sites of inflammation, eosinophils must adhere to, and migrate across the microvasculature. These processes are largely controlled by type 2-immune responses; interleukin (IL)-4 and IL-13 induce the expression of endothelial adhesion molecule vascular cell adhesion molecule-1 (VCAM-1), a representative adhesive ligand for eosinophils, while also stimulating generations of CC chemokines from structural cells, including epithelial cells. VCAM-1 and CC chemokines synergistically induce transmigration of eosinophils to the tissue inflammation site. Another type 2 cytokine, IL-5, prolongs survival, and enhances the effector functions of eosinophils. Recently, accumulating evidence has established that corticosteroid-resistant group 2 innate lymphoid cells are cellular sources for IL-5. Another immunological mechanism that may be contributing to eosinophilic inflammation involves type 1 immune system-associated molecules such as interferons and IP-10. In addition to these immune pathways, lipid mediators, such as cysteinyl leukotrienes, directly provoke the infiltration and activation of eosinophils. Extracellular matrix proteins including periostin also induce the adhesion and activation of eosinophils. Finally, activated neutrophils can also induce eosinophil transmigration. In summary, various mechanisms are involved within eosinophilic inflammation, and effective therapeutic strategies targeting these pathways should be established.

**Keywords:** Eosinophils; Allergy; Allergic inflammation

## INTRODUCTION

Eosinophils are generally believed to play important roles in the pathogenesis of certain allergic or inflammatory disorders. For example, anti-interleukin (IL)-5 treatments that selectively attenuate the number and functional status of eosinophils dramatically improve disease control of severe bronchial asthma with eosinophilia or eosinophilic granulomatosis with polyangiitis [1, 2]. In a murine model of asthma, a lack of eosinophils is sufficient to abolish airway remodeling [3]. Eosinophils play roles within various diseases via the release of inflammatory mediators into tissue sites such as specific granule proteins, cysteinyl leukotrienes, radical oxygen species, and a variety of cytokines or chemokines [4, 5]. Moreover, cytolysis of eosinophils generates nuclear-derived DNA traps that are major

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extracellular structural components in eosinophil-rich secretion and can contribute to viscosity [6]. More recently, Charcot-Leyden crystals, which are formed from the eosinophil granule protein galectin-10, were found to act as an adjuvant to augment the type 2-immune response [7]. For eosinophils to accumulate within the site of allergic inflammation, they must first adhere to and then migrate across the microvasculature [4, 8]. In this review article, the current understanding of the control mechanisms of eosinophilic inflammation within pathological conditions will be discussed.

## “CLASSICAL” TYPE 2-IMMUNITY

It is historically well established that eosinophil adhesion to and their transmigration across endothelial cells are largely controlled by type 2-immune pathways. The representative type 2 cytokines IL-4 and IL-13 induce the expression of adhesion molecule vascular cell adhesion molecule-1 (VCAM-1), but not intercellular adhesion molecule-1 (ICAM-1), on endothelial cells [9]. VCAM-1 is representative of a powerful adhesive ligand for peripheral blood eosinophils, but not neutrophils [9].

VCAM-1 induces a higher degree of adhesion of human peripheral blood eosinophils via interaction with alpha 4 integrins, such as alpha 4-beta 1 (CD49d/CD29, very late antigen [VLA]-4) and alpha 4-beta 7, expressed on eosinophil surfaces as compared to ICAM-1, which is constitutively expressed on endothelial cells [10]. Therefore, IL-4 and IL-13 play a role in capturing eosinophils at the level of the vasculature within inflammation sites [10]. The counter-ligands for ICAM-1 expressed on eosinophils are beta2-integrins including alpha L beta2 (CD11a/CD18, lymphocyte function-associated antigen-1) and alpha M beta2 (CD11b/CD18, Mac-1). ICAM-1 is essentially involved in the induction of transendothelial migration of eosinophils with its counter ligand beta2 integrins expressed on eosinophils [11]. In the presence of type-2 inflammation, another endothelial adhesion protein, such as P-selectin, may also play a role in capturing and interacting with eosinophils [12].

The process of interaction with VCAM-1 augment the effector functions of eosinophils. For example, we have observed that adhesion to recombinant human (rh)-VCAM-1 or VCAM-1-expressing endothelial cells upregulate superoxide generation of eosinophils [13, 14]. In terms of the immunological significance of such a respiratory burst of eosinophils, we confirmed that hydrogen peroxide is able to upregulate the function of beta 2 integrins on these cells [15], suggesting that the process of cell adhesion to VCAM-1 facilitates eosinophil interactions with ICAM-1.

IL-4 and IL-13 also result in the generation of CC chemokines from epithelial cells, airway smooth muscle cells, and even airway fibroblasts [16, 17]. CC chemokines and VCAM-1, but not ICAM-1, synergistically and effectively induce eosinophil migration into tissue. We observed that culture supernatants of specific allergen-stimulated peripheral blood mononuclear cells (PBMCs) obtained from atopic asthmatics enhanced eosinophil transmigration across VCAM-1-expressing endothelial cells, and this migration was blocked by anti- $\alpha_4$ -integrin mAb [18]. Furthermore, the enhancement of eosinophil transmigration with the PBMC supernatant was blocked by mAb against CCR3, a major chemokine receptor present on eosinophils [18]. We then confirmed that eosinophil migrations induced by the CC chemokines RANTES (regulated on activation, normal T cell expressed and secreted), eotaxin, eotaxin-2, monocyte chemoattractant protein (MCP)-3, and MCP-4 were all

augmented in the presence of rh-VCAM-1, but not in the presence of rh-ICAM-1 [19]. These observations suggest that the CCR3/CC-chemokine pathway plays an essential role in eosinophil trafficking in the presence of type 2 inflammation.

The type 2 cytokine IL-5 controls the development and maturation of eosinophils in the bone marrow. Following migration into the tissue site, IL-5 prolongs survival and enhances effector functions of eosinophils [20]. It is noteworthy that although IL-5 at physiological concentrations is not a potent chemoattractant for human eosinophils, it does prime the chemotactic response of these cells. IL-5 is generated by Th2 cells, group 2 innate lymphoid cells (ILC2s), mast cells, and even natural killer T cells [21]. Among them, ILC2s secrete tremendous amounts of IL-5 and IL-13 as compared with other cell types [22]. At inflammation sites, the cytokines IL-33, IL-25, and thymic stromal lymphopoietin (TSLP) may be released from a variety of cells—including epithelial cells—and activate ILC2s [22]. There is evidence that IL-33 and TSLP are increased in the lower airways of severe asthmatics [23, 24]. Eosinophils are IL-25-producing cells and hence could activate ILC2s [25]. ILC2s expressing IL-5 mRNA are increased in the sputum of severe asthmatics despite the use of high-dose inhaled corticosteroids (ICS) and thus could be important cellular sources of IL-5 in the airways of these patients [26]. Interestingly, anti-IL-5 treatment, which provides clinical effectiveness for severe “eosinophilic” asthma, is not sufficiently effective within mild to moderate asthma cases, especially in patients not treated with ICS [27]. A mechanism to explain this discrepancy may be contributed to other eosinophil growth factors, such as granulocyte-macrophage colony-stimulating factor (GM-CSF), which strongly prolongs the survival and upregulates the functional status of eosinophils. Eosinophil viability-enhancing activity in sputum from patients with acute asthma is mainly attributed to GM-CSF, not IL-5 [28]. Eosinophils by themselves are capable of spontaneously generating GM-CSF, but not IL-5, during the process of their transendothelial migration [29]. Following exposure to GM-CSF, the expression of the IL-5 receptor (R) is reduced *in vitro* [30]. Finally, airway eosinophils obtained following segmental allergen challenge express GM-CSF-R, but not IL-5-R [31], raising the possibility that GM-CSF plays a role within certain asthmatic conditions, especially in mild atopic situation. Such actions related to GM-CSF may not be seriously problematic in clinical settings, as ICS is usually sufficient to attenuate airway expression of GM-CSF [32].

## POSSIBLE INVOLVEMENT OF TYPE 1 IMMUNITY

During viral infection, both eosinophilic and neutrophilic inflammation are increased in the airways [33]. Type 1 immunity involves production of interferons (IFNs) and CXC chemokines—such as CXCL10/IP-10—with both being related to antiviral immunity [34]. IFN-gamma is upregulated in the lower airways of severe persistent asthma [35, 36]. Furthermore, the IFN-gamma-producing ability of PBMCs is higher in patients with corticosteroid-resistant asthma as compared to those with corticosteroid-sensitive asthma [37]. In the case of IP-10, concentrations of this specific chemokine are increased during viral-acute asthma [38].

We have confirmed that eosinophil adhesion-inducing activity of endothelial cells stimulated with IFN-beta is significantly augmented when TNF-alpha is present [39]. Furthermore, such augmented adhesion was inhibited by anti-alpha 4 integrin or anti-beta 2 integrin antibodies. Finally, IFN-beta enhanced the expression of both VCAM-1 and ICAM-1 on

endothelial cells. These findings indicate that IFN-beta augments the adhesiveness of endothelial cells for eosinophils, primarily via expression of the aforementioned adhesion proteins. We also found that IP-10 significantly enhanced eosinophil adhesion to ICAM-1 and induced eosinophil superoxide anion generation in the presence of ICAM-1 [40]. Finally, we observed that IP-10 concentrations in sputum were higher in asthmatics that exhibited a mixed granulocyte subtype (eosinophils  $\geq 2\%$  and neutrophils  $\geq 40\%$ ) as compared to healthy subjects [41]. Therefore, CXCR3 ligands such as IP-10 may serve as potent promoters for eosinophilic airway inflammation in asthma. Taken together, type 1 immune system-associated molecules such as IFNs and IP-10 could be involved in the development of eosinophilic inflammation within viral-associated or severe persistent asthmatic disease; however, the clinical relevance of these contributing factors should be further investigated.

## ROLE OF LIPID MEDIATORS

When activated, inflammatory cells—including mast cells, basophils, neutrophils, and eosinophils—are capable of releasing lipids as newly generated mediators. Among them, platelet-activating factor (PAF) and leukotriene (LT) B<sub>4</sub> were first described as inducing both eosinophil and neutrophil chemotaxis more than 30 years ago [42]. Subsequently, several lipid mediators—including prostaglandin (PG) D<sub>2</sub>, 5-Oxo-6, 8, 11, 14-eicosatetraenoic acid (5-oxo-E<sub>2</sub>E), and cysteinyl leukotrienes (CysLTs)—were found to be eosinophil chemoattractants [43-45]. Among these mediators, there is evidence that CysLTs clinically contribute to the accumulation of eosinophils within asthmatic airway tissues, such as inhalation of LTE<sub>4</sub> [46]. Regarding this mechanism, we previously observed that the CysLT LTD<sub>4</sub> directly upregulates the expression of  $\beta 2$  integrins on human eosinophils and augments eosinophil adhesion *in vitro*, mainly via CysLT 1 receptor, a receptor for CysLTs expressed on their surface [47]. Moreover, we confirmed that LTD<sub>4</sub> induces transendothelial migration, respiratory burst, and degranulation of eosinophils through  $\beta 2$  integrin and the CysLT 1 receptor [48]. Such enhanced eosinophil functions provoked by LTD<sub>4</sub> are blocked by montelukast, a CysLT 1 receptor antagonist, but not by beta-adrenergic agonist [49]. Even in clinical settings, addition of a CysLT 1 receptor antagonist, but not long-acting beta-agonists, to ICS further attenuates airway eosinophilia in asthmatic patients [50]. Collectively, CysLTs partly contribute to eosinophilic infiltration and activation in asthmatic airways. Chemoattractant receptor-homologous molecule expressed on TH<sub>2</sub> cells mediates prostaglandin D<sub>2</sub> (PGD<sub>2</sub>)-dependent migration of eosinophils; however, the clinical significance of PGD<sub>2</sub> in allergic disease remains to be elucidated.

## ROLES OF EXTRACELLULAR MATRIX PROTEINS

Some extracellular matrix proteins exert promoting effects upon eosinophilic inflammation. For example, fibronectin, an adhesive ligand for VLA-4 that is constitutively expressed on eosinophils, prolongs survival and increases generation of leukotriene C<sub>4</sub> from eosinophils [51]. Similar effects are also observed with laminin [52]. Periostin, an extracellular matrix protein that is highly expressed in the airways of asthmatics in response to Th<sub>2</sub> cytokines such as IL-13, functions as a matricellular protein that binds to receptors and activates cells, including eosinophils. In this context, we confirmed that periostin directly induces eosinophil adhesion, which is comparable to the effect of VCAM-1. Furthermore, periostin induces eosinophil superoxide anion generation and degranulation through the  $\alpha M\beta 2$

integrin *in vitro* [53]. Collectively, such extracellular matrix proteins may contribute to the enhancement of eosinophilic inflammation in certain conditions.

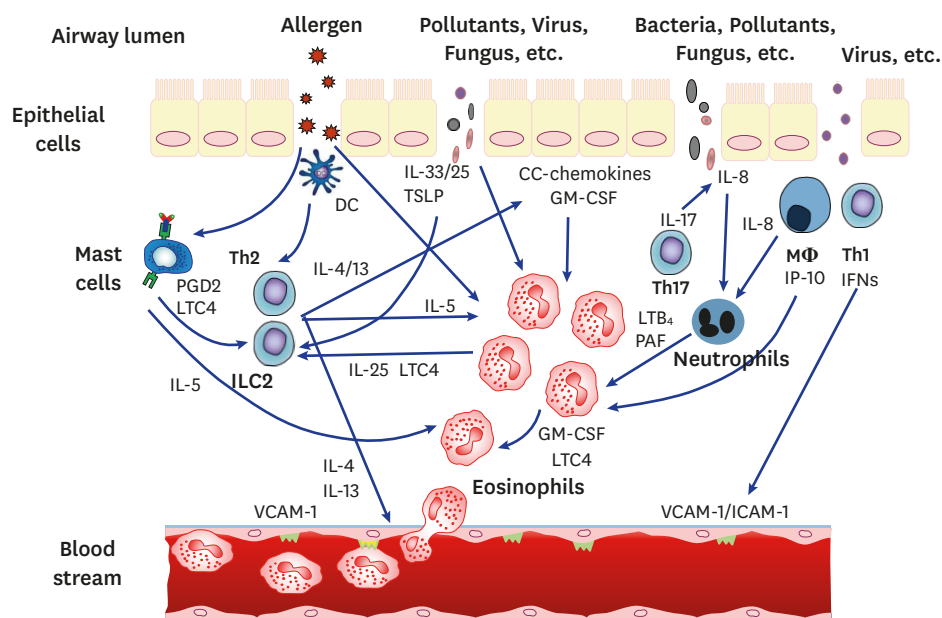
## INTERACTIONS WITH ACTIVATED NEUTROPHILS

Neutrophilic inflammation has been shown to be associated with eosinophilic inflammation in severe asthma. For example, the European Network Study for Understanding Mechanisms of Severe Asthma study showed that severe asthmatics have both a greater sputum neutrophil count and an increased release of eosinophil-derived mediators [54]. There is evidence that IL-8 plays an important role in the accumulation of neutrophils within inflammation sites. For example, we and others confirmed that IL-8 expression is upregulated in the airways of severe asthmatic patients [55,56]. Concerning the relationship between neutrophils and eosinophils within severely asthmatic airways, we observed that neutrophils that had migrated in response to IL-8 strikingly induced the transbasement membrane migration of eosinophils *in vitro*, even without the presence of eosinophil chemoattractant [57]. This neutrophil-induced eosinophil migration is inhibited by either leukotriene B<sub>4</sub> (LTB<sub>4</sub>) or PAF antagonists. Therefore, IL-8-stimulated neutrophils are capable of enhancing eosinophil accumulation in asthmatic airways through release of LTB<sub>4</sub> or PAF from neutrophils.

Lipopolysaccharide (LPS) may play a role in inducing IL-8 or neutrophilic inflammation in the airways of severe asthmatics. In the bronchoalveolar lavage (BAL) fluid of asthmatic children, LPS levels correlate with airway neutrophils or IL-8 [58]. Furthermore, concentrations of LPS in BAL fluid and genes associated with LPS signaling activation are higher in corticosteroid-resistant asthma. A positive correlation is observed between IL-8 mRNA expression in BAL cells and the amount of LPS in BAL fluid [59]. In a study investigating house dust mite (HDM)-sensitive mild asthmatics treated with ICS, inhalation of a combination of LPS and mite allergen-induced activation of eosinophils in the lower airways, while mite allergen alone did not [60]. In this context, we confirmed that LPS-stimulated neutrophils can induce the transbasement membrane migration of eosinophils *in vitro* [61]. Taken together, activated neutrophils, either in the presence of IL-8 or endotoxin, may be involved in inducing eosinophil transmigration.

## ENVIRONMENTAL FACTORS

Environmental factors may also facilitate eosinophilic inflammation. As noted above, LPS is a factor that can augment eosinophil migration via activation of neutrophils and is increased in certain living situations, including within the presence of household pets, cockroaches, or carpeted floors [62]. Some fungi existing within common living environments are also capable of inducing eosinophil activations. For example, aspartate protease activities secreted by *Alternaria* induce activation and degranulation of human eosinophils through protease-activated receptor-2 expressed on the cells [63]. Additionally, *Aspergillus fumigatus* can induce extracellular DNA traps of human eosinophils, which is dependent upon the Syk tyrosine kinase pathway [64]. We recently observed that *Dermatophagooides farinae* extract, a representative HDM, or its major allergen Derf 1 directly induced adhesion, respiratory burst, and release of specific granule protein of eosinophils obtained from normal subjects that were not sensitized against HDM, thereby suggesting that exposure to HDM in the environment might augment eosinophilic inflammation [65]. From this point of view, the



**Fig. 1.** Summary of major contributing mechanisms involved in eosinophilic inflammation. PGD2, prostaglandin D2; LTC4, leukotriene C4; Th2, T-helper 2 cells; ILC2, innate lymphoid cell 2; IL, interleukin; DC, dendritic cells; TSLP, thymic stromal lymphopoietin; GM-CSF, granulocyte-macrophage colony-stimulating factor; LTB<sub>4</sub>, leukotriene B<sub>4</sub>; PAF, platelet-activating factor; VCAM-1, vascular cell adhesion molecule-1; ICAM-1, intercellular adhesion molecule-1.

clinical significance of cleanliness of the home and surrounding environment could be profound in terms of eosinophilic inflammation; however, more study would be required to determine if this is the case.

## CONCLUSION

Both classical and novel mechanisms are involved in eosinophilic inflammation (**Fig. 1**). Further development of effective therapeutic strategies targeting effective control of eosinophilic inflammation is required.

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