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THE ROLE OF EOSINOPHILS IN ASTHMA

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Summary. *Asthma is a common respiratory disease. The prevalence of asthma has been increasing over the past twenty years in most countries, and it affects up to 10% of the population of developed countries. Airway inflammation is a major factor in the pathogenesis of asthma. The presence of peripheral blood eosinophilia and activated eosinophils in the chronic inflammatory infiltrate of the airways is a characteristic of both allergic and non-allergic asthma. This review article describes the mechanisms of eosinophillic inflammation in asthma: eosinophilopoiesis, role of eosinophil growth factors (IL-3, IL-5 and GM-CSF), phases in the recruitment of eosinophils from bone marrow to airway mucosa, activation and eosinophil effector functions in the pathogenesis of asthma.*

Key words: *Eosinophils, asthma, inflammation, cytokines, chemokines*

Introduction

Asthma is a common respiratory disease. The morbidity and mortality of asthma are increasing, making it a global health concern. Symptoms of chest tightness, coughing, wheezing and dyspnea, as well as characteristic physiologic abnormalities of variable airflow obstruction and airway hyperresponsiveness to bronchoconstrictor stimuli characterize asthma (1). The primary underlying abnormality in the disease is a chronic inflammation of the airway mucosa that gives rise to reversible obstruction and hyperresponsiveness. The predominant inflammatory cell in asthmatic inflammation as determined by fiberoptic bronchial biopsy, bronchoalveolar lavage, and induced sputum, is the eosinophil, accompanied by T-lymphocytes, mast cells and basophils (2). Eosinophillic inflammation of the airways, with an increase in activated and degranulated eosinophils is the key feature of both allergic and non-allergic asthma (3,4). Significant correlation exists between the activation of eosinophils and the severity of asthma as reflected in bronchial hyperresponsiveness and asthma symptom (Aas) score (5). Increase in number of activated EG2+ eosinophils in the airways is present even in patients with mild asthma.

Eosinophilopoiesis

Eosinophils are produced in the bone marrow from CD 34^+ pluripotent progenitor cells, which are common to both eosinophils and basophils under the influence of growth factors, including interleukin-3 (IL-3), granulocyte-macrophage colony-stimulating factor (GM-CSF), and IL-5. IL-3 and GM-CSF are active on early precursors, while IL-5 acts as a late factor of differentiation (6). IL-5 has the most cell-specific effects on the differentiation and production of eosinophils, and acts acutely to release a pool of already developed eosinophils from the bone marrow into the circulation.

Mechanisms of the eosinophil recruitment in the asthmatic airways

Seven hours after allergen challenge during the late phase response, eosinophils increase in sputum samples of asthmatics, and this is associated with the appearance of eosinophil- basophil progenitors, and eosinophilia in peripheral blood (7). Progenitor CD 34+ cells bear the IL-5 receptor (IL-5R) with increased responsiveness to IL-5 suggesting they are primed toward the development of eosiniophils (8). IL-5 generated in the inflamed lung tissues in asthma acts hormonally on the bone marrow to increase the production of eosinophils (9). The presence of eosinophil progenitors and eosinophil growth factors IL-3, IL-5 and GM-CSF within the asthmatic lung indicates the potential of local eosinophil differentiation (10). The migration of eosinophils into the airways is initiated by local chemoattractant factors (11). Many chemotactic substances act on eosinophils, including lipid mediators (Leukotriene B4 LTB4 and platelet activating factor PAF), anaphylatoxins (C3a and c5a) and chemokines (Macrophage inflammatory protein-1α MIP-1α, Regulated on activation, normal T-cell expressed and secreted RANTES, eotaxin, eotaxin2, macrophage-derived chemokine MDC, monocyte chemotactic protein-2 MCP-2, MCP-3, MCP-4, IL-8 and IL-16) (12,13,14). The increased number of eosinophils in asthmatic patients is the combination of increased

eosinophilopoiesis and rate of egress from the bone marrow. The eosinophil recruitment results from the complex mechanisms that involve interaction of adhesion molecules on the eosinophils with counter ligands on endothelial cells, extracellular matrix proteins and other tissue structures (15). Among these mechanisms are tethering and rolling on the endothelial surface, firm adhesion and transendothelial migration. The initial reversible tethering and rolling of eosinophils on the endothelium involve the formation of numerous weak reversible bonds between P- selectin and P-selectin glycoprotein ligand-1 (PSGL-1) and very late activation antigen-4 (VLA-4) with vascular cell adhesion molecule-1 (VCAM-1). Preformed P-selectin is stored intracellularly in the Weibel-Palade bodies, from where it is mobilized to the endothelial surface by histamine and PAF (16). The tethering and rolling of eosinophils on the endothelium is followed by the activation step mediated by chemoattractants (17). Chemoattractants direct the migration of the tethered cells, involving crawling along the endothelium where chemokines are deposited in a solid phase, activation, diapedesis, and immigration into the tissue along a gradient of chemotactic signals (18). The activation results in up- regulation of β2- integrins (Mac-1 and leukocyte function-associated antigen-1 LFA-1) and β1- integrin (VLA-4). β2-integrins bind to inetrcellular adhesion molecule-1 (ICAM-1) on endothelium whereas β1-integrin binds to VCAM-1 resulting in the firm arrest that is critical for transmigration (19). RANTES induces transient activation of VLA-4 increasing their adhesiveness to VCAM-1, whereas MCP-3 stimulation results in conformational change of Mac-1 leading to increased ICAM-1 adhesion (20). IL-4 and IL-13 induce expression of VCAM-1, whereas TNF-α and IL-1 induce expression of ICAM-1 on the surface of endothelial cells (21,22,23). Binding of the CC chemokines (eotaxin, eotaxin2, RANTES and MCP-3) to their G-protein-coupled receptors activates $Ca2^{+}$ flux- induced polymerization and breakdown of actin leads to the formation and retraction of lamellipodia, which function like arms and legs of the migrating cells (24,25). Transendothelial migration also requires the function of matrix metalloprotease-9 (MMP-9) that degrades type IV collagen, entactin, proteoglycans, and elastin, permitting eosinophil penetration through basement membrane (26). Eosinophils are richly endowed with MMP-9 in its precursor, with enzyme activation occuring when eosinophils adhere either to endothelial or epithelial cells (27). The extensive secretion of this enzyme with its capacity to degrade epithelial adhesion molecules, epithelial basement membrane collagen and proteoglycans acts as a component of the airways remodelling (28). After migration through the endothelium, eosinophils come into contact with extracellular matrix (ECM) proteins, that are likely to play important roles in the regulation of eosinophil activation (29).

Eosinophil morphology

The mature eosinophil is usually round or ovoid, having a diameter of 12-17 μ, and a bilobed nucleus. There are three types of granule in the eosinophil: specific, small and primary (30). Judged by electron microscopy, specific granules have a distinct structure, being composed of a electrodense, crystalloid core and an electrolucent matrix. These granules are characerized by their marked affinity for the reddish-orange colloured acid dye eosin. Primary granules are round and develop early in eosinophil maturation. Small granules contain acid phosphatase and arylsulphatase (31). Another constituent of eosinophils is the Charcot-Leyden protein, (bipyramidal crystals) often found in sputa of asthmatic patients. This protein possesses lysophospholypase activity (32). In healthy, non-allergic persons, circulating eosinophils comprise 1-2 % of white blood cells. eosinophilia is classified as mild (351-1500 cells per cubic millimeter), moderate (> 1500 to 5000 cells per cubic millimeter) or severe(> 5000 cells per cubic millimeter) (19)

Pathogenetic role of eosinophils in asthma

The recruitment of eosinophils into bronchial mucosa in which allergic inflammation occurs is a critical contributor to the late asthmatic reaction of congestion and mucus hypersecretion (33) (fig.1). When these cells arrive they degranulate and perpetuate underlying airway inflammation. Eosinophils are a rich source of cytotoxic proteins, lipid mediators, oxygen free radicals and cytokines (34). In asthmatic patients, after transendothelial migration, eosinophils transmigrate and adhere to bronchial epithelium where they degranulate and release substances (eosinophil cationic protein ECP, major basic protein MBP, eosinophil peroxidase EPO and superoxide) which are toxic for epithelial cells (35). Damage and desquamation of cells, cilliostasis, and epithelial secretion (36) manifest the toxicity to airway epithelium. MBP is a selective, allosteric antagonist for M2 muscarinic receptors (autoreceptors) (37). The loss of M2 muscarinic receptor function results in increased airway tone due to increased release of acetylcholine and potentiation of vagally mediated reflex bronchoconstriction and bronchial hyperresponsivenss (38). MBP also stimulates histamine release from basophils and mast cells.

Lipid bodies (intracellular lipid rich domains) are induced to be developed in the activated eosinophils, and are the sites for enhanced synthesis of both lypoxygenase and cyclooxygenase-derived eicosanoids (39). Eosinophils are capable of producing significant quantities of cysteinyl leukotrienes (especially LTC-4). Cysteinyl leukotrienes contract airway smooth muscle (100-1000 fold more potent bronchoconstrictors than histamine), increase vascular permeability, stimulate mucus secretion, decrease mucocilliary clearance, stimulate

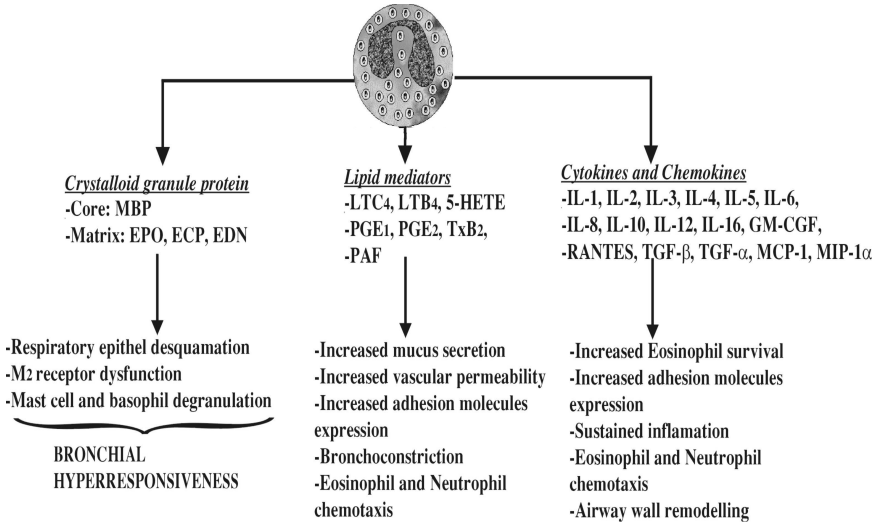

Figure1. Role of eosinophils in the late asthmatic reaction

eosinophil and neutrophil recruitment into the airways (40), stimulate smooth airway muscle proliferation and cause neuronal dysfunction (41).

Eosinophils have the potential to synthetize and release a number of cytokines and chemokines. Cytokines produced by eosinophils include the autocrine-eosinophil active growth factors (IL-3, IL-5, GM-CSF), immunoregulatory cytokines (IL-2, IL-4, IL-1, TGF-β, IFN-γ), proinflammatory cytokines (IL-1, IL-6, TNF-α, IL-16) and chemokines (IL-8, MIP-1α, RANTES) (42). Transforming growth factor-β (TGF-β) is an immunoregulatory factor with a direct effect on growth of some cell types (stimulation on fibroblast growth and inhibition of epithelial cell growth) and upregulation of the synthesis of ECM proteins, inflammatory mediators and cytokines, making it an important factor in the remodelling process (43).

Activation of the eosinophils

After eosinophils arrive at inflammatory site in the airways, they become activated with hypodense phenotype (44). Activated eosinophil expresses a number of receptors for cytokines, chemokines, immunoglobulins and complement. During the inflammatory response, locally produced complement-derived anaphylatoxins C3a and C5a bind to specific cellular receptors and activate respiratory burst in eosinophils. C5a acts as a chemoattractant for neutrophils and eosinophils and represents a major metabolic activator for eosinophils inducing the release of granule proteins and free oxygen radicals that cause damage to the airway tissue (45). Secretory IgA-IL-8 complex is a potent eosinophil chemoattractant and stimulus for degranulation. Its potency is mediated by the secretory component (46). The three cytokines that simulate eosinophilopoiesis (IL-3, IL-5, GM-CSF) can activate their effector functions too. These cytokines bind to the receptor that has a common β-chain and different α-chains (47). IL-3, IL-5, GM-CSF prime eosinophil response to chemoattractants, increases their degranulation, prolongs their survival, and increases free oxygen radical production. IL-5 also activates eosinophils to express the EG2 epitope (activated $EG2^+$ eosinophils) (48). The mechanism of prolonged eosinophil survival by these cytokines is by eliminating apoptosis.

Eosinophil apoptosis

The signaling pathways involved in antiapoptotic effect of the cytokines involve the Lyn, Jak2, Raf1, transduction of survival and death signals (49). Corticosteroids induce eosinophil apoptosis with programmed cell death (an eosinophil dies and doesn't release harmful inflammatory mediators) (50). Treatment of asthmatics with inhaled corticosteroids leads to the depletion of mucosal eosinophils, which is associated with clinical improvement (51). Inhaled corticosteroids have many effects on eosinophilopoiesis including: abrogation of cytokine production by airway tissues, reduction in peripheral blood eosinophil-basophil progenitors after corticosteroid inhalation during asthmatic exacerbation and decreasing or phenotypic changes in human bone marrow progenitor after corticosterod inhalation (52).

Theophylline induces apoptosis by functioning as phosphodiesterase (PDE) inhibitor on the activated eosinophil to increase intracellular cyclic adenosine monophosphate (cAMP), and indirectly through the inhibition of cytokine (IL-5,GM-CSF) production (53).

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ULOGA EOZINOFILA U ASTMI

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Kratak sadržaj: *Astma je česta respiratorna bolest. Tokom poslednjih dvadeset godina u većini zemalja prevalencija astme beleži porast, i zahvata do 10% stanovništva u razvijenim zemljama. Zapalenje disajnih puteva je glavni faktor u patogenezi astme. Prisustvo eozinofilije periferne krvi i aktivisanih eozinofila u hroničnom zapalenjskom infiltratu disajnih puteva, je karakteristično i za alergijski i nealergijski oblik astme. Ovaj pregledni članak opisuje mehanizme eozinofilne inflamacije u astmi: eozinofilopoiezu, ulogu faktora rasta eozinofila (IL-3, IL-5 and GM-CSF), faze u prelasku eozinofila iz kostne srži do sluzokože disajnih puteva, aktivaciju i efektorske funkcije eozinofila u patogenezi astme.*

Ključne reči: *Eozinofili, astma, zapalenje, citokini, hemokini*