

ASTHMA: CYTOKINES INVOLVED IN INFLAMMATION

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Abstract: Asthma has been defined as a chronic inflammatory disorder of the airways that is associated with recruitment of inflammatory cells and the clinical development of wheezing, shortness of breath, chest tightness, and cough. The causes of asthma are multifactorial and include a complex mix of environmental, immunological and host genetic factors. Allergic asthma is a disease characterized by intermittent airway obstruction that causes difficulty in breathing and, in the most severe cases, death from asphyxiation. Ultimately, airway obstruction is mediated by hyper-responsive bronchial smooth muscles, secreted airway glycoproteins and inflammatory debris produced by airway goblet cells and other cells, as well as edema or swelling of the airway wall. Interaction of allergen with T-cells is associated with patterns of cytokines release by immuno-competent cells characterized as T-helper Th1 or Th2 T-immune responses. The Th2 pattern of inflammation induced by this cytokines release is associated with allergic diseases.

The molecular mechanisms underlying allergic inflammation are the signals for immunoglobulin (Ig) E production and the activation of mast cells and eosinophils. The TH2 cells secrete a highly characteristic cytokines that includes interleukin4 (IL-4), IL-5, IL-9, and IL-13, all of which contribute to the various manifestations of allergic inflammation and disease. This review examines the current understanding of the molecular mechanisms of allergic inflammation and includes a discussion of the roles of T-lymphocytes, immunoglobulin (Ig) E, mast cells, and eosinophils in the pathogenesis of allergic disease.

I. INTRODUCTION

Allergic asthma is a chronic airway disease that affects millions of people in the developed world. The disease is characterized by concurring airway inflammation, cytokines production, airway hyper-responsiveness (AHR) to inhaled antigen, and pulmonary fibrosis. Several cell types, including epithelial cells, eosinophils, neutrophils, smooth muscle cells, alveolar macrophages, dendritic cells, mast cells, and T lymphocytes, have been implicated in the pathogenesis of asthma. Most of the definitions for asthma have emphasized the characteristics of fluctuations over times in bronchoconstriction and the reversible nature of the disease. As the patho-physiological basis of asthma became clearer, definitions began to include a statement about the pathological characteristics. The 2006 revision of GINA

guidelines proposes an operational description of asthma as: *“A chronic inflammatory disorder of the airways in which many cells and cellular elements play a role. The chronic inflammation is associated with airway hyperresponsiveness that leads to recurrent episodes of wheezing, breathlessness, chest tightness, and coughing, particularly at night or in the early morning. These episodes are usually associated with widespread, but variable, airflow obstruction within the lung that is often reversible either spontaneously or with treatment.”*

During an asthma attack, smooth muscles located in the bronchioles of the lungs constrict and decrease the flow of air in the airways. Inflammation or excess mucus secretion can further decrease the amount of air flow. Direct bronchoscopy reveals that the airways of asthmatic patients are often reddened and swollen, indicating the acute inflammation. Lavage has revealed an increase in numbers of lymphocytes, mast cells, eosinophils and evidence for activation of macrophages in comparison with non-asthmatic controls. Biopsies have revealed evidence for increased numbers and activation of mast cells, macrophages, eosinophils and T-lymphocytes.

The fundamental causes of asthma are not completely understood. The strongest risk factors for developing asthma are a combination of genetic predisposition with environmental exposure to inhaled substances and particles that may provoke allergic reactions or irritate the airways, such as: indoor allergens (for example, house dust mites in bedding, carpets and stuffed furniture, pollution and pet dander), outdoor allergens (such as pollens and moulds), Tobacco smoke, Chemical irritants in the workplace, Air pollution. Other triggers can include cold air, extreme emotional arousal such as anger or fear, and physical exercise [1]. Even certain medications can trigger asthma: aspirin and other non-steroid anti-inflammatory drugs, and beta-blockers (which are used to treat high blood pressure, heart conditions and migraine [2]).

The treatment of asthma is internationally agreed upon and guidelines have been developed for the management of asthma. All guidelines focus on the treatment of inflammation although there are differences between them. Management should take into account that asthma is a condition associated with the following: acute symptoms that can be quickly reversed by bronchodilators; exacerbations caused by chronic inflammation which can be prevented or more slowly reversed by anti-inflammatory drugs; and the process of airway wall remodeling, for which there no defined treatment is yet fully validated [3]. Thus, asthma should be seen as a continuum from symptoms to airway wall remodeling, but the sequence and/or the severity of these events is highly variable

from patient to patient. Asthma has significant genetic and environmental components, but since its pathogenesis is not clear, much of its definition is descriptive [4].

II. T-CELL RESPONSES IN ALLERGIC DISEASE

T lymphocytes are the prominent cells in both normal and asthmatic airway mucosa, which are activated in response to antigen stimulation or during acute asthma exacerbations and produce high levels of cytokines. According to their surface cell markers and distinct functions T lymphocytes are divided into two broad subsets the CD4⁺ (T helper) and the CD8⁺ (T-cytotoxic) cells. CD4⁺ cells are further subdivided into TH1 and TH2 cells, depending on the type of cytokines that they produce. Other cells involved in the pathogenesis of asthma include mast cells, basophils, macrophages, and eosinophils. IL-2, IL-12, tumor necrosis factor (TNF) α , and interferon (IFN) γ -, and are involved in the elimination of intracellular pathogens which produced by Th1 cells. Th2 cells produce granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-4, IL-5, IL-6, and IL-13. Presence of Th2 cytokines modulates the balance of Th1/Th2 cells and also plays a important role in the development of the allergic response. The production of interleukin IL-4 and IL-5 by helper-inducer lymphocytes characterizes allergic inflammation. At the site of allergen exposure, inflammatory mediators of the early and late-phase allergic responses cause mucus secretion, airway smooth muscle contraction, and mucosal edema.

III. EOSINOPHILS

Eosinophils develop in the bone marrow from CD34⁺ progenitor cells under stimulation of IL-3, IL-5, and GM-CSF. The genes encoding these cytokines are closely linked and are found on chromosome 5. Although all three cytokines promote eosinophilopoiesis, IL-5 uniquely promotes the development and differentiation of eosinophils. IL-5, a product of Th2 cells, also plays an important role in the mobilization of eosinophils from the bone marrow and into the bloodstream. Mature eosinophils first detach from the bone marrow extracellular matrix and then migrate across the bone marrow sinus endothelium. The eosinophils are then released from the endothelium and into the bloodstream or are retained in the medullary cavity of the marrow. IL-5 can also induce the rapid release of pooled eosinophils from the bone marrow. Eosinophils circulate in the peripheral blood with a normal half-life of 8 to 18 hours [5]. Once they have migrated into peripheral tissues, they persist for several days. The migration of eosinophils from the peripheral blood into tissues is a multistep process mediated by cytokines, chemoattractants, selectins, and integrins. Integrins are a family of cell surface proteins that mediate cell-to-cell and cell-to-extracellular matrix interactions. Both neutrophils and eosinophils migrate into peripheral tissues by rolling along, adhering to, and then passing between endothelial cells. The rolling of eosinophils is primarily mediated by P-selectin and

the rolling of neutrophils is mediated by E-selectin [6]. Eosinophils undergo cellular activation after being exposed to chemoattractants such as the chemokines, platelet-activating factor (PAF), and eotaxin, as well as IL-5. Activated T cells and eosinophils are important pathophysiologic elements in asthma. Mucosal damage in chronic asthma has been shown to be associated with cytotoxic and pro-inflammatory mediator release from activated eosinophils. These products include reactive oxygen species and cytotoxic granule and vesicular proteins: major basic protein (MBP), eosinophil cationic protein, eosinophil peroxidase, and eosinophil-derived neurotoxin, as well as cytokines and chemokines together with phospholipid derived, pharmacologically active mediators. Cytokines released from Th2-type cells, particularly IL-3, IL-5, and GM-CSF, are thought to regulate eosinophil priming, activation, and survival.

IV. CYTOKINES

Cytokines are small protein mediators that play an integral role in the coordination and persistence of inflammation in asthma. Cytokines may also play an important role in antigen presentation and may enhance or suppress the ability of macrophages to act as antigen-presenting cells. IL-3, IL-4, IL-5, and GM-CSF were up regulated in asthmatic patients relative to control subjects [7]. These cytokines were significantly up-regulated after antigen challenge, and their receptors were identified locally on the surface of inflammatory cells. IL-13 is associated not only with IgE synthesis and chemoattraction of eosinophils but also with mucus hypersecretion, fibroblast activation, and the regulation of airway smooth muscle function.

IL-4 is produced by Th2- derived T lymphocytes and certain populations of thymocytes, as well as eosinophils and cells of the basophil and mast cell. Synthesis can also be induced by stimulation of the antigen receptor on T lymphocytes and by IgE Fc receptor cross-linking in mast cells and basophils [8]. Interestingly, corticosteroids enhance the capacity to induce IL-4 synthesis from CD41 T cells. IL-4 has been shown to be expressed by CD41 and CD81 T cells, eosinophils, and mast cells in both atopic and nonatopic asthma. IL-4 appears to be important in the early stages of Th2 cells development. IL-13 is synthesized by activated CD4⁺ and CD8⁺ T cells and is a product of Th1, Th2, and CD4⁺ T cell clones. Both CD4⁺ and CD8⁺ T cell clones synthesize IL-13 in response to antigen-specific or polyclonal stimuli (Zurawski and de Vries, 1994). Increased expression of IL-13 mRNA has been reported in the airway mucosa of patients with atopic and nonatopic asthma. In addition, levels of IL-13 together with IL-4 are increased after segmental allergen challenge of patients with asthma. There is a significant correlation between eosinophils counts and levels of IL-13.

TNF

Two major forms of TNF exist, i.e., TNF- α and TNF- β , which have only 35% amino acid homology but bind to similar receptors. TNF- α may have an important amplifying effect in asthmatic inflammation. There is evidence for increased

TNF- α expression in asthmatic airways, and IgE triggering in sensitized lungs leads to increased expression in epithelial cells in both rat and human lung. Increased TNF- α mRNA expression in bronchial biopsies from asthmatic patients has been reported [9, 10]. TNF- α is also present in the bronchoalveolar lavage fluid (BALF) from asthmatic patients and. TNF- α may be an important mediator in the initiation of chronic inflammation, by activating the secretion of cytokines from a variety of cells in the airways. Several approaches to inhibition of TNF- α synthesis or effects, including the use of monoclonal antibodies to TNF or soluble TNF receptors, in asthma are now under investigation.

V. MAST CELL MEDIATOR

IgE binds to the high-affinity IgE receptor (Fc ϵ _RI) on mast cells, basophils, dendritic cells, eosinophils; and to low affinity IgE receptors (CD23 or Fc ϵ _RII) on monocytes, macrophages, and lymphocytes. The release of mediators of immediate hypersensitivity in a sensitized patient is initiated when allergen binds to the IgE-Fc ϵ RI complex on the surfaces of mast cells [11, 12]. This interaction results in the release of both preformed and newly synthesized inflammatory mediators. IgE may up regulate Fc ϵ _RI expression in mast cells, which permits the mast cells to be activated with lower concentrations of specific allergen. Mast cells develop from a population of CD34⁺ hematopoietic progenitor cells and then mature in peripheral tissues. They reside in connective tissue adjacent to blood vessels and beneath epithelial surfaces [14, 15]. Increased numbers of mast cells are present in tissues affected by chronic inflammation. Mast cells degranulate when IgE antibodies bound to mast cells are cross-linked by allergen. At the site of allergen exposure, these pro-inflammatory mediators cause mucus secretion, airway smooth muscle contraction, and mucosal edema. Preformed mediators stored in cytoplasmic granules include histamine, tryptase, proteoglycans, chymase, carboxypeptidase A, and heparin. These mediators are involved in the immediate clinical responses of vasodilation, edema, bronchoconstriction, and itching [13, 14]. Mast cells contain preformed stores of the cytokines such as TNF and VPF, as well as many other cytokines including IL-2, IL-3, IL-4, IL-13, GM-CSF, and chemokines. Mast cells participate in asthma's inflammatory changes through the elaboration of cytokines. In response to IgE-dependent stimuli, mouse mast cell lines have been shown to produce a profile of cytokines, including IL-3, IL-4, IL-5, and IL-6, similar to the Th-2 profile produced by T lymphocytes. Human lung mast cells have been shown to release IL-4, IL-5, and IL-13 in vitro, and mucosal biopsy specimens from asthmatic persons have revealed positive staining by immunohistochemical means for IL-4, IL-5, IL-6, and TNF- α in mast cells.

VI. LEUKOTRIENES

Leukotrienes are lipid mediators resulting from the catabolism of the arachidonic acid (AA) released from the cell membrane by phospholipase A2 after cell activation.

After its release, AA is metabolized either by the cyclooxygenase pathway, generating prostaglandins and thromboxanes, or by the 5-lipoxygenase (5-LO) pathway, which in association with 5-LO-activating protein as a helper protein produces the leukotrienes: leukotriene B₄, LTC₄, leukotriene D₄

(LTD₄), and leukotriene E₄ (LTE₄), with the last three forming the CysLT group. The CysLTs are produced in eosinophils, monocytes, macrophages, mast cells, basophils, endothelial cells and T lymphocytes. Increased production of CysLTs has been detected in bronchoalveolar lavage (BAL) and urine samples from patients with asthma, especially after allergen challenge or during an acute asthma attack. The CysLTs also have been implicated in the pathophysiology of asthma by way of multiple mechanisms, including mucus hypersecretion, increased microvascular permeability, ciliary activity impairment, inflammatory cell recruitment, edema, and neuronal dysfunction. Most important, these molecules increase airway hyperresponsiveness and cause smooth muscle hypertrophy in both healthy subjects and asthmatic patients. Eosinophils are a rich source of CysLTs that are derived from native AA by the action of phospholipase A₂. Human eosinophils synthesize and release relatively large concentrations of LTC₄ (as much as 70 ng/10⁶ cells) after stimulation with the calcium ionophore A₂₃₁₈₇.

In general, eosinophils obtained from asthmatic subjects appear to produce more LTC₄ than do those from healthy control donors. There are two receptors for CysLTs on smooth muscle cells, CysLT₁ and CysLT₂. The CysLT₁ receptor is the regulator for bronchial smooth muscle contraction and thus is directly relevant to asthma treatment.

VII. BASOPHILS

Basophils possess high levels of the Fc ϵ RI receptor and are capable of an immediate response to allergen. Basophils are not present in healthy airways; they are present in the airways of asthmatic persons under a variety of circumstances [15, 16]. Basophils have been reported in the sputum of patients with symptomatic asthma. Like mast cells, basophils release histamine on activation; unlike mast cells, however, they do not produce PGD₂. The major product of AA metabolism in the basophil appears to be LTC₄. On a per cell basis, basophils produce as much LTC₄ as do mast cells and much more than do eosinophils. Recently, basophils have also been found to be a rich source of IL-4 and IL-13, demonstrating both spontaneous release and response to IgE-mediated stimuli [17, 18].

VIII. MACROPHAGES

Macrophages are the predominant cell recovered by BALF in both non-asthmatic and asthmatic persons. Thus, macrophages are well positioned to respond to and regulate inflammation along the airway. Although the prominence of macrophages along the airway surface and their diverse functions strongly implicate macrophages as playing a role in

asthma, it is unclear whether that role is one of promoting or preventing inflammatory responses. On the one hand, macrophages can perform accessory cell functions by presenting antigen and providing secondary signals (eg, IL-1) required for the differentiation and proliferation of specific lymphocyte responses. These functions may play a role in sensitizing the airway to respond to further exposures. On the other hand, in some systems, alveolar macrophages have been found to be poor antigen-presenting cells, and in the large proportions of macrophages to lymphocytes (5:1 to 10:1) found on the airway surface, macrophages most likely suppress lymphocyte responses. Macrophages also produce other inflammatory mediators, such as platelet-activating factor, prostaglandin F₂ α and thromboxane. These mediators may play important roles in producing broncho-constriction or in causing inflammatory changes, including cell recruitment and altered vascular permeability. Pro-inflammatory cytokines produced by macrophages include IL-1, TNF- α , IL-6, and GM-CSF, which may induce endothelial cell activation, cellular recruitment, and prolonged eosinophil survival. Interleukin-6 and TNF- α may be released by IgE-dependent stimulation. Macrophages also elaborate histamine-releasing factors that appear to act on the basophil and mast cell by way of binding to surface IgE.

IX. ROLE OF SMALL MOLECULES IN ASTHMA

A. Reactive Oxygen Species

There is increasing evidence that oxidative stress and reactive oxygen species (ROS) are involved in inflammatory airway diseases, including asthma (Barnes, 1990; Repine et al., 1997), although relatively few studies have been undertaken in humans. This is partly because of the difficulties of measuring oxidative stress in the airways in vivo and partly because of the relative inefficacy of currently available antioxidants. However, new non-invasive techniques have been developed to assess oxidative stress in the airways, making it possible to reassess the role of oxidative stress in asthma. In rats, oxidative stress increases airway mucus secretion, an effect that is blocked by COX inhibitors [19, 20]. In rat airways, oxidant stress increases cholinergic nerve-induced broncho-constriction, an effect that may be the result of oxidative damage to acetyl-cholinesterase. Oxidants also activate NF- κ B (which orchestrates the expression of multiple inflammatory genes that undergo increased expression in asthma), thereby amplifying the inflammatory response (Barnes and Karin, 1997). Many of the stimuli that activate NF- κ B appear to do so via the formation of ROS, particularly hydrogen peroxide. ROS activate NF- κ B in an epithelial cell line [21] and increase the release of proinflammatory cytokines from cultured human airway epithelial cells.

B. Nitric Oxide

There is increasing evidence that endogenous NO plays a key role in physiological regulation of airway functions and is

implicated in airway diseases, including asthma. NO is a gas that is derived from the amino acid L-arginine by the enzyme NOS, of which at least three isoforms exist. There are two cNOS forms; one was first described in brain and is localized to neural tissue [neuronal NOS (nNOS) or NOSI], and the other is localized to endothelial cells [endothelial NOS (eNOS) or NOSIII], although it has become apparent that both enzymes are also expressed in other cells, such as epithelial cells. Both enzymes are activated by increases in [Ca²⁺]_i and produce small amounts of NO, which serve a local regulatory function. In contrast, iNOS (NOSII) is not normally expressed but is induced by inflammatory cytokines and endotoxin. This enzyme form is less dependent on increases in [Ca²⁺]_i, because calmodulin is tightly bound to the enzyme; when the enzyme is induced it is activated and produces much larger amounts of NO than do cNOS isoforms. NO produced by cNOS is involved in physiological regulation of airway function, whereas NO produced by iNOS is involved in inflammatory diseases of the airways and in host defenses against infection.

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