EDITORIAL

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Blood molecular biomarkers of the inflammatory phenotypes of asthma

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Asthma is a heterogenous disease characterized by chronic airway inflammation and reversible airflow limitation. The various asthma subtypes have similar clinical symptoms, including shortness of breath, wheezing, and cough. Multiple biologic pathways underlie the inflammatory responses in the airway of patients with asthma. Since 1990, much research has focused on characterizing the subtypes of asthma in terms of their clinical and pathophysiological features. Identifying asthma endotypes, which have distinct pathogenic mechanisms, is a major challenge but could improve treatment outcomes [1].

Asthma can be classified in clinical, inflammatory, immunologic, and molecular terms. The clinical factors include disease onset and severity, presence of atopy, treatment response, comorbidities (such as aspirin intolerance and obesity), and aggravating factors (such as exercise and certain occupations) [1]. Phenotyping based on immunologic and molecular analyses enables the identification of patients likely to respond to biologics targeting type 2 (T2) inflammation, especially in severe asthma. The T2 inflammatory phenotype (T2-high) is characterized by the production of the cytokines interleukin (IL)-4, IL-5, and IL-13 by type 2 helper T cells during an allergic response. It may be also activated by viruses or other environmental factors, which stimulate innate immune cells such as type 2 innate lymphoid cells (ILC2s) and mast cells by promoting the production of IL-25, IL-33 and thymic stromal lymphopoietin by epithelial cells [2]. The T2 phenotype is associated with eosinophilic inflammation, which manifests clinically as elevated blood or sputum eosinophils or a high level of fraction of exhaled nitric oxide (FeNO). By contrast, non-T2 phenotypes (T2-low) are mediated by neutrophilic inflammation, which is activated by Th1 or Th17 cells. The inflammatory phenotypes have different inflammatory features; there is also a "non-specific inflammatory phenotype" that cannot be classified and has no inflammatory features [2,3].

Because asthma is an airway inflammatory disease, the inflammatory phenotype can be evaluated based on the sputum inflammatory cell profile. However, collection and analysis of induced sputum cells is arduous for both the patient and practitioner; moreover, regular quality control checks are required to prevent inadequate sample collection and ensure reliable results [3]. Therefore, easily measurable biomarkers of airway inflammation are needed. Although the blood eosinophil count and FeNO level are commonly

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used markers of eosinophilic airway inflammation, they are not always correlated with the sputum eosinophil count and are affected by other factors, such as comorbidities, diet, drugs, and environmental factors. Furthermore, no clinical biomarkers of non-T2 or non-eosinophilic inflammatory phenotypes are available [1,2]. In the latest issue of the *Korean Journal of Internal Medicine*, Hur et al. [4] reported serum biomarkers of sputum inflammatory cell profiles in adult patients with asthma. They classified the sputum cellular profile as eosinophilic, neutrophilic, mixed, or paucigranulocytic; this is the most commonly used system for categorizing airway inflammation in asthma. They evaluated four potential serum biomarkers—periostin, eosinophil-derived neurotoxin (EDN), $S_{100}A_9$, and folliculin—which are reportedly associated with the pathogenesis of asthma, in terms of their ability to reflect the type of inflammation. The findings suggested that serum periostin and EDN indicate eosinophilic inflammation, S100A9 indicates neutrophilic inflammation, and folliculin indicates paucigranulocytic inflammation.

T2 asthma is discriminated based on clinical and laboratory characteristics, including a large number of blood or sputum eosinophils, a high FeNO level, a high total immunoglobulin E (IgE) level, and clinical features indicating allergen-induced asthma [2,3]. These features are important for identifying candidate biologics targeting T2 inflammation, such as anti-IgE, anti-IL5, and anti-IL4R monoclonal antibiotics. Biologics targeting other Th2 cytokines and their receptors are also under development. Biomarkers of specific molecular or cytokine pathways would be useful, because the T2 inflammatory and T2-high phenotypes of asthma show heterogeneity [1,3].

The findings of Hur et al. [4] suggest that periostin and EDN could serve as T2 biomarkers, and thus facilitate the diagnosis of eosinophilic phenotypes and prediction of the response to T2-targeted treatments. Periostin, a blood biomarker for T2 asthma, is a downstream molecule of the IL-13 pathway produced and released mainly by airway epithelial cells. Periostin acts both as an extracellular matrix protein and as a matricellular protein during cell activation by binding to receptors on the cell surface [5]. The action of IL-13 is important for inducing goblet cells and enhancing airway hyperresponsiveness and tissue remodeling. Periostin is one of the best biomarkers of IL-13 activation in asthma [2,5]. Serum levels of EDN, which is a degranulation protein released by eosinophils, reportedly reflect eosinophil activation in adult and childhood asthma. EDN may better reflect asthma control status than the total eosinophil count, and may be a useful biomarker for asthma severity [6,7]. In the previous and current reports, EDN and periostin showed similar sensitivity and specificity for blood and sputum eosinophilia [4,7]. Moreover, the ability to diagnose the eosinophilic inflammatory phenotype was enhanced by use of two biomarkers in combination [4]. EDN is directly associated with eosinophil, which is stimulated by activity in the IL-5 pathway, while periostin is associated with airway hyperresponsiveness or remodeling induced by the IL-13 pathway [2,3,6]. IL-13 does not activate eosinophils directly, but is closely related to T2 inflammation because it is mainly produced by Th2 cells, ILC2s, and mast cells. These cells also release other Th2 cytokines, such as IL-4 and IL-5, thereby activating the allergic response and eosinophilic inflammation [2]. This finding suggests that combinations of biomarkers are needed for phenotyping of T2 asthma, because they reflect different aspects of T2 inflammation.

Notably, this study identified serum biomarkers for neutrophilic and paucigranulocytic asthma. In contrast to eosinophilic and T2 asthma, few serum biomarkers for these subtypes of non-eosinophilic and non-T2 asthma have been discovered [2]. Treatment options for these phenotypes are scant, partly due to a lack of specific biomarkers to guide diagnosis and treatment. In real-world practice, there is no useful biomarker for neutrophilic asthma other than sputum neutrophilia [1,2]. Confirming the diagnosis of neutrophilic asthma is difficult because airway neutrophilia is affected by respiratory infections, smoking, and environmental factors. Factors such as IL-6, IL-8, IL-17, and YKL-40 are considered putative biomarkers of neutrophilic asthma [2,8], but their clinical utility is unknown. $S_{100}A_9$ and $S_{100}A_8$ are small calcium-binding proteins released by stressed cells as alarmins, which are endogenous danger signals that promote and exacerbate the inflammatory response [9,10]. $S_{100}A_9$ activates Toll-like receptor 4, neutrophil chemotaxis, and neutrophilic inflammation [9]. $S_{100}A_9$ has been suggested as a biomarker of inflammatory diseases such

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as rheumatoid arthritis, inflammatory bowel disease, and cystic fibrosis [10]. However, $S_{100}A_9$ is a universal marker of neutrophilic inflammatory diseases rather than being specific for neutrophilic asthma.

The paucigranulocytic phenotype of asthma is the least well-known phenotype in terms of its pathophysiologic mechanism. Air flow limitation and airway hyperresponsiveness, which are major characteristics of asthma, are generally induced by airway inflammation, but some symptoms are associated with airway structural changes and remodeling independent of inflammation. Airway epithelial cells, smooth muscle cells, myofibroblasts, and neuronal cells are the major structural cells involved in this process [3,4]. Folliculin is a cytoplasmic protein present in various cell types, including airway epithelial cells. Folliculin maintains the integrity of the epithelial barrier by regulating cell to cell adhesion in intercellular junctions. The release of folliculin from epithelial cells is associated with the pathogenesis of aspirin-induced asthma and toluene-diisocyanate-induced asthma [11,12]. Although the pathophysiologic mechanism of paucigranulocytic asthma is unclear, findings suggest folliculin as a biomarker for this phenotype; it also has the ability to discriminate non-inflammatory phenotypes in other types of asthma. Further studies are warranted, including of biomarkers for non-T2 asthma.

Inflammatory cell profiles can be affected by medication, infection, environmental factors, and exacerbation, so may vary according to the timing of sample collection. Reliable and adequate sputum samples are not always available in real-world practice. Identifying stable surrogate biomarkers of the inflammatory phenotypes of asthma is critical to realize personalized medicine. The ideal biomarker is easily measurable, sensitive, specific, reproducible, cost-effective, provides prognostic information, and is mechanistically linked to the therapeutic target [3]. Although this study evaluated the ability of candidate biomarkers to reflect inflammatory phenotypes, their sensitivity and specificity were insufficient for diagnosis of asthma phenotype. The use of combinations of biomarkers may overcome this limitation. Furthermore, the discovery and development of novel biomarkers is needed. Genomics, epigenomics, transcriptomics, proteomics, metabiologics, and metagenomics can provide unbiased molecular data, which could be used to refine endotypes and facilitate the discovery of new biomarkers. Also, multi-omics analysis is helpful for determination of asthma endotypes and optimal treatments based on the phenotype and underlying mechanism. Such analyses could enable precision medicine for asthma.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

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