Neutrophils in Asthma

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Asthma is a complex disease with a significant inflammatory component. Multiple cell types are involved in its pathophysiology. The presence of eosinophils, the cell usually associated with allergic diseases, does not fully explain the inflammation found in asthma. Neutrophils are present in the airway of the patient with asthma in special circumstances and may represent different asthma phenotypes. Neutrophils are activated and are able to release mediators that promote and prolong asthma symptoms. Increasing evidence suggest that neutrophils may be central players with an important role in the pulmonary inflammatory process present in asthma.

Introduction

Asthma is clinically defined by reversible airflow obstruction and/or bronchial hyperresponsiveness associated with mucus hypersecretion and the classic symptoms of cough, wheeze, and dyspnea. It is an inflammatory disease with a complex immunopathology involving several different cell types and mediators. Eosinophils have been considered the most important cells in the pathophysiology of asthma and other allergic diseases. However, eosinophils are not always found in the airway [1], with less than 50% of asthma cases having airway eosinophilia [2]. Interestingly, most cases of noneosinophilic asthma are associated with increased numbers of neutrophils, although a "pauci-immune" phenotype has also been described, in which neither eosinophils nor neutrophils are seen in airway secretions (eg, induced sputum) [3]. In addition, nonatopic asthma is associated with elevated mean neutrophil count when compared to atopic asthma [4]. The contributing role of neutrophils in the pulmonary allergic inflammatory process has been noted, particularly in patients with status asthmaticus who had sudden death within 24 hours of exacerbation onset [5]. In a cohort of Japanese children with primary autoimmune neutropenia (AIN), the incidence of asthma was below the normal rate in Japan [6]. In fact, none of the children with AIN developed asthma while having neutropenia, but some developed asthma after neutropenia was resolved, suggesting that neutrophils may also contribute to the onset of asthma.

There is no question that neutrophils are present in the airway of patients with asthma, but do they play an active role in its pathophysiology? Increasing evidence seems to suggest that neutrophils are an active participant. Neutrophils also seem to predominate in special circumstances, and may represent discrete phenotypes of asthma [7]. Obviously, identifying these different asthma phenotypes is of great importance for evaluating and appropriately treating patients, and for developing future therapies.

Neutrophils in Acute Asthma Exacerbations

Almost half of the patients who present with an acute exacerbation of asthma have no sputum eosinophilia [1], suggesting that other cells may be involved in this airway inflammatory process. Neutrophils were first noted to be present in lung tissue of patients who died within 24 hours of the asthma exacerbation's onset [5]. This finding is supported by the sixfold increase in sputum neutrophil count seen in asthmatic children with acute exacerbation requiring an emergency department visit [8]. In this particular report, no attempt was made to identify the cause of the asthma exacerbation, although in 68% of cases it was attributed to clinical viral infection. Other studies confirm that neutrophils are the predominant cell in the sputum of patients with acute asthma exacerbation due to respiratory tract infection [9].

However, neutrophils are also present in asthma exacerbations due to causes other than infection. High neutrophil counts are observed in bronchial lavage fluid of patients with noninfectious acute exacerbations of asthma requiring mechanical ventilation [10]. Neutrophils are also the first cell to enter the airway in response to an allergen challenge, but have a transient residence time [11–13]. Activation of neutrophils in acute asthma may be explained because neutrophils from asthmatic individuals express the high-affinity receptor for immunoglobulin (Ig) E (FcεRI), whereas neutrophils from nonasthmatic individuals do not [14]. The engagement of FcεRI leads to the release of interleukin-8 (IL-8), a potent neutrophil

chemotactic factor and activator, recruiting more neutrophils to the area. Increased levels of IL-8 have been noted in noneosinophilic asthma [3]. This autocrine effect of IL-8 on neutrophils may prolong the inflammatory process started by an acute exacerbation.

Neutrophils in Acute Lung Injury Leading to Bronchial Hyperresponsiveness

Insults to the airway generate an inflammatory response and an influx of neutrophils into the airway, reactions associated with nonallergic triggers of asthma. Environmental tobacco smoke (ETS) is known to be such an insult; however, there is conflicting evidence suggesting that ETS correlates with levels of airway neutrophils. An increase in sputum neutrophils was observed in a group of asthmatic patients who were either current or past smokers [4]. Conversely, another study showed no difference between the sputum cell counts of patients with asthma who were former smokers or had never smoked, and no difference in neutrophil counts [3].

Other environmental pollutants can induce airway inflammation when present in adequate concentrations, even in persons with no history of asthma. Nitrogen dioxide (NO_2) and ozone seem to be involved in this process [15,16]. Following ozone exposure, a ninefold increase in neutrophil counts in bronchoalveolar lavage fluid has been reported, and this influx was associated with bronchial hyperresponsiveness [15]. Significantly elevated levels of myeloperoxidase, and a trend for increased IL-8 levels, were also observed after ozone exposure [16]. Enhanced airway reaction to allergens was noted after exposure to high ambient levels of nitrogen dioxide, as evidenced by decreased pulmonary function, and was associated with increased myeloperoxidase levels [17]. Increased levels of neutrophils and matrix metalloproteinase (MMP)-9 were observed in sputum of underground miners who were exposed to dust and diesel exhaust [18]. Together, these results suggest that increased numbers of airway neutrophils may be associated with an asthmalike phenotype in patients who do not have asthma, and may contribute to exacerbation in patients with asthma who are exposed to pollutants, highlighting the importance of neutrophils in these processes.

Association of Asthma Severity with Airway Neutrophilia

Identifying a patient with severe asthma can be a daunting task due to the heterogeneity of the disease and the multitude of factors that may interfere with the appropriate classification [19]. The intricacies of asthma's pathology have not been completely elucidated, which contributes to the difficulty of the undertaking. Patients with severe steroid-dependent asthma consistently have more airway neutrophilia when compared with patients who have well-controlled or milder forms of asthma. Severe asthma alone, independent of corticosteroid therapy, has been associated with an increase in airway neutrophils [20]. Again, this may be a result of increased IL-8 levels, which appear to be constitutively elevated in patients with stable asthma, independent of exacerbation. In subjects with moderate and severe asthma (when compared with mild asthma), elevated IL-8 levels are associated with greater severity of disease; moreover, IL-8 levels in such patients remain elevated despite corticosteroid treatment [20].

Another factor that may contribute to the increase in neutrophil influx to the airway of patients with asthma is tumor necrosis factor (TNF)-α. TNF-α is released in IgE-mediated responses by mast cells and eosinophils; because it is chemotactic for neutrophils, it ultimately leads to influx of neutrophils to the local area [13]. In patients with severe asthma, elevated TNF-α levels correlate with the increased number of airway neutrophils [21]. Several reports show a predominance of neutrophils, compared with eosinophils, in the airway lumen, bronchial wall, and alveolar walls of steroid-dependent severely asthmatic individuals, as measured in bronchoalveolar lavage fluid and in bronchial and transbronchial biopsy samples [22,23]. Further characterization of patients with severe steroid-dependent asthma has demonstrated two distinct pathologic subtypes based on the presence or absence of airway eosinophils; regardless of the subtype, airway neutrophil counts were persistently increased [24].

The European Network for Understanding the Mechanism of Severe Asthma (ENFUMOSA) study also showed increased neutrophil counts in the sputum of patients with severe asthma, as compared to those with well-controlled asthma [25]. In this study no difference was seen in the numbers of other inflammatory cells (eg, eosinophils, lymphocytes, macrophages, epithelial cells, and metachromatic cells). The finding of airway neutrophilia only in patients with a severe form of chronic obstructive pulmonary disease is intriguing and may suggest that neutrophils are associated with disease severity [26]. However, there is evidence of increased neutrophil counts in sputum of patients with persistent asthma—not only severe persistent asthma, but also mild and moderate persistent asthma [3]. Preliminary data suggest that increased neutrophil numbers are also seen in milder forms of asthma. Nguyen et al. [27•], reporting on a small cohort of nonasthmatic subjects, noticed that the numbers of neutrophils obtained by bronchial biopsy were elevated in the airway of subjects with mild to moderate asthma when compared with a normal nonasthmatic cohort. Another fascinating fact is that the increased levels of neutrophils are not a function of prolonged disease, because they do not differ among severe asthmatics with early onset disease when compared with late onset [28]. Nocturnal asthma is also associated with elevated numbers of pulmonary neutrophils, and this correlates with symptom severity [29].

Neutrophil Involvement in Airway Remodeling

Airway histologic and structural changes seen in asthma, and leading to an ultimate alteration of airway function, are recognized as an important aspect of airway remodeling [30]. This process is not completely understood, and several cells and mediators appear to be involved. Activated neutrophils are recruited to the airways, where they release their inflammatory mediators and proteases. One important factor released by neutrophils in the airway is transforming growth factor (TGF)-β [31,32]. This mediator is closely linked with airway remodeling because its expression correlates with basement membrane thickness [33], and causes mesenchymal cells to adopt a myofibroblast phenotype [34]. Peripheral blood neutrophils, and neutrophilic myelocytes in the bone marrow [33], constitutively express TGF-β in both asthmatic and normal individuals, although neutrophils from asthmatic patients are able to release higher levels of TGF-β when compared with neutrophils from nonasthmatic individuals [35]. In addition, TGF-β expression is significantly increased in severely asthmatic patients [24].

Another important factor linking neutrophils to airway remodeling in asthma is their ability to release proteases, including MMP and elastase. MMPs have been implicated in angiogenesis, smooth muscle hyperplasia, selective degradation of extracellular matrix components, and trafficking of inflammatory cells [36–40]. Among the MMPs, MMP-9 is one of the best characterized in asthma. Levels of MMP-9 are elevated in asthmatic individuals when compared with nonasthmatic controls [41], and active MMP-9 is prevalent in asthma with eosinophil predominance [42]. However, neutrophils also produce MMP-9 in high quantities during asthmatic exacerbations. MMP-9 levels appear to be associated with disease severity, and higher levels are seen in severe asthma when compared with mild asthma [43]. The number of sputum neutrophils correlates with levels of both total and active sputum elastase [44], which may lead to proteolytic lung degradation and the disruption of the bronchial elastic fiber network [45]. Neutrophil elastase and MMP-9 work synergistically to enhance IL-8 function, where neutrophil elastase induces IL-8 production [46], and MMP-9 cleaves IL-8 to a more potent form [42]. As a result, IL-8 will attract more neutrophils to the area of inflammation. Not only are neutrophil elastase concentrations increased in asthma, but they show an inverse correlation with predicted forced expiratory volume in 1 second (FEV_1) [44], which suggests that activated neutrophils are important in the airway inflammatory process. In addition, neutrophil elastases are able to inactivate tissue inhibitor of metalloproteinase-1 (TIMP-1, an inhibitor factor for MMP-9), and hence contribute to increased MMP-9 levels [42]. An imbalance exists between neutrophil proteases and antiproteases, with an increase in both MMP-9 and neutrophil elastase when compared with their inhibitors—TIMP-1 for MMP-9, and α_1-antitrypsin (α_1-AT) and secretory leukocyte protease inhibitor (SLPI) for neutrophil elastase. However, it is not clear if this imbalance is a function of increased neutrophil protease production or an inherent defect of the inhibitors. There is an unmistakable potential for a self-perpetuating mechanism of inflammation, which may lead to an increase in airway structural changes.

Role of Corticosteroids in Airway Neutrophilia

It is well known that corticosteroid therapy reduces the rate of neutrophil apoptosis, thus prolonging their survival [47]. Consequently, it is natural to attribute the increase of neutrophil numbers in patients with severe asthma to associated corticosteroid use. In the ENFUMOSA study, patients with severe asthma had increased sputum neutrophilia; this finding was independent of regular use of corticosteroids [25]. However, another study reported reduced airway neutrophil numbers in patients with severe asthma who were treated with oral corticosteroids [23]. In a different trial, patients with stable but severe asthma were treated with placebo or with a single injection of triamcinolone (to assure compliance), with no change observed in airway neutrophilic inflammation when compared with baseline in either the patients who received systemic steroids or the patients who received placebo [48]. This effect may be because systemic steroids do not increase airway neutrophil influx, or it might be due to a response to systemic steroid that has already reached a maximum. Furthermore, sputum neutrophilia has been reported in steroid-naive patients with asthma. In fact, these patients did not have a good response to corticosteroids when this treatment was attempted [4]. Whether inhaled corticosteroids were associated with increased numbers of neutrophils was explored in a previous report, which suggested that inhaled corticosteroid therapy is associated with elevated numbers of neutrophils in the airways, but only when used in high doses [23].

This finding is in contrast with a more recent report, which suggested that inhaled corticosteroid alone would not contribute to elevated numbers of neutrophils in the asthmatic airway, whereas oral corticosteroids appeared to do so [27•]. This report discussed two randomized, double-blind, placebo-controlled trials where patients with stable mild-to-moderate asthma underwent full characterization, including bronchial biopsies [27•]. As part of the enrollment criteria, subjects did not receive any corticosteroid therapy, either inhaled or oral, for at least 3 months before enrollment. In one protocol, subjects were randomized to receive either inhaled corticosteroid or placebo for 4 weeks; in the second protocol, subjects were randomized to receive either oral corticosteroid or placebo for 7 days. The subjects receiving oral corticosteroid had a significant increase in the airway neutrophil

count when compared with placebo, whereas no change was observed in the subjects who received inhaled steroids or placebo. In summary, there is no clear answer whether corticosteroid use in patients with asthma results in, or is the reason for, the increase in airway neutrophilia. More studies, with better trial design, are needed to explore this issue further.

Conclusions

Interest in the neutrophil as an important component of asthmatic airway inflammation has been renewed because the presence of airway eosinophilia does not fully explain this pathologic process, for several reasons: 1) only about 50% of patients with asthma have airway eosinophilia; 2) airway eosinophilia is not present in all asthma exacerbations; 3) in some instances, intense eosinophilic inflammation in the airway fails to induce asthma symptoms; 4) in vivo antieosinophilic therapies were not efficacious; and 5) animal models with eosinophil deficiency have little impact on airway pathology in response to allergen [7]. On the other hand, a strong association is being established between airway neutrophilic inflammation and several different phenotypes of asthma. There is no doubt that neutrophils are present in asthmatic airway inflammation, and that they are activated. The confusion stems, in part, from the different methods and outcome measures employed by different investigators seeking to determine the extent of neutrophil involvement in asthma. As an example of this quandary, some investigators choose bronchoalveolar lavage, but others choose bronchial biopsy to approach this question.

Furthermore, we continue to group patients with asthma together even though it is well known that asthma is a heterogeneous disease and may represent a set of syndromes. Only when we are able to recognize the different phenotypes clinically and pathologically will we be able to clearly define the role of each inflammatory cell. Perhaps the airway inflammatory process is like an orchestra where each musician knows his part so well that a conductor is not required. Depending on when we arrive at the concert hall, we hear (capture) one musician (neutrophil) or another (eosinophil), and therefore believe that the one we "heard" is the dominant and most important component. To continue the orchestra analogy, it is quite possible that the airway inflammatory process is a continuum, like a symphony, with each cell playing its part, then fading into the background, so that being present at the correct time is necessary to experience the process fully. More comprehensive studies are currently being conducted through the Severe Asthma Research Program (SARP) and the ENFUMOSA group in an attempt to establish the different asthma phenotypes, and finally enhance our understanding of the asthmatic airway inflammatory process.

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