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Eosinophilic Asthma

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Abstract

Eosinophilic asthma is known as a main phenotype of asthma classified on the basis of immune cells involved in inflammatory response in the respiratory airway. Eosinophilic asthma can be related to increased severity of asthma, allergic sensitization, adult onset, and increased resistance to corticosteroids. The prevalence of eosinophilic asthma is 32–40% among asthmatic patients. Different cells and cytokines are involved in its pathogenesis including eosinophil, mast cells, type 2 helper T cells, innate lymphoid cells, IL-4, IL-5, and IL-13. Eosinophil count in induced sputum and bronchoalveolar lavage is the yardstick for recognizing and distinguishing eosinophilic asthma from non-eosinophilic asthma, while various tests which are noninvasive such as fractional exhaled nitric oxide and periostin are arising as possible substitutes. Novel and advanced therapies new and advanced therapies and more convenient biological drugs, Leads to high requirement for particular endotype- and phenotype-related treatment plans. Identification and knowledge of the specific pathophysiology of eosinophilic asthma have great association with disease management and chances for better patient prognosis.

Keywords: eosinophilic asthma, phenotype, mast cells, Th2 cell, ILCs, interleukin-5

1. Introduction

Asthma is a Greek word which means “labored breathing.” Asthma is a common disease which is characterized by reversible airway inflammation, chronic airway blockage, hyperresponsiveness, wheezing, and cough arising spontaneously and in reaction to nonspecific environmental factors. It affects an approximately 358 million people worldwide, causing a significant burden on healthcare systems. The highest prevalence of asthma has been found in the United Kingdom (15%) followed by Australia (14.7%), Canada (14.1%), and the United States (10.9%). In Asia the highest incidence of asthma has been recorded in Japan (6.7%), followed by Iran (5.5%), Pakistan (4.3%), Bangladesh (3.8%), and India (3%) and lowest in China (2.1%) [1]. It is a complex multifactorial disorder with various predisposing factors in environment and genes in which genetics of the individual plays a vital role [2]. Genome-wide studies have reported different loci that are associated with asthma. Asthma is associated directly with genes such as heterogeneity in Fc epsilon receptor 1 (FcεR1) on 11th and q region of 5th chromosome 11, while some other gene polymorphisms have no direct link with asthma.

Asthma usually starts in infancy or young age. Wheezing in early childhood does not always lead to asthma in late childhood. As a matter of fact, wheezing in infancy is commonly related to those children whose airways are relatively small than normal children. They will likely wheeze when they have viral bronchitis. On the other hand, pulmonary function starts off at normal range in children who frequently

progress to asthma. After asthma development, their lungs will not develop due to continuous inflammation of their disease.

After genetics, another factor is the environment in which atopy is the most critical cause of asthma. Most of the asthmatic persons have had skin allergy in childhood followed by nasal allergy which leads to asthma. This series of events is called allergic march. Other factors in environment such as construction designs of houses, pollution, dust mites, molds, pet danders, particles of cockroach waste, tobacco smoke, inhalation of cold and dry air, food and infection are trigger factors to cause asthma. Today, our residence and daily activities have changed such as homes are more heated as well as isolated. Taking a bath and showers more frequently leads to more moisture inside the homes. These changes have made our house environment friendlier for house dust mites. Diet has also changed such as seasonal fruits and vegetables switched to artificially ripened fruits. This simulated ripening of fruits may alter their chemical structure and antigenicity [3]. Air pollutants that have been rising due to vehicular traffic are ozone, particulates, and nitrogen oxide. Air pollutant affects asthma by increasing IgE production, imposing oxidative stress on airways directly and indirectly, functioning as a vector for allergens, and enhancing release of IL-4 and histamine from basophils [4] (**Figure 1**).

There is a close relationship between infections and asthma exacerbation. Increased exposure to infection of respiratory viruses is protective against asthma development. This is called hygiene hypothesis. Different researches on children revealed protective effect of infections in farming communities. Infants who drink unpasteurized milk or are taken to the animal house have a reduced chance of allergy and asthma, but there is no protective effect of infections if children are exposed only after 1 year of age. Once asthma is developed, viral diseases can

Figure 1.
The role of atopy and other environmental factors in asthma.

exacerbate its symptoms because viruses increase airway inflammation linked with asthma. Bacterial and parasitic infections can minimize the risk of allergy by reducing IgE sensitization and weakening the airways' response to allergen [5].

1.1 Classification of asthma

Asthma is a complex heterogeneous disease with variety of phenotypes. A disease phenotype gives information about clinical and morphological characteristics of disease, triggers, and therapy response but does not describe about pathogenesis of disease. Due to this reason, the classification of asthma has been further clarified with the development of endotypes, which is based on pathological mechanisms and treatment responses of asthma.

There is an overlap in this classification. Each endotype of asthma can have several phenotypes, just as a specific phenotype may be linked with more than one endotype [6]. These phenotypes have distinct subtypes based on symptoms, triggers, age at onset of disease, severity of disease, and underlying inflammation. Traditionally, asthma has been classified into extrinsic/atopic and intrinsic/nonatopic asthma. Atopic asthma starts in children who have family members with history of allergy and good treatment response. Atopic asthma usually begins after allergen exposure. On the other hand, intrinsic asthma is developed in adult age, and family history is absent in this type of asthma. Intrinsic asthma is a nonallergic type of asthma caused by cold, humidity, strong smells, infections (viral-induced asthma), and chemicals in smoke and cigarette. Nonallergic asthma occurs in 10–33% of asthmatic patients [7].

Asthma can also be divided into early-onset and late-onset asthma according to age of presentation of disease. Symptom-based asthma includes chronic asthma, acute severe asthma, brittle asthma, nocturnal asthma, and exercise-induced asthma. On the basis of frequency and severity of symptoms, the Global Initiative for Asthma (GINA) has classified asthma into intermittent, mild persistent, moderate persistent, and severe persistent asthma [8]. The American Thoracic Society and European Respiratory Society have also classified asthma into refractory asthma and “difficult/therapy-resistant asthma” based on the medication plan to achieve good control on asthma [9]. The World Health Organization (WHO) divided severe asthma into untreated severe asthma, difficult-to-treat asthma, and treatment-resistant severe asthma [10]. Based on etiology and underlying inflammation, asthma has also been classified into eosinophilic and non-eosinophilic (neutrophilic and paucigranulocytic) asthma [11].

1.2 Eosinophilic asthma

Eosinophilic asthma is a specific phenotype of asthma that is defined by inflammation of the basement membrane in the airway mucosa and high eosinophil levels in sputum and blood compared with non-eosinophilic asthma where no typical thickening of the basement membrane has been seen. Repeated asthma exacerbations are more noticeable in patients of eosinophilic than non-eosinophilic asthma [12]. Even though the exact incidence of eosinophilic asthma is not known, among patients with severe asthma who show about 5–10% of the asthmatic people, sputum eosinophilia ($\geq 2\%$) or blood (≥ 300 cells/ μl) can be observed in 32–40% of population which are linked with recurrent asthma exacerbations, as well as disease severity [13]. A subgroup of patients of eosinophilic asthma maintains constant airways and sputum eosinophilia even with conventional corticosteroid therapy called steroid-resistant eosinophilic asthma. In different studies, the levels of eosinophil in sputum are high in asthmatics with severe disease [14].

Eosinophilic asthma has three distinct presentations. The first phenotype of eosinophilic asthma is termed as allergen-exacerbated asthma in whom patients show allergen sensitization (atopy), accompanied with allergic rhinitis, present with exacerbated symptoms on allergen exposure and common in early-onset asthma [7, 15]. The second phenotype of eosinophilic asthma comprises those individuals in whom the eosinophilic inflammation is a prominent pathology, but these patients are nonatopic and can present at any age especially in adult age. This phenotype is called idiopathic eosinophilic asthma [7, 16]. Aspirin-exacerbated respiratory disease is the third phenotype of eosinophilic asthma with distinct features comprised of the presence of severe rhinosinusitis with nasal polyps and aspirin sensitivity. Like idiopathic eosinophilic asthma, aspirin-exacerbated respiratory disease is also presented in adulthood and nonatopic patients. However, different studies have documented that a small number of patients who developed asthma early in life showed 36% tissue eosinophilia, in comparison with the late-onset asthma which had 63% eosinophil level [17].

2. Pathophysiological mechanism

Asthma is a complex disease characterized by different pathological mechanisms including inflammation, hyperresponsiveness, remodeling, and angiogenesis of airways (**Figure 2**).

2.1 Airway inflammation

Eosinophilic airway inflammation is the main pathophysiological mechanism of eosinophilic asthma. Eosinophilic asthma develops from complex immunologic and pro-inflammatory mechanisms, mainly driven by T helper 2 (Th2) cells, which is a part of adaptive immunity release interleukins (IL-5, IL-4, and IL-13). Besides being orchestrated by mechanisms of adaptive immunity, Th2-mediated airway eosinophilia can be also linked with innate immunity, which relied on intercellular connection comprising of dendritic cells, bronchial epithelial cells, and innate lymphoid cells. As a result, airway eosinophilia arises due to the biological activity of both type 2 helper T (Th2) and type 2 innate lymphoid (ILC2) cells, which are critically participating in the pathogenic process of type-2 inflammation in eosinophilic allergic and nonallergic asthma [18]. These mechanisms are linked with increased IgE expression. In eosinophilic asthma patients, eosinophils collect in the respiratory tract. Differentiation of Th2 lymphocytes needs the association of various promoting elements, including costimulatory molecules and interleukins released by dendritic cells and inflammatory cells.

Eosinophilic allergic asthma is caused by aeroallergen like pollen and house dust mite which have proteolytic characteristics and also have small amount of bacterial components like lipopolysaccharides (LPS) [19]. Thus, on entrance into the respiratory epithelial membrane, allergens can attach with the Toll-like receptor (TLR), a receptor which is involved in innate immunity. Upon TLR activation, epithelial cells produce cytokines including thymic stromal lymphopoietin (TSLP), IL-25, and IL-33 which are capable of developing adaptive immune response of Th2 type. Moreover, TLR activation also evokes the secretion of chemokines such as CCL2 and CCL20, which increase the maturation of dendritic cells [20]. These dendritic cells move into the lumen of airways, take aeroallergens, and break them in the cytoplasm, leading to the generation of peptide fragments of allergen. These fragments are presented by class II HLA molecules on dendritic cells that move to regional lymph nodes where these antigen fragments are presented to T lymphocytes [21].

Figure 2.
Pathogenesis of eosinophilic asthma. Asthma arises from interaction between genetic and environmental factors including allergens and viruses. Allergens or viruses can be caught by dendritic cells (DCs) located in the epithelium, which process and present antigen to naïve (Tho) T helper cells. This allergen activates Tho to Th2 cells which produce IL-4 and IL-13. These cytokines activate B cell for class switching to IgE immunoglobulin. Further, Th2 cells also secrete IL-5, which activates and recruits eosinophil. IgE-dependent degranulation of mast cells secretes both immediate and newly formed mediators like leukotriene, prostaglandin cytokines, etc. other important cells contributing to asthma pathobiology are type 2 innate lymphoid cell (ILCs2), producing IL-13 and IL-5 which cause eosinophil recruitment and expansion in nonallergic eosinophilic asthma.

After activation of T-cell receptors by antigenic peptides, sensitization and stimulation of adaptive immune system take place. Stimulation of naïve T lymphocytes needs the attachment of their costimulatory molecules (CD28, ICOS, and OX40) with their ligand present on dendritic cells (CD80/B7.1, CD86/B7.2, ICOS ligand, and OX40 ligand). Differentiation of T lymphocytes is critically dependent on the cytokine environment [22]. Th2 polarization requires high levels of IL-4 and low concentration of IL-12. IL-4 is secreted by mast cells and basophils. GATA3 is the main transcription factor present in type 2 helper T cells that promote the production of Th2-type cytokines including IL-4, IL-5, IL-9, and IL-13. These interleukins cause eosinophils and mast cells' maturation and recruitment, promoting immunoglobulin class switching to IgE production. As a result, cytotoxic products released by degranulation of eosinophils induce airway epithelial injury, mucus hyperproduction, bronchial hyperresponsiveness, impaired ciliary movement, and an increase in smooth muscle size [23].

The late-onset type of eosinophilic asthma that is usually nonallergic arises in the absence of stimulation of Th2 lymphocytes. Recent researches suggest that the main role in the development of eosinophilic nonallergic asthma is played by

ILC2s which is activated by IL-25, by IL-33, and by prostaglandin D2 (PGD2) [24]. Consequently, these two distinct pro-inflammatory routes driven by either Th2 lymphocytes or innate ILC2s produce IL-5 which is mainly involved in eosinophilic inflammation of airways in asthma.

2.2 Airway hyperresponsiveness

Chronic inflammation of airways in asthma leads to more rapid contraction of smooth muscles of airways than in normal person in effect of broad range of stimuli, a condition termed airway hyperresponsiveness [25]. Airway hyperresponsiveness is a result of eosinophil infiltration mediated by T lymphocyte-secreted factor called eosinophil chemotactic factor (ECF-L). Hyper responsiveness of the air ways is caused by the decrease in function of neuronal M2 muscarinic receptor on parasympathetic nerves in the lungs due to eosinophil's major basic protein which is a protein released from granules of eosinophils. Schwartz et al. reported a direct relationship between eosinophil count in the airways, sputum and peripheral blood, and airway hyperresponsiveness [26].

2.3 Airway remodeling

Airway remodeling is the permanent cellular and structural modification in the airways primarily due to repair mechanisms in reaction to chronic inflammation. In a broad term, the airway is modified so that it acts in a different manner when allergens or nonspecific factors like exercise, cold air, perfume, and smoke are induced into the patient and it leads to irreversible change of lung functions [27]. There are various changes in structural and physiological characteristics which are different in every asthmatic patient. Most noticeable structural change is thickening of basement membrane of airway which is due to accumulation of type III collagen produced by myofibroblast. These myofibroblastic cells are stimulated and controlled by growth factors secreted by the epithelial cells and various cytokines (transforming growth factor- β (TGF- β), IL-10, and IL-17) released by T lymphocytes and eosinophils that have profibrotic responses while at the same time down-regulating the function of T and B lymphocytes [28].

Previously, it was thought that the airways' epithelial membrane is an innocent sufferer, becoming injured and lost due to the effect of toxic agents secreted by eosinophils and other inflammatory cells. But now, it has been reported that growth factors and interleukins (IL-8) secreted by the cells of epithelial membrane perform an active role in remodeling. Metalloproteases and epidermal growth factors released from matrix on inflammation stimulate these chemokines. On chemokine activation, neutrophils and other immune cells attracted to the area of damage cause structural alterations in the airways. Other structural changes including mucus metaplasia and increased angiogenesis have also been observed in asthmatic patients [29].

2.4 Angiogenesis

There is a rise in the number of blood arteries in the medium and small respiratory airway submucosa. It may help in physiological changes in airways of patients with asthma, including asthma due to exercise. Several studies have been documented that vascular endothelial growth factor (VEGF) may contribute in angiogenesis. High expression of VEGF has been observed due to hypoxia and several cytokines and growth factors such as epidermal growth factor, TGF- β , IL-1 α , and IL-6. VEGF expression is decreased by other interleukins including IL-10 and IL-13 [30].

2.5 Role of eosinophil in pathogenesis

Eosinophils are granulocytes in blood produced in the bone marrow with other white blood cells making about 1–3% of white blood cells. Eosinophil plays multiple functions and is an important component of allergic and asthmatic type 2 immune responses. Allergens on exposure starts a group of processes by Th2 cytokine-producing cells, resulting in eosinophils' attraction to the airway through the action of IL-5, and eotaxin research reported that Clara cells of the airway epithelium are the main source of eotaxin in the lung [30].

During asthma attack, eosinophils are stimulated to release proteins from granules including major basic protein, eosinophil peroxidase, eosinophil cationic protein, and eosinophil-derived neurotoxin, all of which are toxic to the epithelial cells of airway. Furthermore, eosinophils secrete plenty of inflammatory mediators like cytokines (interleukins IL-13 and IL-5), platelet-activating factor, growth factors (TGF- α and TGF- β), leukotrienes, thromboxane, and prostaglandins. The secretion of all these mediators results in enhancement of the inflammatory process, airways' epithelium cell injury, airway hyperresponsiveness, mucus hypersecretion, and airway remodeling and bronchospasm [31]. Eosinophils control the allergen-dependent Th2 pulmonary immune responses activated by dendritic cells and T cells as well as decrease Th1 responses [32].

2.6 Role of IL-5 in pathogenesis

Although various bioactive proteins such as IL-3 and granulocyte-macrophage colony-stimulating factor affect the life cycle of eosinophils, eosinophils react mainly to IL-5. Th2 cells, ILCs2, mast cells, natural killer T (NKT) cells, and eosinophils produce IL-5 within respiratory air passage of sufferer with eosinophilic asthma. In asthmatic patients, the bone marrow responds to environmental irritant by rising eosinophil production, and in asthmatics presenting both acute and late asthmatic reactions, this event is related with increased IL-5 mRNA proportion than persons having only early bronchial reactions. Apart from the effect of IL-5 on the bone marrow, it has also been observed that IL-5 enhances eosinophil maturation in airways of allergic patients [33].

IL-5 can also promote eosinophilic infiltration in bronchial airways due to synergetic effect of IL-5 with other chemoattractants of eosinophils such as eotaxins. The IL-5 role in eosinophil recruitment within the bronchial airways is due to its antiapoptotic action on eosinophils [34]. IL-5 exerts its effect by attachment with IL-5 receptor expressed on eosinophils and basophils. IL-5 receptor is composed of an IL-5-specific α subunit (IL-5R α) and a nonspecific β c chain that react with IL-5, IL-3, and GM-CSF [35]. The level of IL-5R α is expressed three times higher on eosinophils than basophils [36].

2.7 Role of IL-33 in pathogenesis

IL-33 is the newly discovered member of cytokine of IL-1 group. Schmitz et al. described IL-33 as a promoter of various type 2-related responses, including cytokine (IL-4, IL-5, and IL-13) and IgE production. In addition to type 2-related response, ST2, the IL-33 receptor, is present on several types of cells engaged in type 2 effector function, including Th2 cells, mast cells, basophils, eosinophils, and ILC2s [37]. Studies in asthma described the supporting role of IL-33 on monocyte development and eosinophil differentiation from the bone marrow [38].

2.8 Role of mast cell pathogenesis

Mast cells are the source of the Th2 cytokines including IL-4 and IL-5 that regulate antibodies' class switching to IgE and eosinophil production, respectively. Mast cells have been observed in higher frequency in asthmatic airways and stimulated by allergen exposure. On activation, mast cells degranulate and secrete their mediators such as histamine and leukotrienes, causing bronchospasm and acute bronchoconstriction by allergen. On the other hand, leukotriene is an essential mediator in airway inflammation and remodeling specifically in symptoms induced by exercise in intrinsic asthma. The granule proteases including tryptase are also released by mast cells. Tryptase is involved in airway remodeling and releases pro-inflammatory chemokine from intracellular matrix [39].

2.9 Role of ILCs in pathogenesis

Innate lymphoid cells (ILCs) are newly discovered immune cells that have lymphoid morphology but deficient in antigen receptor. Type 2 innate lymphoid cells (ILC2) are non-B/non-T cells that release IL-5 and IL-13 on activation by IL-25 and IL-33 and expressed MHC class II high and CD11cdull on their surface. Several studies reported that ILC2 originates from common lymphoid progenitor cells and not from either myeloid or erythroid progenitors, confirming that these cells are of lymphoid origin. ILCs have three different types, ILC1s, ILC2s, and ILC3s, on the basis of identical cytokine profile associated with the helper T subsets Th1, Th2, and Th17, respectively [40]. ILC2s are known to produce type 2 cytokines including IL-4, IL-5, and IL-13 on exposure to allergen, IL-25 and IL-33, and are therefore probable new member in Th2 cell-independent innate type 2 responses. ILC2s can be stimulated by several cytokines especially epithelial cell-derived cytokines IL-25, IL-33, prostaglandin, and leukotriene which have been observed to start ILC2 reaction in both animals and humans [41].

3. Diagnosis

Eosinophilic asthma diagnosis is considered essential in primary, secondary, and tertiary treatments. Typically, general practitioner uses this diagnosis to determine the initialization of inhaled corticosteroids (ICSs). A patient with signs of eosinophilic inflammation is likely to respond to ICSs; however, patients should not be treated with ICSs in the absence of airway eosinophilia. In addition, it is essential to recognize if a patient has airway eosinophilia because those with chronic eosinophilia are susceptible to severe problems and airway remodeling in spite of inhaled or oral corticosteroid treatment. Therefore it must be completely examined [42]. Significantly, all available resources and information are used in all settings to better presume if a person has eosinophilic asthma.

Eosinophilic asthma analysis depends on the confirmation of eosinophilic inflammation in airways of asthmatics, though there is no common diagnostic method. Many procedures can be utilized to diagnose airway eosinophilia in the airways that include induced sputum, bronchial biopsies, blood, and exhaled breath. Generally, airway biopsies or bronchoalveolar lavage (BAL) is principally observed for the analysis of airway inflammation. But for daily clinical use, this method is very invasive. Hence, to determine airway inflammation aseptically in an appropriate and cheap manner. The best recognized and the most common method for testing eosinophilic asthma is the identification of eosinophils in induced sputum [43].

3.1 Bronchial mucosal and BAL eosinophils

The histocytology of a biopsy sample of bronchial tissue could be a diagnostic test to determine the appearance of eosinophils in the submucosa and epithelial cells of air passage. But in daily clinical use, it is impossible to take patients' biopsy due to an invasive method. The interaction between eosinophils is poor in different airway areas because BAL represents eosinophils in the peripheral air passage, while sputum wash and bronchial wash produce a variety of small and adjacent large air passages. Additionally, the analysis of bronchial submucosal and BAL eosinophils is not consistent, so it is difficult to relate results of these tests between laboratories. Roughly, if the tissue and BAL express sufficient amount of eosinophil, possibly they are also increased in sputum. This observation may not be true. More importantly, the number of eosinophils in sputum (airway luminal) is more associated with clinical guidelines for asthma control, like the worsening of symptoms than the numbers of eosinophils in tissue section. This association may not be surprising, provided that eosinophils are triggered as they pass through different areas and are further induced in the lumen of air passage than in tissues [44].

3.2 Eosinophil count in sputum

The advance applications of methods to carefully and accurately induce and assess the sputum have allowed the possibilities to investigate the features of inflammatory process in airway in asthmatics. This brings attention to the heterogeneity of airway inflammation in asthma [45]. Currently, sputum analysis is essentially an extensive and aseptic method for testing the airway inflammation. The analysis of sputum with hypertonic solution of saline is reliable in asthmatics who have just 0.9 L forced expiratory volume in the first second (FEV1) and is effective in almost 80% of asthma patients [46]. The test for the collection, preparation, and determination of cell counts of sputum is easily characterized and organized, and its stability, responsiveness, and validity were explained. The normal values for sputum cell counts were determined, and on the basis of sputum examination, guidelines are available to improve the treatment. However, the eosinophil count in non-asthmatics is 1.2%, while 3% or more sputum eosinophil is usually believed as clinically important. Further investigation is required apart from the complete cell differentiation, probably the levels of biomarkers, like eosinophil-free granules, or the level of protein released from granules (e.g., eosinophil peroxidase) is precise and more significant [47].

3.3 Peripheral blood eosinophil

Eosinophilic counts in a peripheral blood are easily collected and mostly convenient, and still it is deficient in both accuracy and susceptibility. However, some asthmatics perhaps reveal that blood eosinophils rise in those patients who have peripheral eosinophilia. So a proposed association is found with acute asthma signs and decreased pulmonary activity as examined by FEV1 [48]. But in asthma, blood eosinophil counts were not recognized to safely associate with increased eosinophils in sputum. It was shown that eosinophils' quantity ($>300/\mu\text{L}$) in blood had just 50% positive predictive value in finding the phenotype of an asthma that is on the basis of eosinophil in sputum ($>2\%$). Altogether, these studies show that peripheral blood eosinophilia perhaps is a sign of severe condition in asthma but not constantly associated with sputum eosinophilia.

3.4 Pulmonary function test (PFT)

PFT evaluates volume and rate of airflow that breathe in and out. The FEV₁ of exhalation is assessed and compared to the total air volume during forced expiration (forced vital capacity [FVC]). It is an early test for diagnosis of asthma to evaluate airway blockage, disease severity, and reversibility of symptoms. Reduced FEV₁, blockage in airflow (lower level of FEV₁/FVC), and concavity in FEV loop are expected in patients of asthma [49]. Other PFTs include bronchodilator responsiveness (BDR) test which is predictive of adult-onset asthma. Specific airway resistance (sRaw) analyses by body plethysmography may also be an indicator of early airflow blockage. Hastie et al. reported multiple parameters such as FeNO level, reduced FEV₁, persistent airflow obstruction, total IgE, and blood eosinophil counts in diagnosing eosinophilic asthma [50].

3.5 Exhaled breath condensate (EBC)

EBC is a new, noninvasive test of identifying biological markers, predominantly secreting from the lower part of the airway. EBC is obtained at the time of quiet respiration, as a result of cooling and liquefaction of the air droplets that breathe out [51]. It is a distinct method in detecting molecular pathways related to the respiratory tract. Antus et al. reported lower EBC pH in asthmatic compared with control subjects [52]. Hydrogen peroxide (H₂O₂), an indicator of oxidative stress, was elevated in EBC of patients with asthma. Furthermore, EBC-H₂O₂ concentration is associated with asthma severity and prognosis [53]. Other biomarkers such as CysLTs (LTD₄, LTE₄, and LTC₄), eicosanoids (8-isoprostane and prostaglandin E₂), interleukins (IL-4), and high-sensitivity C-reactive protein (hs-CRP) are found in increased levels in asthma with exercise-induced bronchoconstriction. Serum hs-CRP and fractional exhaled nitric oxide (FeNO) concentration were significantly associated with EBC-hs-CRP levels in patients of asthma [54, 55].

3.6 Fraction of exhaled nitric oxide (FeNO)

Nitric oxide synthase helps in the synthesis of nitric oxide, a reactive molecule that is shown on cells in airway epithelium. In asthma, FeNO analysis by breath assays is usually treated as an aseptic sign of airway inflammation. FeNO analysis is simple, rapid, and noninvasive in contrast to the bronchoscopy and sputum induction. Significantly, it was shown that FeNO quantification perhaps is helpful as a clinical instrument for administering the asthma and managing the disease, but different findings result in some controversy about FeNO efficacy [56]. In a study, more than 90 asthma patients were examined by Smith et al., and they identified that FeNO acts as an effective tool for the withdrawal of inhaled corticosteroid treatment. Tseliou et al. also studied that >19 parts per billion FeNO levels were due to sputum eosinophilia with 78% sensitivity and 73% reactivity in individuals who had mild to acute asthma, while few of them relied on prednisone. Differently, Nair et al. in a clinical trial performed with mepolizumab described that FeNO levels and sputum eosinophil percentages are not associated with asthmatics who relied on prednisone [57].

3.7 Total IgE

IgE plays an important part in allergic asthma. IgE antibodies produced by allergic patients are specific for antigens like pollens and house dust mite, attached with IgE-specific receptors on basophils and mast cells. The connection of IgE

molecules stimulates the release of intermediates (arachidonic acid metabolites and histamine) and cytokines (IL-4, tumor necrosis factor alpha, and IL-5) that are important for early- and late-stage allergic response and the associated penetration of eosinophils in the airway. Different findings which have determined a relation between levels of IgE in serum, airway eosinophilic asthma, and anti-IgE treatment were explained, closely related with a remarkable decrease in tissue eosinophils. But in spite of these findings, it is not suggested to use IgE as a biomarker for eosinophilic inflammation. Latest meta-analysis by Korevaar and his fellows, they have described low validity and inadequacy for this biomarker in comparison with FeNO to find sputum eosinophilia [58]. The results were not valid, when comparing blood eosinophils with IgE. Hence, to find eosinophilic asthma, IgE appears to be less effective of all currently available biomarkers.

3.8 Periostin

Periostin is an interleukin-13-regulated matrix protein which is present outside the cells. It was described that periostin promotes the recruitment of allergen-induced eosinophils to the lungs, leading to eosinophil binding to fibronectin. Additionally, it was shown that periostin affects the durability of lung cancer cells due to Akt/PKB pathway; though it has not been examined, maybe it could improve the survival of eosinophils [59].

Generally, periostin is available as an essential biomarker for the detection of eosinophil levels in air passage in asthma patients because of its function in the recruitment of eosinophils in tissue. Jia et al. conducted a study on different parameters that include age, BMI, gender, blood eosinophils, and levels of IgE, FeNO, and periostin in the serum of 59 acute asthmatic cases and demonstrated that airway eosinophilia was best determined by periostin in the serum. The level of periostin (>25 ng/mL) in serum had 93% positive predictive value and 37% negative predictive value for $>3\%$ eosinophils in sputum or tissue eosinophilia. In asthma the exact function of periostin is not observed. In addition to function in eosinophilia, animal models propose that perhaps periostin is associated with airway remodeling through growth factor- β switching and can also have supportive part in airway hyperresponsiveness induced by allergen [60].

4. Treatment

The present eosinophilic asthma treatment is introduced with common guideline-based therapy that consists of ICS and bronchodilators that have been thoroughly studied elsewhere [61]. Usually the eosinophil appearance has been linked with susceptibility to corticosteroids, while some eosinophilic asthma patients were identified with subsequent steroid refractory.

Eosinophilic asthma treatment consists of elevated dose of ICS and oral corticosteroids. ICS are primarily used to decrease airway inflammation and mucus hypersecretion, beginning with the reduced strong dosage and increasing to high-dose ICS due to increased intensity. Several severe asthmatics become addicted to corticosteroids. Depending upon toxic corticosteroids for long-term maintenance, treatment perhaps impairs the individuals and may result in corticosteroid resistance [62].

Perhaps many methods which are considered for corticosteroid-resistant asthma have been described in addition to the activation of p38 mitogen-activated protein kinase and inflammatory genes controlled by transcription factor-kB. A p38 mitogen-activated protein kinase is significant to trigger GATA3 (the master

Th2 cytokine transcription factor). Moreover, phosphoinositide 3-kinase (PI3K) controls inflammatory pathways and activates the PI3K δ isozyme through oxidative stress that can reduce the corticosteroid susceptibility by decreased histone deacetylase 2 (an enzyme marked by theophylline). Further steroid refractory asthma can comprise elevated expression of the alternatively linked variant of the glucocorticoid receptor and elevated formation of macrophage migratory inhibitory factor that can arrest the anti-inflammatory outcomes of corticosteroids [63].

Other factors are under examination for the management of asthma comprised of antagonists focusing on thymic stromal lymphopoietin, IL-25, IL-33, GM-CSF, and chemokine receptor 3 that are expressed on eosinophils [61].

4.1 Biologic therapies

The treatment of refractory eosinophilic asthma includes the drugs that specifically target T helper 2 cytokines as well as anti-IgE, anti-IL-5, and anti-IL-13 monoclonal antibodies [64].

4.1.1 Omalizumab

An IgG1 recombinant humanized monoclonal antibody against IgE is omalizumab. Omalizumab binds with IgE Fc portion, recognizing Fc ϵ R1, IgE high-affinity receptors on the top of basophils, and mast cells that result in the downregulation of receptor and suppress the release of inflammatory intermediates. An important function of IgE is to act in allergic response pathophysiology, while omalizumab impairs both early- and late-phase inhaled allergen responses in asthmatics [65]. The previous studies showed a remarkable decrease in eosinophils in airway tissue and induced sputum (8 at baseline in contrast to 1.5 posttreatment) in asthmatics that were treated with omalizumab. Later, it was reported that treatment for 16 weeks reduced the number of eosinophils in blood from 6.2 to 1.3% at baseline [66]. Thus total serum IgE is not applicable for eosinophilic asthma as a diagnostic marker. So, the levels of total IgE in serum should be applied for examining anti-IgE therapy.

The therapy against IgE is effective to eosinophilic asthma treatment in spite of IgE levels. One reason for the observed paradox is that the no response of IgE levels may be associated with the downregulation of Fc ϵ R1 by anti-IgE on the surface of basophils, dendritic cells, and mast cells. A decrease in cells that express Fc ϵ R1 reduces the intermediate responses of allergen-induced IgE, suppressing the discharge of cytokine and the induction of eosinophil into the airway [67]. Moreover, anti-IgE treatment may assist to reduce the numbers of airway dendritic cells that result in the reduction of Th2 cell differentiation and Th2 cytokines that are required for the recruitment and survival of eosinophils. Thus total IgE in serum may not be related to clinical response or eosinophilic asthma, while omalizumab is useful in the treatment of asthma and decreases the airway eosinophils.

It was studied by Noga et al. that omalizumab is also important as it may have proapoptotic effects on eosinophils [68]. The reduced number of mast cell mediators helps in the stability of eosinophil that may lead to eosinophil apoptosis in individuals that were tested with omalizumab. Particularly, omalizumab is also found as a corticosteroid-sparing drug in persistent eosinophilic pneumonia, a condition that is identified by symmetric lung penetration and the remarkable eosinophil recruitment in blood and BAL fluid [69]. Hence, the outcomes of anti-IgE therapy on lung eosinophilia give more understandings about allergic inflammation mechanisms, which can assist in improving the phenotype-specific analysis.

4.1.2 Targeting IL-5 and interleukin-5 receptor α

The key function of IL-5 in tissues is to stimulate the growth, recruitment, activation, and differentiation of eosinophils. Initial studies described the elevated IL-5 expression in BAL fluid and bronchial biopsies in asthmatic patients. Moreover it was shown that following the allergen confront, IL-5 mRNA was regulated in bronchial mucosa, and the levels were associated with the disease activity. After anti-IL-5 treatment, airway hyperresponsiveness and airway eosinophil assembly after allergen challenge were reduced in animal models [70]. So, there is enough explanation for selecting IL-5 in asthmatics to particularly decrease the eosinophil migration, maturation, and stability that can cause many features of asthma pathogenesis.

4.1.2.1 Mepolizumab

An IgG1-humanized noncomplement-fixing monoclonal antibody is mepolizumab that is specific for human IL-5. Mepolizumab prevents the binding of human IL-5 to the alpha chain of IL-5 receptor complex that is expressed with high affinity on the surface of eosinophil cell. It was shown that in the bronchial mucosa of atopic individuals, anti-IL-5 therapy causes maturational blockage of eosinophil progenitors in the bone marrow and reduces the eosinophil precursors (CD34+ IL-5R α +) [71]. It is interesting that mepolizumab has different effects in different tissues which results in the complete reduction of eosinophils in sputum and blood exclusively 55% decrease in the bronchial mucosa. It was proposed by Flood-Page et al. that different levels of tissue infiltration could be due to the improved expression or downregulation of IL-5 receptor. Once assembled into the tissue, probably the survival of airway eosinophils depends on IL-3, GM-CSF, or eotaxins.

Two latest findings demonstrate that there could be useful outcome of mepolizumab in certain groups of eosinophilic asthma patients. It was found that double-blind placebo-controlled research consists of 61 cases with a history of chronic acute exacerbations and refractory eosinophilic asthma; following 1-year monthly injections of mepolizumab, a remarkable decrease in exacerbations and recovery in symptom scores were observed in patients treated with mepolizumab [72].

4.1.2.2 Reslizumab

Reslizumab is an anti-IL-5 humanized monoclonal antibody (IgG4), also provided to the eosinophilic asthma patients that were poorly managed [73]. A latest study described a remarkable decrease of eosinophils in sputum, and the respiratory activity improved while relating with inactive drug following monthly 15 weeks of reslizumab therapy (3 mg/kg). The useful results of reslizumab were mostly marked in nasal polyp patients and in those patients who had a maximum level of eosinophils in sputum and blood. Significantly besides the level of eosinophils, the appearance of nasal polyposis can recognize asthma patients that were treated with anti-IL-5.

4.1.2.3 Benralizumab

Benralizumab is an anti-IL-5R α afucosylated humanized monoclonal antibody, identified on eosinophils and nowadays in Phase II clinical trials. In a prospective Phase II study, the result of one shot of benralizumab (1 mg/kg) that was given intravenously related to the monthly three shots (100 or 200 mg) given

subcutaneously or placebo in adult patients of eosinophilic asthma was studied [74]. It was described that following final dose of benralizumab through intravenous and subcutaneous passage helped in the reduction of eosinophil levels in sputum and airway mucosa as well as complete eosinophil count arrest in peripheral blood and bone marrow for up to 28 days.

4.1.3 Targeting interleukin-4 and interleukin-4 receptor α

IL-4 and IL-13 are essential cytokines in the pathogenesis of atopic disease and allergic asthma. These are expressed by basophils, innate lymphoid cells, mast cells, and Th2 cells. IL-4 is important for various asthma characteristics that include mucus formation, switching of B-cell isotypes, and differentiation of Th2 cells. IL-4 and IL-13 transmit signal inside the cells by two different overlapped heterodimeric receptors which are part of IL-R α [75]. Receptor attachment is triggered by a typical signaling pathway, signal transducer and activator of transcription 6 (STAT-6), that is important for the production of Th2 inflammation, an asthma feature. Significantly, eotaxins help in eosinophilic induction as well as rely on IL-4 or IL-13 for the stimulation of STAT-6. At present many drugs are under examination that use IL-4/IL-13/STAT-6 pathway.

4.1.3.1 Pascolizumab

Pascolizumab is a human-based IL-4 monoclonal antibody that was considered in animal studies as well as Phase I and II clinical trials. Pascolizumab was strongly accepted in Phase I clinical trial with mild to moderate asthma in adult patients; anyhow following Phase II trial on a large scale was stopped because it was unsuccessful to express the clinical results in symptomatic individuals who were steroid immature [76].

4.1.3.2 Altrakincept

Altrakincept is an artificial humanized antagonist IL-4R α that inhibits the penetration of airway eosinophils and hypersecretion of mucus in a mouse model when managed during allergen challenges. One dose of the medicine improves the pulmonary activity and disease problems in Phase I and II trials [77].

4.1.3.3 Pitrakinra

Pitrakinra is an antagonist, which targets the heterodimeric receptor of IL-4 and IL-13 cytokines, comprises the subunits IL-4R α and IL-13R α 1. Pitrakinra suppressed the early-stage and late-stage reactions produced by allergen when managed by the subcutaneous or inhaled passage [78].

4.1.3.4 Dupilumab

A humanized monoclonal antibody to the IL-4R α subunit is dupilumab, currently described in a follow-up study analysis [79]. It was studied that 104 subjects with mild to acute persistent asthma and eosinophilia were separated to gain subcutaneously a single dose (300 mg) of dupilumab or placebo in a week for 12 weeks. In the treated group, this study developed a remarkable recovery in lung function related to the decrease in asthma inflammation as long-acting beta-agonists, and received steroids were absorbed. In addition, the significant modifications from basic standards in Th2-related indicators, as well as FeNO,

IgE, chemokine ligand 17, and chemokine ligand 26 (eotaxin-3), were found in the group of dupilumab by 12 weeks. The levels of blood and sputum eosinophils were not dissimilar following dupilumab therapy, while there were less number of people who give sputum, so statistical examination was excluded. Generally, identifying the IL-4R α signaling (that also stimulates IL-13 signaling) acts as a good therapeutic approach for eosinophilic asthma.

4.1.4 Targeting IL-13

An important part of IL-13 in airway eosinophilic induction in a way depends on the combined function of IL-5 and eotaxin in mouse models. Additionally, many studies demonstrate that IL-13 is important for corticosteroid protection in asthma. In a study on animals, IL-13 inhibition procedures have described reduction in airway hyperresponsiveness, inflammation caused by environmental immunogen, and remodeling of airways [80]. Thus nowadays, pharmaceuticals that target this cytokine are under examination in those who have refractory eosinophilic asthma due to steroids.

4.1.4.1 Anrakinzumab

Anrakinzumab is a complete human IL-13-targeted antibody. In Phase II clinical trial, its effects have shown a decrease in late asthmatic responses produced by allergen after two doses (2 mg/kg) that were given subcutaneously for 2 weeks [80].

4.1.4.2 Lebrikizumab

Lebrikizumab is a humanized anti-IL-13 monoclonal antibody. In a latest study, lebrikizumab was investigated in 219 adults with weakly controlled asthma against long-acting beta-agonists and ICSs [81]. Consequently, the treated group after 12 weeks of therapy has improved FEV₁, while high pretreatment with serum periostin levels has more good effects in patients. In post hoc examination, it was interesting that high FeNO and Th2 markers which include CCL13 (human monocyte chemoattractant protein-4), peripheral eosinophilia, CCL17, and total IgE levels were further related with a significant decrease in the levels of acute problems in lebrikizumab-treated cases relative to placebo.

4.1.4.3 Tralokinumab

Tralokinumab is another antibody against IL-13, also effective in Phase II study in improving the lung activity of individuals with moderate to acute asthma [81].

5. Conclusions

In conclusion, asthma is a heterogeneous condition with several phenotypes and endotypes on the basis of different immunopathogenic mechanisms such as underlying inflammation, environmental factors, and disease severity. Understanding of distinct phenotypes with specific pathophysiology is essential for management of patients with eosinophilic asthma. Categorization of asthma into eosinophilic and non-eosinophilic subphenotypes depends on the difference in cells involved in inflammation of respiratory airway. Generally, eosinophilic inflammation has been linked with extrinsic (allergic) asthma with Th2-type response, but now eosinophils have also been observed in the airways of nonallergic (intrinsic) asthma. The

development of new biological therapies like monoclonal immunoglobulin and small particles that block IgE, interleukins of Th2 type, and particular inflammatory factors has improved the knowledge about the immunopathogenesis of this phenotype and emphasizes the significance of individual-directed treatment. For doctors, it is essential to early recognize eosinophilic patients because this phenotype may need patient-directed therapies to prevent worsening of asthma symptoms.

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Acronyms and abbreviations

FcεR1	Fc epsilon receptor 1
GINA	Global Initiative for Asthma
WHO	World Health Organization
IL	interleukin
Th2 cells	type 2 helper T cells
ILCs2 cells	type 2 innate lymphoid cells
LPS	lipopolysaccharides
PGD2	prostaglandin D2
TGF	transforming growth factor

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