REVIEW ARTICLE

Airway inflammation in chronic obstructive pulmonary disease

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ABSTRACT

Chronic obstructive pulmonary disease (COPD) is an inflammatory airway disease whose incidence and mortality increases every year. It is associated with an abnormal inflammatory response of the lung to toxic particles or gases (usually cigarette smoke). A central role in the pathophysiology has been shown to play a chronic inflammation of the airways that is expressed primarily by hypersecretion of mucus, stenosis of the smaller airways and the establishment of pulmonary emphysema. There is an increasing trend for assessing the inflammatory pattern of inflammatory airway diseases through mediators measured by noninvasive techniques. Markers in biological fluids and exhaled air have been the subject of intense evaluation over the past few years, with some of them reaching their introduction into clinical practice, while others remain as research tools. Of particular interest for the scientific community is the discovery of clinically exploitable biomarkers associated with specific phenotypes of the disease. Studying the effects of therapeutic interventions in these biomarkers may lead to targeted therapy based on phenotype and this is perhaps the future of therapeutics in COPD.

KEYWORDS

Chronic obstructive pulmonary disease (COPD); pathophysiology; airway inflammation; biomarkers; non-invasive techniques

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Chronic obstructive pulmonary disease (COPD) is a common respiratory condition involving the airways and characterized by airflow limitation (1,2). It affects more than 5 percent of the population and is associated with high morbidity and mortality (3). It is the third-ranked cause of death in the United States, killing more than 120,000 individuals each year (4). COPD is the only one of the six leading causes of death in the U.S. whose mortality is increasing. It is estimated that 2020 will be the third leading cause of death worldwide after ischemic heart disease and cerebrovascular disease (5,6). As a consequence of its high prevalence and chronicity, it causes high resource utilization with frequent clinician office visits, frequent hospitalizations due to acute exacerbations, and the need for chronic therapy (e.g., supplemental oxygen therapy, medication) (1).

Correct diagnosis of COPD is important because appropriate management can decrease symptoms (especially dyspnea), reduce the frequency and severity of exacerbations, improve health status, improve exercise capacity, and prolong survival (7). The Global Initiative for Chronic Obstructive Lung Disease (GOLD) defines COPD as follows: COPD, a common preventable and treatable disease, is characterized by airflow limitation that is usually progressive and associated with an enhanced chronic inflammatory response in the airways and the lung to noxious

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particles or gases. Exacerbations and comorbidities contribute to the overall severity in individual patients (8).

Central role in the pathophysiology of COPD has been shown to play a chronic inflammation of the airways that is expressed primarily by increased numbers of goblet cells, mucus gland hyperplasia, fibrosis, narrowing and reduction in the number of small airways and airway collapse due to the loss of tethering caused by alveolar wall destruction in emphysema (9). Pulmonary emphysema is associated with increased activity of proteolytic enzymes (MMP-2, MMP-9, MMP-12, cathepsin K, L and S and neutrophil elastase) which are activated due to inflammation and oxidative stress occurring in COPD. Mucus hyper secretion, which can be associated with an increased number of exacerbations, possibly radiate inflammatory lesion around the submucosal glands and severity of inflammation in most peripheral airways (10,11).

Macrophages play a key role in inflammation of COPD. Smoking and other inhaled irritants cause inflammatory response in the peripheral airways and lung parenchyma. The activated macrophages release of inflammation mediators and chemotactic factors, including tumor necrosis factor-a (TNF-a), interleukin IL-6, interleukin IL-8, monocyte chemotactic peptide (MCP)-1, leukotriene LTB4 and reactive oxygen species, and secrete proteolytic enzymes (especially MMP-9, MMP-12) whose action contributes to the establishment of pulmonary emphysema. Under the influence of secreted chemotactic factors (particularly IL-8 and LTB-4), neutrophils move directly to the respiratory tract and cause the over-stimulation of submucosal mucous glands and goblet cells by proteinases (especially neutrophil elastase, cathepsin-G, proteinase-3) secreted from these (12-16).

Bronchial biopsies from COPD patients also showed infiltration of the bronchi by CD4+ and CD8+ predominantly T lymphocytes (17-22). There is a correlation between the number of T-lymphocytes, the extent of alveolar destruction and the rate of airway obstruction (12). The role of CD8+ lymphocytes is not fully understood. It is believed that CD8+ lymphocytes have the ability of lysis and apoptosis of alveolar epithelial cells through the secretion of perforins, granzyme-B and TNF-a. There is a correlation of the CD8+ lymphocytes with increased apoptosis of alveolar cells in emphysema (23).

Remarkable role in COPD inflammation are playing dendritic and epithelial cells. Dendritic cells play a central role in initiating immune response activating a variety of inflammatory and immune cells including macrophages, B and T-lymphocytes and neutrophils (24). They form a rich network into airways and lung parenchyma, which is located near the surface, so that it can distinguish inhaled toxic substances (25,26). The epithelial cells of the airways and alveoli are a major source of inflammatory mediators and proteases. Triggered by smoking, they secrete various factors such as TNF-a, the TGF-b, interleukins IL-1b and IL-8 and GM-CSF (granulocyte-macrophage colony stimulating factor), which cause activation of fibroblasts and small airways fibrosis (27-29). The epithelial cells contribute to the defense of the body through the production of mucus which traps bacteria and through substances, such as defensins and cationic proteins, which have antimicrobial properties (30,31). The effect of harmful factors on their surface results in their squamous metaplasia, disruption of their action and the submucosal gland and goblet cell metaplasia (13).

Finally, dominant position in the pathophysiology of COPD holds oxidative stress which is installed when the production of active oxygen radicals overcomes the antioxidant defense mechanisms of the body. This results in serious damage to lipids, proteins and cell DNA (disturbed protease-antiprotease balance) (32-34). The reactive oxygen species are produced by cells such as neutrophils, macrophages, eosinophils and epithelial cells, when activated by the inflammation in the respiratory tract (33). Main indicators of oxidative stress in the body are the hydrogen peroxide (H_2O_2) and 8-isoprostaglandin F2a (8-isoprostane) which is elevated in exhaled air (35-39).

Assessment of airway inflammation was performed until recently only with invasive techniques such as the bronchoalveolar lavage (BAL), or intrabronchial and transbronchial biopsy. However, such techniques are not always feasible to use nor are measures of daily clinical practice (40). The need for noninvasive assessment of airway inflammation is imperative, since inflammatory airway diseases, are usually characterized by variation in their clinical presentation throughout their course. Moreover, there is an increasing trend for assessing the inflammatory pattern of inflammatory airway diseases through mediators measured by noninvasive techniques (41). Markers in biological fluids and exhaled air have been the subject of intense evaluation over the past few years, with some of them reaching their introduction into clinical practice, while others remain as research tools (42). The most studied and used non-invasive techniques are induced sputum, biomarkers measurement in exhaled air [mainly represented by exhaled nitric oxide (NO)] and exhaled breath condensate (EBC) (40).

Induced sputum is a noninvasive technique that enables clinical researchers to elucidate the course of several inflammatory airway infections, including COPD. Its main advantage lies in making, processing and analysis fully thought out methodology. Inhalation of isotonic or hypertonic solutions administered by nebulisation has been demonstrated to induce a small amount of airway secretion that can be expectorated and analyzed. The

Table 1. Inflammatory process of chronic obstructive pulmonary disease (COPD).

Inflammation cells	Stimulators	Inflammatory mediators	Increased inflammatory mediators
Macrophages	Inhaled irritants, dendritic cells	TNF-a, IL-6, IL-8, MCP-1, LTB4 and reactive oxygen species, MMP-9, MMP12	Oxidative stress ↑H_2O_2 ↑8-isoprostane
CD8+ T lymphocytes	Inhaled irritants, dendritic cells	Perforins, grazyme-B, TNF-a	
Dendritic cells	Inhaled irritants	Macrophages, B and T-lymphocytes and neutrophils	
Epithelial cells	Inhaled irritants	TNF-a, the TGF-b, interleukins IL-1b and IL-8 and GM-CSF	
Neutrophils	Macrophages, dendritic cells	Neutrophil elastase, cathepsin-G, proteinase-3	

mechanisms by which this occurs are not known, but both direct and indirect mechanisms are likely to be involved. It is believed that the increased osmolarity of the airway lining fluid increases vascular permeability in bronchial mucosa and induces production of mucus by submucosal glands (43). Induced sputum is a reproducible method, sensitive and reliable whose value is not limited to detection of sputum inflammatory mediators. The main clinical use is associated with the recognition of eosinophilic inflammation and the utilization of eosinophils as a tool for guiding treatment and monitoring the course of patients with asthma and COPD (44,45).

The main and most important representative of the measured biomarkers in exhaled air is the fraction of exhaled nitric oxide (FeNO), which is the most studied biomarker today. NO in orally exhaled air mainly originates from the respiratory epithelium. NO is produced by inducible NO synthase (iNOS), which is regulated by signal transducer and activator of transcription (STAT)-1 under the influence of homeostatic interferon-c (46). The main limitation to the use of FeNO lies in the fact that smoking has a negative effect on its concentration. The main application is in asthma which provides fast information on eosinophilic inflammation in the airways of asthmatic individuals. It was also recognized early on that FeNO is increased in at least some subgroups of patients with COPD (47,48). It was later suggested that this increase correlated with bronchial reversibility to salbutamol and thus could represent an asthma-like component of COPD (49). In recent years is investigated the potential role of FeNO in predicting response to treatment in acute exacerbations of COPD, and the likelihood that FeNO be an indicator of treatment response to corticosteroids in patients with COPD (50-53).

The assessment of markers of airway inflammation/oxidative stress in EBC has been suggested as a simple, rapid, safe and noninvasive technique that is suitable for assessment of airway inflammation in humans of all ages (41,54-56). EBC is formed by cooling of exhaled breath of patients in a condenser system (54,55). Whereas volatile mediators of airway inflammation/oxidative stress are mainly recovered in the gas phase of exhaled breath, nonvolatile biomarkers from the epithelial lining fluid of the airway epithelium are released in aerosol particles, which can be measured in EBC (54,55). Several markers in EBC can be assessed simultaneously. Potentially, noninvasive biomarkers in EBC may be of help in the discrimination between different inflammatory phenotypes, the assessment of the severity of airway inflammation, to monitor treatment and disease control, and to predict disease exacerbations at an early stage (41,54,56). Besides these potential "pearls" of EBC measurements, there are a number of pitfalls: (I) the concentration of markers is low, thus ultrasensitive techniques are required for analysis; (II) many factors may have an influence on biomarkers in EBC (Table 1); and (III) there is variation in results between different centers. Molecules that are measured in EBC are H_2O_2, derivatives of NO, prostanoids (prostaglandins and thromboxanes), leukotrienes, 8-isoprostane, EBC ph and other biomarkers such as cytokines (interleukins and TNF-a) and various proteins (56,57).

The main therapeutic option for the treatment of chronic inflammation of asthmatic patients is corticosteroids. However, despite proven inflammatory background of COPD, corticosteroids do not seem to have any effect on disease's inflammation. No changes in neutrophilic inflammation and no decrease in inflammatory mediators and proteases are observed after their use. There is an opposite effect of corticosteroids in granulocytes with decreased survival of eosinophils and prolongation of life of the neutrophils. This has led to the failure of treatment to stop the course of COPD and demonstrates the need for discovery of new therapeutic interventions (13).

The need for noninvasive evaluation of airway inflammation and the discovery of targeted therapeutic interventions is imperative since COPD is characterized by variable clinical manifestations during the course of and resistance to standard

anti-inflammatory therapy. The biomarkers in biological fluids and exhaled air were the subject of intense study in previous years and others than those used today in clinical practice and others are still under investigation. The central question is whether the information obtained from measurements of inflammatory mediators currently fulfills the conditions required to characterize them as appropriate biomarkers. An imaginary biomarker should be evaluated by a standard protocol, must have an acceptable reproducibility, should demonstrate the specificity of the disease and must have the ability to detect either changes attributable to therapeutic interventions or changes in the health status (e.g., an exacerbation of the disease). With the techniques available today is difficult to detect imaginary biomarker that meets the above conditions (58).

COPD is a heterogeneous entity that includes a variety of obstructive diseases that differ considerably on their mechanisms of action and response to treatment. Hence the need to recognize the different phenotypes of the disease within the range of these syndromes is vital for patient management. The ideal approach is not measuring the levels of a particular biomarker, but the effort to recognize the specific phenotype associated with specific biomarker and underlying pathophysiology. The combination, in fact, of different biomarkers can approach more effectively the classification of a particular phenotype. The knowledge of the pathophysiology of epidemic diseases, such as COPD, is essential for the management of patients and to evaluate the effects of new treatments for them and non-invasive diagnostic techniques help in this direction. Based on the current literature shows that combining different non-invasive diagnostic methods by which samples are taken from different compartments of the airways is possible to assess in detail the different mediators of inflammation and to monitor the effectiveness of treatment. In the future, the exploitation of this specialized monitoring of inflammation may allow each phenotype of the disease to have a characteristic profile of exhaled biomarkers and studying the effects of therapeutic interventions in these biomarkers may lead to targeted therapy based on phenotype and this is perhaps the future of therapeutics in COPD (58).

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