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Neutrophil recruitment by allergens contribute to allergic sensitization and allergic inflammation

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Abstract

Purpose of review—To discuss the presence and role of neutrophils in asthma and allergic diseases, and outline importance of pollen and cat dander-induced innate neutrophil recruitment in induction of allergic sensitization and allergic inflammation.

Recent findings—Uncontrolled asthma is associated with elevated numbers of neutrophils, and levels of neutrophil-attracting chemokine IL-8 and IL-17 in BAL fluids. These parameters negatively correlate with lung function. Pollen allergens and cat dander recruit neutrophils to the airways in a TLR4, MD2 and CXCR2-dependent manner. Repeated recruitment of activated neutrophils by these allergens facilitates allergic sensitization and airway inflammation. Inhibition of neutrophil recruitment with CXCR2 inhibitor, disruption of TLR4, or siRNA against MD2 also inhibits allergic inflammation. The molecular mechanisms by which neutrophils shift the inflammatory response of the airways to inhaled allergens to an allergic phenotype is an area of active research.

Summary—Recent studies have revealed that neutrophil recruitment is important in development of allergic sensitization and inflammation. Inhibition of neutrophils recruitment may be strategy to control allergic inflammation.

Keywords

Cat dander; MD2; Neutrophils; Pollens; Toll like receptor 4

Introduction

Asthma is a common inflammatory disease of the airways that affects 10 percent of the population in North America [1]. In patients with asthma, only a small 5–10% subset have uncontrolled asthma [2]; yet this subset accounts for a remarkable 30–50% of morbidity from this disease [3]. The type of inflammatory cells in the airways in uncontrolled asthma

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Conflicts of interest

There are no specific conflicts of interest regarding the content of this article.

is different from controlled asthma, with controlled asthma having more eosinophils and Th2 cytokines than uncontrolled asthma [4–11]. The percentage of neutrophils in the sputum in uncontrolled asthma is higher compared to controlled asthma [6,7]. Subjects with acute severe asthma have higher percentage of neutrophils in their tracheal aspirate compared to a control patients undergoing nonpulmonary surgical procedures [8]. Likewise, patients intubated for status asthmaticus have a higher percentage of neutrophils in their BAL fluid compared to that from patients with stable controlled asthma [9]. Sudden-onset fatal asthma is characterized by more neutrophils in the airways than eosinophils in peribronchial lung tissues, which is different from slow-onset fatal asthma which demonstrates a preponderance of eosinophils in the airways [5]. Neutrophils have long been viewed as terminally differentiated cells that clear extracellular pathogens like bacterium. However, a growing body of literature indicates that neutrophils have numerous additional effects that regulate innate and adaptive immune responses [12,13]. Exposure to allergens can rapidly induce an innate inflammatory response consisting of recruitment of neutrophils to the airways [14–17]. However, the molecular mechanisms by which allergens induce rapid recruitment of neutrophils are not well understood well. In this review we summarize recent data describing the molecular mechanisms that control allergen-induced neutrophil recruitment, and the contribution of these cells to allergic sensitization and allergic airway inflammation.

Uncontrolled asthma is associated with elevated level of IL-8 and IL-17

IL-8, a chemokine that attracts neutrophils, is associated with uncontrolled asthma. The concentration of IL-8 in sputum of subjects with uncontrolled asthma is higher than controlled asthma [6]. The levels of IL-8 in tracheal aspirates from the subjects intubated for acute uncontrolled asthma are higher compared to patients undergoing surgical procedures unrelated to the lung [8]. The patients intubated for status asthmaticus have higher levels of IL-8 in BAL fluid compared to controlled asthma [9]. We evaluated a panel of 48 cytokines and chemokines in BAL fluid from healthy control subjects and subjects with controlled and uncontrolled asthma, and reported that neutrophils and IL-8 are the only inflammatory components in BAL fluids that distinguish controlled asthma from uncontrolled asthma, and both correlate inversely with FEV_1 [18].

IL-17 is also another chemokine that attracts neutrophils. Like IL-8, it is associated with uncontrolled asthma. The levels of IL-17 are elevated in the subjects with uncontrolled asthma compared to subjects with controlled asthma or normal controls [19]. Subjects with asthma have greater percentages of IL-17A positive PBMC compared with healthy control subjects [20]. Supernatants from patients with steroid resistant asthma have more IL-17A compared to patients with steroid sensitive asthma [20]. Asthma exacerbations in atopic severe asthma increase IL-17F and IL-17A expression in lamina propria in the airway and stimulate neutrophil recruitment to the airways [21]. The number of bronchial IL-17F positive cells correlate with number of bronchial neutrophils and FEV1 (%predicted) [21]. The levels of IL-17 in BAL fluid correlate negatively with FEV1 in asthmatic subjects [22]. Together these studies suggest a role of IL-8 and IL-17 in the pathogenesis of uncontrolled asthma.

Neutrophil recruitment stimulates development of allergic cutaneous inflammation

Neutrophil recruitment to the skin can also stimulate allergic skin inflammation [23]. Neutrophil recruitment to the skin is stimulated by tape-stripped in WT mice. This tape-stripped neutrophil recruitment is reduced in Leukotriene B4 (Ltb4)r1−/− mice. Ltb4r1−/− mice mount reduced ovalbumin (OVA)-induced allergic skin inflammation [23]. Transfer of neutrophils from WT mice to Ltb4r1−/− recipients mice exacerbates allergic skin inflammation, indicating that expression of Leukotriene B4 Receptor 1 (BLT1) on neutrophils is important for acceleration of allergic skin inflammation [23]. Intradermal administration of neutrophils from donor WT mice to Ltb4r1−/− recipients mice enhances allergic skin inflammation [23]. Taken together, recruitment of neutrophil is crucial to develop allergic skin inflammation through their LTB4 production and their expression of BLT1. Another group reported a requirement of neutrophils for sensitization and elicitation of contact hypersensitivity. Treating the skin with 2,4,6-trinitrochlorobenzene (TNCB) stimulates infiltration of neutrophils [24]. Neutrophil depletion by anti-Ly6G antibody during the phase of sensitization and mice with a myeloid-specific conditional deletion of the antiapoptotic Mcl-1 protein (Mcl-$1^{\Delta Myelo}$ mice) inhibits the TNCB -induced inflammatory skin response [24]. Together, these studies suggest that neutrophils are important in facilitating allergic cutaneous inflammation.

Pollen allergenic extracts stimulate CXCL chemokines and recruit neutrophils to the airways

Several *in vitro* and *ex vivo* studies have shown that diverse allergenic extracts induce secretion of IL-8 from airway cells [14,25–27]. Thus, microarray analysis revealed that Timothy grass pollen extract induces elevation of IL-8 expression in NCI-H292 cells, a mucoepidermoid carcinoma cell line derived from human lungs [25]. Human airway epithelial cell secrete IL-8 following incubation timothy grass pollen allergen [26], and this IL-8 secretion in bronchial epithelial cells from not only subjects with uncontrolled asthma, but healthy volunteers [27]. Allergenic pollens have intrinsic NADPH oxidases, and these NADPH oxidases raised oxidized glutathione (GSSG) and 4-hydroxynonenal (4-HNE) in BAL fluids of challenged mice. These oxidases stimulate tyrosine phosphorylation of p38 MAPK and secretion of IL-8 [14]. Intranasal challenge with ragweed pollen NADPH oxidase induces neutrophil recruitment to the airway in naïve mice and mice lacking T cells, B cells or mast cells [14]. Likewise, in human subjects with asthma or seasonal allergic rhinitis, allergen challenge increases the level of IL-8 [28–30] and neutrophil recruitment [15–17] in the airways.

TLR4, MD2 and CXCR2 mediate pollen and cat dander-induced innate neutrophil recruitment

We have recently reported that ragweed pollen extract can bind and stimulate CXCL-8 secretion from cells that express both Toll like receptor 4 (TLR4) and Myeloid differentiation protein-2 (MD2), but not cells that only express TLR4 [31]. Suppression of

Md2 in human bronchial epithelial cells by siRNA inhibits ragweed pollen extract, Amb a 1, the main IgE-inducing allergenic protein in ragweed pollen extract, Bermuda, rye, firebush, pigweed, and cat dander extract-induced CXCL8 secretion [31]. Challenge of mice with ragweed pollen extract induces TLR4, CXCR2 and NF-κB -dependent recruitment of reactive oxygen species (ROS)-generating neutrophils to the airways [32]. Suppression of *Md2* mRNA by siRNA administration inhibits ragweed pollen extract and cat dander extract-induced neutrophil recruitment in naïve mice [31]. One of the major allergenic components of house dust mite, Der p2, has structural homology with MD2 that facilitates allergic inflammation by enhancing TLR4 inflammatory cascade [33,34]. Because there is no similarity of structure between Amb a1 and Der p2, its ability to stimulate CXCL8 suggests a distinct mechanism from the structural mimicry of MD2 used by Der p2 [33]. These data indicate that TLR4 and MD2 together regulate pollen extract and cat dander-induced NF-κB activation, CXCL 1/2 secretion, and recruitment of neutrophils to the airway, likely utilizing a mechanism that is distinct from molecular mimicry.

TLR4, MD2 and CXCR2 mediate pollen and cat dander-induced allergic sensitization and allergic airway inflammation

We have reported that MD2, TLR4 and CXCR2 mediated neutrophil recruitment is crucial for induction of allergic sensitization and allergic inflammation [31,32]. Thus, blocking ragweed pollen extract-induced recruitment of activated neutrophils by deletion of TLR4 inhibits induction of allergic airway inflammation [32]. Likewise intranasal administration of CXCR2 inhibitor during repeated intranasal administration of ragweed pollen extract to the airway reduces allergic sensitization and inflammation [32]. Furthermore, forcing recruitment of neutrophils by administration of superoxide generator in *Tlr4* KO mice reconstitutes ragweed pollen extract-induced allergic inflammation [32]. Direct intranasal administration of neutrophils from wild type donor mice together with ragweed pollen extract into *Tlr4* KO recipient mice reconstitutes ragweed pollen extract-induced allergic sensitization and airway inflammation [32]. In mice treated with siRNA to *Md2*, repeated challenge with ragweed pollen extract induces minimal allergic sensitization and airway inflammation [31], indicating MD2 plays an important role in induction of allergic sensitization to ragweed pollen extract. Since diverse allergenic extracts require both MD2 and TLR4 to induce innate inflammation [31,32], competition of LPS and allergens to bind MD2/TLR4 might explain why exposure to endotoxin reduces the risk of developing allergies [35,36]. Together these results strongly suggest that ragweed pollen extract and cat dander extract-induced innate recruitment of neutrophils stimulates allergic sensitization and allergic airway inflammation

Molecular mechanisms by which neutrophils shifts to an allergic phenotype

The molecular mechanisms by which neutrophils stimulate allergic inflammation is an active area of research. Recent several studies have shown that neutrophils can regulate the induction of allergic inflammation through their innate responses. Neutrophil are a major source of oxidative stress by their respiratory chain. Because mice deficient in gp91phox

showed less Th2 cytokine secretion and allergic inflammation [37], recruitment of ROS-generating neutrophils after allergen challenge may have an important role in subsequent allergic inflammation. Several studies have shown that chronic oxidative stress can worsen allergic asthma [38–41], alter DC function, and modify Th1/Th2 balance [42,43]. By contrast, administration of anti-oxidants such as ascorbic acid, N-acetyl-cysteine, tocopherol, or lactoferrin with antigen suppresses antigen-induced allergic airway inflammation [44–46]. Since neutrophils recruited by intranasal allergen challenge generates sustained ROS in the airways, it is likely that this property of neutrophil promotes allergic inflammation through ROS generation. This is suggested by reports that mice deficient in gp91phox, the dominant superoxide generating enzyme in neutrophils, have decreased ROS, and mount an attenuated allergic inflammatory response to allergen challenge [37]. LTB4 generation from recruited neutrophil to the skin stimulates accumulation of effector $CD4^{+}$T cells to OVA-challenged skin, thereby facilitating allergic skin inflammation [23]. Neutrophils may have contributed to allergic inflammation by increasing microvascular permeability [47], inducing proinflammatory cytokines [48], matrix metalloproteinase 9 (MMP-9) [49], and MUC5AC [50].

Small molecule inhibitors of neutrophil recruitment inhibit allergic inflammation

Neutrophil recruitment from blood to a sterile extravascular site has been shown to be dependent specifically on LTB4 [51]. In addition, rrecruited neutrophil-derived LTB4 is crucial for induction of subsequent allergic skin inflammation [23]. Pharmacologic blockade of LTB4 synthesis by best at in suppresses allergic skin inflammation elicited by cutaneous antigen challenge in sensitized mice [23]. Because the level of LTB4 is elevated in BALF from the patients with asthma [52], suppression of LTB4 synthesis or using an LTB4 inhibitor could be effective in inhibiting allergic airway inflammation. However, since intranasal ragweed pollen extract challenge does not induce a significant increase of LTB4 in BALF in allergic model mice [32], further studies about the role of LTB4 in induction of allergic airway inflammation are required.

CXCR2 is the receptor for CXCL1 and CXCL2. Prior murine and human studies suggests an importance of CXCR2 signaling in induction of asthma and allergic inflammation. Thus, treatment of mice with anti-CXCR2 mAb inhibits late-phase airway obstruction, airway hyperresponsiveness, eosinophilic inflammation, and goblet cell hyperplasia [53]. Furthermore, subjects with uncontrolled asthma treated with the CXCR2 inhibitor SCH 527123 have been reported to have fewer mild exacerbations [54]. We recently reported that this receptor plays a critical role in recruitment of pollen-induced neutrophils to the airways and pollen-induced allergic sensitization and allergic airway inflammation [55]. SB225002 is a small molecule inhibitor of ligand binding to CXCR2 and inhibits CXCR2-mediated neutrophilic recruitment [55]. Administration of SB225002 before intranasal ragweed pollen extract challenge inhibits superoxide generating neutrophil recruitment to the airway [32]. Administrating SB225002 prior to each of repeated ragweed pollen extract challenge suppresses ragweed pollen extract-induced allergic inflammation, accumulation of mucin in epithelial cells, production of ragweed pollen extract-specific IgE in serum, and secretion of

IL-5, IL-13, TSLP, and IL-33 in BALF [32]. Future studies will have to validate the results of these studies in humans.

Conclusions

An increasing body of literature indicates that neutrophils and neutrophil-attracting-chemokines like IL-8 and IL-17 are increased in uncontrolled asthma. Recent studies have shown that neutrophils are closely associated with not only severity, but initiation of allergic inflammation and allergic sensitization. The innate immune responses stimulated by exposure of the airways to pollen and cat dander allergenic extracts recruits neutrophils to the airways, that in turn stimulate allergic sensitization and inflammation (Fig. 1). The mechanisms by which recruited neutrophils contribute to the induction of allergic sensitization and inflammation is not yet understood, but could be due to its ability to produce ROS, leukotrienes, cytokines like IL-33 or TSLP, or other factors that stimulate allergic inflammation. Further basic and translational research is required to characterize the role and contribution of neutrophils in induction of allergic sensitization and allergic airway inflammation. Future studies should evaluate targets like TLR4/MD2, CXCR2 or LTB4 to inhibit recruitment of activated neutrophils as a novel strategy to prevent development of allergic sensitization and allergic airway inflammation.

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Bullet point sentences

- Uncontrolled asthma is associated with increased numbers of neutrophils in the airways and elevated levels of neutrophil-attracting chemokine IL-8 and IL-17. These parameters negatively correlate with lung function.
- Neutrophils recruitment is crucial for development of skin allergic inflammation.
- Pollen allergenic extracts and cat dander innately recruit neutrophils to the airway in a TLR4, MD2 and CXCR2-dependent manner.
- Repeated recruitment of activated neutrophils following exposure to pollen allergenic extracts or cat dander facilitates allergic sensitization and airway inflammation.
- The molecular mechanisms by which repeated recruitment neutrophils shifts the airway inflammation to an allergic phenotype is an active area of research
- Inhibition of CXCR2, LTB4, MD2 and TLR4 may serve as strategies to inhibit induction of allergic sensitization and inflammation.

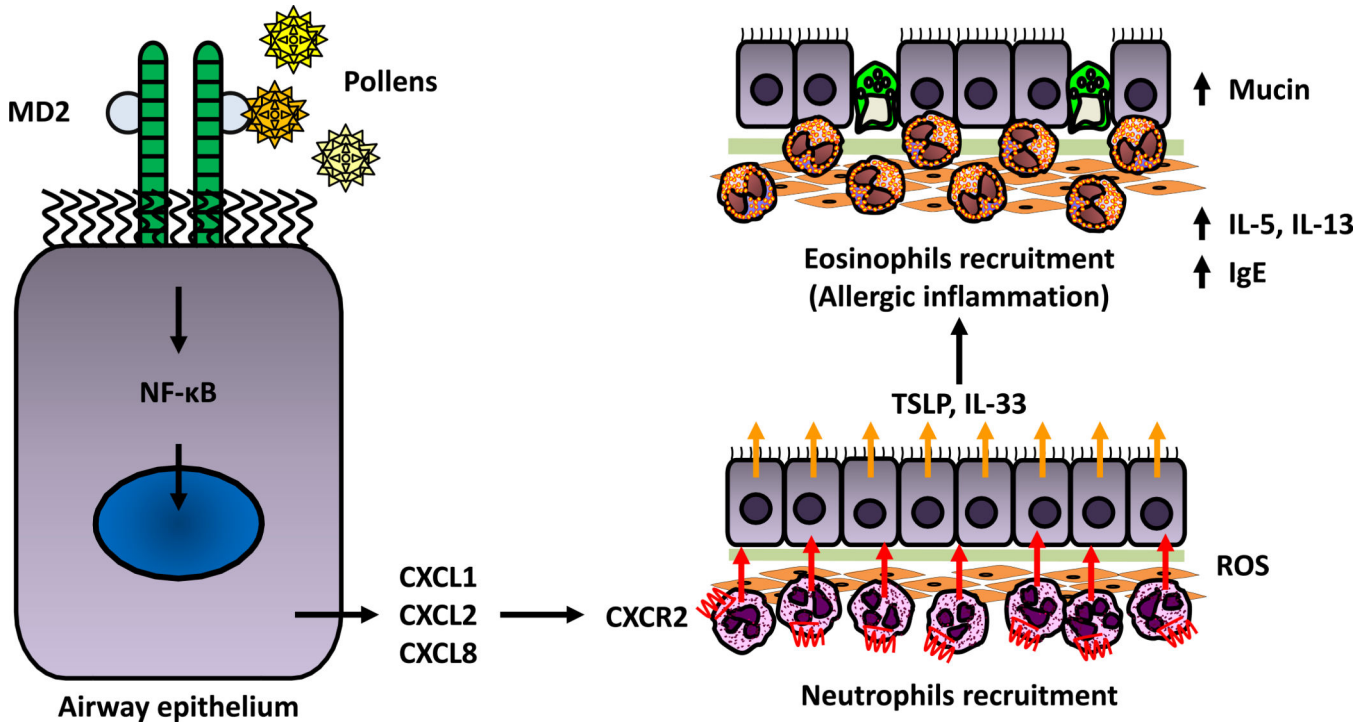

Figure 1. Schematic diagram showing allergen-induced innate neutrophil recruitment is critical for induction of allergic sensitization and airway inflammation

Recognition of pollens and cat dander by MD2 in the context of TLR4 activates NF-κB mediated secretion of CXCL1, CXCL2, and CXCL8 from the airway epithelium. These chemokines recruit ROS-generating neutrophils to the epithelium via CXCR2. The neutrophil-activated airway epithelium secretes TSLP and IL-33 upon subsequent contact with the same allergens, thereby facilitating allergic sensitization and allergic airway inflammation.

ROS; Reactive oxygen species