EOSINOPHILS AND EOSINOPHIL PRODUCTS IN ASTHMA

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Eosinophils are known to be an indirect marker of airway inflammation in asthma. It is known since long that the total eosinophil count reflects asthmatic activity and is useful for regulating steroid dosage and for early detection of exacerbations. Eosinophils are currently regarded as the effector cells responsible for much of the pathology of asthma. Eosinophil-mediated damage to the respiratory epithelium is a major pathogenetic mechanism in asthma. This article is a review of the latest works about the relationship of eosinophil and eosinophil products with asthma.

INTRODUCTION

Asthma is an inflammatory disease of the airways characterized by its reversible, reactive nature. Asthma is not progressive, but is rather notable for episodes of exacerbations and remissions. Its symptoms are a result of airflow obstruction and include dyspnea, cough, wheezing, and chest tightness. The classic triad consists of cough, wheeze, and dyspnea. Traditionally, symptoms are worse at night or early in the morning [1]. A number of hypotheses about the pathogenesis of asthma have been suggested that propose totally different mechanisms of causality at the biological level. These can be grouped into allergic, inflammatory, neurogenic, and physical mechanisms with current evidence in favour of a combination of allergic and inflammatory processes.

The obstruction of airways in acute fatal asthma is due to contraction of bronchial smooth muscle, thickening of airway wall and intraluminal mucus and debris. The wall thickening is characterised by submucosal oedema, vasodilatation, with cellular infiltrates, predominantly of lymphocytes and eosinophils. The lumen contains mucus plugs, shed epithelium (Creola bodies) and Charcot-Leyden crystals (crystallised eosinophil derived major basic protein). Bronchoscopic biopsies show presence of significantly more active eosinophils in the submucosae of mild and even asymptomatic asthmatics compared to controls. T lymphocytes are also present in increased numbers. Airway inflammation is present during an acute exacerbation of asthma, and is characterized by infiltration and activation of both eosinophils and neutrophils [2].

Lavage fluid from the lumen of the asthmatic airway contains numerous mediators including histamine, prostaglandins (predominantly PGD2 and PGF2), leukotrienes, kinins, kallikrein, and eosinophil derived major basic protein. In the current model of asthma as an allergic disease, two cell types are central. These are the lymphocyte, especially in the role of T-cell as a regulator of immune processes, and the eosinophil, as effector cell causing tissue damage [3,4].

EOSINOPHILS

Eosinophils are involved in defence against parasites but are best known for their role in allergic diseases and asthma[5]. A prominent feature of the eosinophil is the presence of many spherical or ovoid granules in their cytoplasm. Eosinophilic granulocytes are so named because they are stained intensely by eosin. Under the light microscope, a bilobed nucleus is typically seen in a normal healthy eosinophil[6]. Eosinophils are uniquely endowed with an arsenal of enzymes that enables them to generate an array of reactive oxidants and diffusible radical species. Although these leukocytes constitute an essential component of the effector limb of host defenses, they also are implicated in contributing to inflammatory tissue injury[7].

The regulatory mechanisms of activation and degranulation of eosinophils occur in three stages. The first phase is the regulation of proliferation and differentiation in the bone marrow, which is consistent and occurring at a low level in the absence of immunological stimulation. When Th2 lymphocytes are activated to produce cytokines, such as IL-5, this increases the production of eosinophils in the bone marrow and promotes release of these cells into the circulation[8]. The second step is the migration of eosinophils from the circulation to various tissues. The migration of eosinophils depends on chemoattractants such as eotaxin, which is an eosinophil specific chemokine[9]. The third step comprises the release of granule proteins, which occurs when eosinophils become exposed to soluble mediators for example PAF[10], immuncomplexes[11] or solid particles[12].

Four distinct populations of granule (primary granules, secondary or specific granules, small granules, lipid bodies) have been recognized in eosinophil[13]. The effector functions of eosinophils are of twofold. First, the release of highly toxic granule proteins stored preferentially in secondary granules. The primary granules contain lysophospholipase, associated with eosinophilic infiltrations[14], and the secondary, or specific granules, contain major basic protein (MBP), eosinophils cationic protein (ECP), eosinophil peroxidase (EPO) and eosinophil protein X or eosinophil derived neurotoxin (EPX/EDN)[15,16]. The second effector function of the eosinophil is to produce and release inflammatory mediators such as PAF[17], LTC4[17], IL-5[18] and eotaxin[9], which amplify the inflammatory response by recruiting and activating leukocytes and epithelial cells.

Eosinophils develop from bone marrow (BM) progenitors, and interleukin-5 (IL-5) and eotaxin may act in expansion and mobilisation of eosinophils in asthma. The data suggest that IL-5 may act early in eosinophil development, and that eotaxin has the capacity to mobilise an eosinophil pool in asthma. The migration of eosinophils depends on chemoattractants such as eotaxin, which is an eosinophil specific chemokine[9]. Eotaxin represents the principal chemoattractant for the recruitment of the pulmonary eosinophils[19]. Eosinophils derive from CD34(+) bone marrow progenitor cells under the influence of haematopoietic growth factors, subsequently migrating to the airways under the cooperative influence of interleukin (IL)-5 and chemokines, including eotaxin[4]. There may be a critical role for Interleukin-13 in accumulation of intraepithelial eosinophils in chronic asthma, as well as in epithelial and subepithelial remodelling[20].

EOSINOPHILS IN ASTHMA

Blood eosinophils are known to be an indirect marker of airway inflammation in asthma[21]. It is known since long that the total eosinophil count reflects asthmatic activity and is useful for regulating steroid dosage and for early detection of exacerbations[22,23]. Eosinophils are currently regarded as the effector cells responsible for much of the pathology of asthma[24]. Eosinophil-mediated damage to the respiratory epithelium is a major pathogenetic mechanism in asthma[25]. Atopy in general and asthma in particular are associated with blood and lung eosinophilia. The eosinophil mediates damage to the respiratory epithelium and is the prime effector cell in the pathophysiology of asthma[16]. Eosinophils play a major role in the onset and maintenance of bronchial inflammation and tissue injury in asthma[26]. Eosinophilia of lung and blood associated with injury to the mucociliary escalator and excessive shedding of bronchial epithelium are hallmarks of both allergic and nonallergic asthma[16].

There is a 50-100 fold increase in the number of eosinophils relative to neutrophils in the bronchial mucosa in asthma. This is the result of the cumulative and sequential effects of increases in selective eosinophil versus neutrophil migration occurring at a number of stages in the life cycle of the eosinophil[27]. Blood eosinophil counts have for several decades been viewed as a valuable tool for indicating disease severity as they reflect the degree and extent of inflammation in the asthmatic lung[28]. Studies have reported a correlation between the number of blood eosinophils and the severity of asthma[29,30]. It has been reported that in the patients with asthma and pronounced eosinophilia the lung function of the patients is principally related to the number of circulating eosinophils[29].

It has been determined that the degree of eosinophilia is proportional to the severity of asthma, as measured by clinical grading or pulmonary function[22,29,31]. Specific bronchial challenge, experimental and nonexperimental in sensitive individuals is associated with a short term rise in eosinophil count. Large numbers of bronchial submucosal and lumenal eosinophils are pathognomic in acute severe asthma refractory to therapy (status asthmaticus). Eosinophils are increased in bronchoalveolar lavage fluid from asthmatics compared to nonasthmatics[29,32] as are subepithelial (and to a lesser extent deeper submucosal) eosinophils on bronchial biopsy[31,33,34]. In biopsy specimens from patients with symptomatic asthma, eosinophils are found in high numbers in the airway epithelium and in deeper bronchial tissues[35]. Two-thirds of people with mild to moderate asthma are reported to have increased sputum eosinophilia[36].

Mechanism of exercise-induced asthma is undetermined however eosinophils have been suggested as playing a role in its occurrence. The influx of eosinophils to the airway in patients who develop exercise-induced asthma can be partially explained by the leukotrienes in the airways of those patients[37]. Eosinophils have been considered to play an important role in the pathogenesis of virus-induced asthma exacerbations[38].

In the clinical management of patients with bronchial asthma blood eosinophilia is considered a risk factor, indicating deterioration and exacerbation[39]. However, patients with asthma may have a normal number of eosinophils in their blood[40], similarly bronchial biopsy specimens from patients who have died of status asthmaticus do not always reveal eosinophils[6] and histological studies performed after bronchoscopy in patients with asthma have not always found eosinophils in the bronchial mucosa[41]. Based on these results and the inconsistent findings of other investigators, we cannot depend on the eosinophilic count to monitor disease activity[28-30,41].

PATHOPHYSIOLOGY

The role of the eosinophil in the pathophysiology of allergy and asthma has been the focus of intense interest during the last two decades. While the presence of eosinophils in humans with allergy and asthma

is well established, the precise role of this cell in humans and in animal models is less clear. However, recent developments in research on many organ systems have provided novel insights into the possible underlying role of the eosinophil in both allergic and nonallergic inflammation[42]. The main role of the eosinophil seems to be a cytotoxic one, and it acts effectively both against host cells and parasites such as protozoa.

There are many potential mechanisms through which eosinophils may contribute to tissue injury and oxidative modification of biological targets in asthma[7]. Eosinophils may contribute to airway hyper responsiveness in asthma through the effects of eosinophil derived granular proteins in the bronchial epithelium[43].

Eosinophils are a source of cytokines within the airways of asthmatic individuals that may exert an important immunoregulatory influence[44]. Eosinophils are a potential source of IL-12. Eosinophil-derived IL-12 may contribute and modulate the local allergic inflammatory responses[44]. There is evidence of increased expression of the CD4+ cell chemoattractant interleukin (IL)-16 in bronchial biopsies of atopic asthmatic subjects compared to normal controls. IL-16 immunoreactive cells were identified. T cells, eosinophils and mast cells comprised are the potential cellular sources of IL-16 in asthmatic airways[45].

Like other leukocytes, eosinophils present in excessive numbers in inflamed tissues are removed by apoptosis. This phenomenon, also called 'programmed cell death', allows elimination of dangerous or redundant cells, thereby ensuring maintenance of tissue homeostasis. It has been suggested that a defect in eosinophil apoptosis would participate in the development and persistence of allergic airways inflammation in asthma[26]. A defect in apoptosis might contribute to the chronic tissue eosinophilia associated with asthma[45]. Apoptotic eosinophils are detectable in induced sputum of allergic patients. Apoptosis could be an important mechanism in the control of acute eosinophilic inflammation in patients with asthma exposed to the sensitizing antigens. It appears that the apoptotic mechanism could be less effective in controlling tissue eosinophilia in asthmatic patients with chronic eosinophilic inflammation[46].

Eosinophil apoptosis, as well as the expression and function of various molecules determining this process, are closely regulated by various stimuli, including cytokines, lipid mediators and growth factors released by various cell types and by the eosinophil itself, as well as exogenous molecules, such as glucocorticoids[26].,

There are increased numbers of circulating CD34(+) progenitor cells for eosinophils in patients with atopic asthma, with a further increase following allergen exposure or spontaneous worsening of asthma[47]. In addition, they suggest that in chronic asthma, IL-13 may be capable of signalling via a pathway that does not involve IL-4Ralpha[20].

When activated, eosinophils undergo degranulation causing epithelial damage in the airway, desquamation and increased airway hypersensitivity[6]. Mechanisms leading to eosinophil degranulation are not well understood. At least three discrete processes have been defined that result in the release of granule contents from eosinophils: secretion, piecemeal degranulation and cytolysis (necrosis)[13].

EOSINOPHIL PRODUCTS

Eosinophil granules contain several proteins, which are secreted into the extracellular environment following stimulation. They include basic proteins, cytokines, chemokines, lipid mediators and oxygen radicals[13]. Charcot-Leyden crystal (CLC) protein (or lysophospholipase) makes up to 10% of the total protein in eosinophils. Its function is unknown, although it has been speculated that CLC may protect eosinophils from the toxic effects of lysophospholipids. The most distinguishing feature of the eosinophils is its high content of highly charged cationic proteins, eosinophil cationic protein (ECP), eosinophil protein X (EPX) and major basic protein (MBP). These proteins together with eosinophil peroxidase (EPO) have been implicated as final effector molecules in eosinophil-mediated tissue damage. All these proteins are shown to be highly toxic to cells of the respiratory epithelium[6].

A number of studies have indicated that the assessment of eosinophil-derived proteins in various body fluids could be used for monitoring disease activity of asthma. Due to the relationship between levels of eosinophil proteins in serum/urine samples and lung function, as well as significant concentration differences between symptomatic and asymptomatic asthmatics, the assessment of eosinophil proteins in serum or urine samples appear to be more appropriate in monitoring disease activity.

In addition, recent reports have provided convincing evidence that bronchial inflammation and elevated levels of eosinophil granule associated proteins are even present in the bronchoalveolar lavage fluid of patients whose asthma is stable, suggesting that ongoing recruitment and activation of inflammatory cells may also be occurring in asymptomatic asthmatic individuals[31]. The potency of the eosinophil to release these mediators can be greatly enhanced by eosinophil mobilizing and activating factors such as granulocyte macrophage colony stimulating factor, interleukins 3 and 5, platelet-activating factor, and perhaps leukotriene B4[40].

Major basic protein (MBP) is regarded as the most important out of these. This 31 kD protein is highly basic, and negatively charged in solution, and exhibits potent cytotoxic activity. Eosinophilic cationic protein (ECP) and eosinophil-derived neurotoxin (18-21 kD) are highly homologous proteins and exhibit ribonuclease activity. Both lead to exfoliation of respiratory epithelium. Both are highly basic. ECP also stimulates mast cell degranulation, inhibits T-cell activity, and shortens the coagulation time. Eosinophil peroxidase is cytotoxic in itself, but more so in the presence of hydrogen peroxide and halides. It too can cause mast cell degranulation [6].

ECP AND ASTHMA

Eosinophil cationic protein (ECP) is an eosinophil granule protein which is highly cytotoxic and is released by activated eosinophils[10]. Measurement of serum ECP is helpful in determining asthma activity and deciding the use of anti-asthma drugs[48]. There is a correlation of ECP and the eotaxin level in asthma patients[49]. Measuring serum ECP levels has the advantage over eosinophilic count in that it reflects not only the number of eosinophils but also their degree of activation and is therefore a better inflammatory marker[50]. In asthmatics, sECP is associated with PEF variability and symptom severity[51]. Although the ECP values are significantly more elevated in atopic than in non-atopic asthma, elevated serum ECP are not specific for atopic asthma[52].

Measurement of serum ECP is helpful in determining asthma activity and deciding the use of anti-asthma drugs[48]. ECP has also been suggested to be a helpful indicator of persistent infantile wheeze[53]. There is a significant correlation between sECP level and blood eosinophil count as well as between sECP level and serum tIgE. There is a significant correlation of asthma attack frequency with sECP level. Serum ECP level seems to reflect the allergy activity level, especially two weeks prior to and after the measurement[54].

ECP levels, however, may not be elevated in some asthmatic patients, even when they are symptomatic. It may not be useful in patients who are older, have longer disease duration or possibly have thicker airway walls[55].

Levels of salivary ECP are elevated in patients with asthma and associated with presumed activity of disease as recorded by alteration of taken dose of inhaled corticosteroid[24]. The serum level of ECP seems to be a good clinical marker of monitoring the disease. Serum levels of ECP in the patients with asthma during attack were significantly higher than those of the controls and stayed high up to the 7th day[1]. Serum ECP may be used to predict a response to corticosteroid therapy in adult patients with asthma[56].

MBP AND ASTHMA

Eosinophil granule major basic protein (MBP) is present at sites of epithelial damage in cases of asthma; this protein is toxic to respiratory epithelium and its levels are raised in the sputum of patients with asthma. MBP in the eosinophil granule is released into respiratory tissue of patients with severe asthma and that it is associated with tissue damage. In vitro, MBP causes shedding of respiratory epithelium. In vivo, levels of MBP in the sputum of asthmatics reflect severity of current disease, while immunofluorescent studies show that MBP is present in the respiratory epithelial defects seen in asthmatics' bronchi[57]. In vitro, the eosinophil granule major basic protein (MBP) is toxic to the MBP-mediated damage to the respiratory epithelium consists of desquamation and frank destruction of ciliated cells[16]. Increased sputum MBP concentration is a good marker for asthma, and patients treated for acute asthma have high levels of MBP in their sputa, which decrease after treatment. In the lungs of patients who had died of asthma, MBP has been localized outside of the eosinophil in association with damage to the epithelium[16].

EDN/EPX AND ASTHMA

Eosinophil-derived neurotoxin (EDN), also called eosinophil protein X (EPX), has been suggested to be a useful marker of eosinophilic inflammation. EDN levels in serum, plasma and urine from the patients significantly correlated with the number of peripheral blood eosinophils, but not total serum IgE levels[58]. Eosinophil protein X (EPX) has been used to assess eosinophil activity and monitor inflammation in bronchial asthma[59].

Recently there has been a lot of interest in urinary EPX, which can be obtained easily and that may be helpful in diagnosing both asthma and atopy in children. However, there is a great overlap between controls and symptomatics[60]. Urinary EPX is a useful marker of airway inflammation and can be helpful in guiding asthma management[3,59].

It has been suggested that urinary eosinophil protein X (U-EPX) can be used to monitor bronchial inflammation in childhood asthma. It may reflect differences in eosinophil involvement and activation between children with atopic and non-atopic asthma[61]. A statistically significant correlation was found between urinary concentration of eosinophil protein X (U-EPX) concentrations and the severity of attacks in asthmatic children[60].

EOSINOPHILS IN DIAGNOSIS AND MANAGEMENT OF ASTHMA

Eosinophil blood counts and eosinophil cation protein (ECP) serum level are often used as markers of clinical monitoring of the disease activity[25]. Correct prediction of clinical response in an individual patient, however, remains poor with our currently used clinical and inflammatory parameters[62]. The proportion

of eosinophils in sputum is a more sensitive marker of asthmatic airway inflammation than the proportion of eosinophils in blood[63]. Eosinophil count and/or ECP and EPO levels can be used to estimate the short-term risk of deterioration and the need for corticosteroid treatment in cases of mild and moderate allergic asthma[64]. The development of commercial kits for other markers is still in the process.

Treatment decisions in asthma are based on assessments of symptoms and simple measures of lung function, which do not relate closely to underlying eosinophilic airway inflammation[65]. Corticosteroids are considered to be one of the most effective medicine for asthma by suppressing airway inflammation and Prednisolone is considered to be effective medicine for asthma by suppressing eosinophil activation through IL-5[66]. A treatment strategy directed at normalisation of the induced sputum eosinophil count reduces asthma exacerbations and admissions without the need for additional anti-inflammatory treatment[65].

FUTURE RESEARCH

The above review indicates that the relationship of eosinophils and asthma is very important for diagnosis and management of asthma. There is a need to work on all the aspects of this relationship that includes finding correlation of symptoms of asthma with markers of eosinophil activity, identifying the substances attracting, activating or developing eosinophils and developing drugs to neutralize these substances. Works are now underway to develop asthma therapy leading to inhibition of eosinophil priming of cytotoxic mechanisms in vivo[67]. For the development of more effective anti-asthma drugs it, therefore, seems relevant to unravel and interfere with the steps of eosinophil activation[40]. There are now attempts to inhibit eosinophil differentiation at bone marrow level[68].

Eosinophilic inflammation is a feature of asthma. However, serological markers to indicate eosinophil activation in this process are not fully defined[56]. Markers of airway inflammation are needed for prediction of asthma deterioration and evaluation of disease severity[64]. It is, therefore, important to find a marker of disease activity, ideally one that is simple to measure, reliable and inexpensive. As yet no such marker has been found for asthma. Therefore, there is a need for assessing different eosinophil products to develop a serological marker of airway inflammatory activity in asthma.

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