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Review

Biomarkers of *in vivo* fluorescence imaging in allergic airway inflammation

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ABSTRACT

Airway inflammation is a central component of the manifestation of asthma but is relatively inaccessible to study. Current imaging techniques such as X-ray CT, MRI, and PET, have advanced noninvasive research on pulmonary diseases. However, these techniques mainly facilitate the anatomical or structural assessment of the diseased lung and/or typically use radioactive agents. *In vivo* fluorescence imaging is a novel method for noninvasive, real-time, and specific monitoring of lung airway inflammation, which is particularly important to gain a further understanding asthma. Compared to conventional techniques, fluorescent imaging has the advantages of rapid feedback, as well as high sensitivity and resolution. Recently, there has been an increase in the identification of biomarkers, including matrix metalloproteinases, cathepsins, selectins, folate receptor-beta, nanoparticles, as well as sialic acid-binding immunoglobulin-like lectin-F to assess the level of airway inflammation in asthma. Recent advances in our understanding of these biomarkers as molecular probes for *in vivo* imaging are discussed in this review.

Contents

1. Introduction

Asthma is a common chronic inflammatory disease characterized by a variable degree of bronchial obstruction, airway hyperresponsiveness and mucus hypersecretion. More than 300 million people worldwide suffer from asthma and this number is growing steadily [1]. Therefore, asthma is still a major public health concern that affects the quality of life and causes a huge financial and social burden. Chronic allergic airway inflammation is the central component of asthma. The airways exhibit a strong or untimely contraction reaction (airway hyperresponsiveness) to various stimulating factors due to a long-term local inflammatory responses [2]. Finally, the continuous chronic injury and repair process in the airways is characterized by changes in the composition,

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quantity, and structure of cells and molecules referred to as irreversible airway remodeling [3]. Therefore, all of the clinical manifestations of asthma, including the onset, progress, treatment, and prognosis of the disease are directly or indirectly related to the changes in the levels of the airway inflammation. Controlling the degree of airway inflammation is the primary goal of asthma treatment. Evaluation of airway inflammation is an important means of asthma management and, of great significance for determining the treatment options [4].

Studies in humans have enabled us to extend our understanding of allergic airway inflammation, however, technical and ethical difficulties associated with *in vivo* human studies have limited research aimed at elucidating the inter-relationship between the main cell types involved in the pathology of the airway disease and their role in disease development [5]. As a result, the widespread use of animal models has greatly contributed to our understanding of the cellular and molecular pathways underlying human allergic asthma [6].

Until recently, techniques routinely used in humans to diagnose asthma are lung function tests, CT, exhaled nitric oxide (FeNO), as well as blood and sputum eosinophil count. However, pulmonary function tests are insensitive and often normal or unchanging in patients [7], and the CT of most asthma patients are also normal. Similarly, FeNO, levels have not exhibited a superiority for the treatment of asthma compared with clinical symptoms and spirometry/peak flow and cannot be routinely recommended for clinical practice [8]. In patients with asthma, blood and sputum eosinophil counts are often, but not always, elevated [9]. The best characterized new asthma biomarkers derived from a variety of biological sources including exhaled gases, induced sputum, serum and urine have been summarized elsewhere [10], are not discussed here. Likewise, we often use invasive or terminal procedures, such as pulmonary function, bronchoalveolar lavage (BAL), and histology of excised tissue, to assess airway inflammation in animal models of asthma, particularly in rodents and guinea-pigs [11]. Although these animal models provide valuable insight into inflammatory cell and mediator changes in the context of asthma, they are terminal procedures that preclude the possibility of a repeated, longitudinal assessment of test subjects and are not in real-time [12,13]. Current imaging techniques, such as X-ray CT, MRI, and PET, have advanced noninvasive research on pulmonary diseases [14]. However, these techniques mainly facilitate the anatomical or structural assessment of the diseased lung and/or typically involve the use of radioactive agents. In contrast, optical molecular imaging provides a novel, more specific approach which can perform a real-time assessment of inflammation by combining specific biological molecules with optical sensors *in vivo*. Presently, matrix metalloproteinases (MMPs), cathepsins, selectins, folate receptor-beta (FR-β), sialic acid-binding immunoglobulin-like lectin-F (Siglec-F), and nanoparticles are biomarkers for airway inflammation. In addition, the use of optical probes with near-infrared fluorescence (NIRF) allows for improved photon penetration through tissue and minimizes the effects of tissue autofluorescence, resulting in increased sensitivity [15,16]. Importantly, NIRF imaging lacks radioactivity and is therefore considered to be an alternative to nuclear imaging, the current gold standard for clinical functional imaging [17]. The development of near-infrared fluorophores and nanomaterials over the past decade has facilitated the translation of fluorescence imaging from the microscopic (e.g., epifluorescence, confocal and multifocal microscopy, and mesoscopic optical projection tomography) to macroscopic imaging (e.g., fluorescence molecular tomography and, fluorescence reflectance imaging) [15]. Therefore, all of these biomarkers are NIRF-labeled probes. Recent advances in fluorescence imaging have been reviewed elsewhere in-depth [18]. This short review is not intended to be a comprehensive discussion of all aspects of *in vivo* fluorescence imaging, but instead focuses on the application of imaging probes during allergic inflammation.

2. Proteinases

2.1. MMPs

MMPs are secreted by a variety of pro-inflammatory cells, including neutrophils, macrophages, eosinophils, and mast cells that are characteristically present in asthma. MMPs are composed of 20 types of highly conserved zinc and calcium-dependent protein enzymes and can degrade most of the proteins in the extracellular matrix [19]. MMPs play a significant role during different stages of asthma and are closely linked to the pathogenesis of the disease. MMPs can also degrade tissue extracellular matrix; promote the recruitment, proliferation, and survival of inflammatory cells; and participate directly in bronchoconstriction and airway remodeling [14]. In addition, MMPs may play a role in the loss of lung elastic recoil exhibited in a subset of patients with chronic persistent asthma [20]. Of the MMPs, MMP-2 and MMP-9 are of particular relevance to asthma. Genetic knockout animal models have demonstrated that MMP-2 and MMP-9 are important in asthma pathogenesis [21,22]. Moreover, studies have confirmed that the increase of MMP2/9 is associated with asthma, and the concentrations of MMP-9 correlate with the number of neutrophils in the airways [14,23–27]. Based on these preceding findings, MMPs can serve as possible biomarkers to detect airway inflammation. Cortez et al. [14] described the use of an injectable MMP-targeted optical sensor that specifically and quantitatively resolved the eosinophil activity in asthma. In addition, Csilla et al. [28] applied MMP-targeted molecular imaging to detect the level of airway inflammation of asthma. The elevated *in vivo* MMP-2/9 protease activity corresponded to the areas of high inflammation surrounding the airways. The elevated activity was also maintained throughout the early stages of airway remodeling, and the elevated levels of MMP-2/9 in murine asthma were in agreement with those generated in the studies by Kumagai et al. [29] and Kim et al. [30].

Studies on the use of MMPs as molecular imaging biomarkers of asthma are abundant. Nevertheless, the immediate application of the current fluorescence design to clinical use requires administering fiberoptic bronchoscopy to detect asthma in the clinic, which can be applied to image up to the fourth-order bronchi. Optical sensors may also detect inflammation unrelated to eosinophils or other inflammatory cells. Their insufficient specificity in asthma, however, limits the clinical application.

2.2. Cathepsins

Cathepsins are the primary members of the cysteine protease family, the largest subfamily among the proteases [31]. More than 20 cathepsins have been discovered in living organisms, including 11 human cathepsins, which are related to many human diseases, such as tumors, osteoporosis and arthritis. Cathepsins have recently received much attention as important drug targets. Eosinophilia is the main feature of asthma inflammation. Cathepsins are active in the intracellular lysosomes of inflammatory eosinophils, providing intracellular accumulation of the imaging agent and, thus, a selective quantification of eosinophils [32]. Cathepsins could provide greater specificity in analysis than MMPs since: (1) cathepsins are present within the cell, which permits the specific internalization of the imaging agent in cells, whereas MMPs are primarily secreted into the environment outside the cell; and (2) MMPs also contribute towards the pathogenesis of asthma due to the role in eosinophil functionality, which can complicate the analysis the results from noninvasive imaging procedures [33]. Korideck et al.