



Review

Biomarkers of *in vivo* fluorescence imaging in allergic airway inflammationFa-Ping Wang^a, Ying-Qi Fan^a, Su-Yun Li^b, Hui Mao^{a,*}^a Department of Respiratory Medicine, West China Hospital, Sichuan University, Chengdu 610041, China^b Department of Respiratory Medicine, First Affiliated Hospital of Henan College of Traditional Chinese Medicine, Zhengzhou 450000, China

ARTICLE INFO

Article history:

Received 21 October 2015

Received in revised form

17 February 2016

Accepted 17 February 2016

Available online 20 February 2016

Keywords:

Asthma

Fluorescence imaging

Airway inflammation

Biomarkers

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.

© 2016 Elsevier Ltd. All rights reserved.

Contents

1. Introduction	100
2. Proteinases	101
2.1. MMPs	101
2.2. Cathepsins	101
3. Selectins	102
4. FR- β expression on lung macrophages	102
5. Nanoparticles	102
6. Siglec-F expression on eosinophils	103
7. Summary	103
References	103

1. Introduction

Asthma is a common chronic inflammatory disease characterized by a variable degree of bronchial obstruction, airway hyper-responsiveness 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,

* Corresponding author.

E-mail address: merrymh@126.com (H. Mao).

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.

successfully used quantitative fluorescence molecular tomography paired with a cathepsin-activated imaging agent (ProSense 680) to noninvasively visualize pulmonary eosinophilia in mice. This study reported an excellent correlation between BAL eosinophilia and the assessment of ProSense 680 in quantifying disease severity [33].

3. Selectins

Selectins, are type I membrane molecules that bind carbohydrates and are involved in the recruitment of eosinophil, leukocyte-endothelial, and cell-platelet specific adhesion processes [34]. Selectins decisively contribute to the pathology of asthma because they mediate the migration of inflammatory cells and promote the recruitment of inflammatory and airway hyperresponsiveness [35]. Selectin family members consist of L-selectin, E-selectin, and P-selectin, in which L, E, and P represent the initial obtainment of these three selectins on leukocytes, endothelial cells, and platelets, respectively. L-selectin was discovered on lymphocytes as a homing receptor and was later found to be expressed on a variety of other leukocytes. P-selectin is stored in alpha-granules of platelets and endothelial cells and is translocated to the cell surface of activated endothelial cells and platelets [36]. Selectin-mediated adhesion of leukocytes to the vascular endothelium is a critical early event in the initiation of the inflammatory response [37]. L-selectin appears to play a key role in inducing endothelial leukocyte accumulation and the homing of inflammatory cells to sites of airway inflammation [38]. Pitchford et al. showed that P-selectin plays an important role in pulmonary eosinophil recruitment [39]. Momose et al. demonstrated that L-selectin played a crucial role in the infiltration of eosinophils, and dexamethasone and theophylline significantly decreased L-selectin expression on eosinophils [40]. Research has also indicated that L-selectin-deficient mice have significantly abrogated airway hyperresponsiveness [41]. The role of selectin-ligand interactions in allergic asthma is well established, making them an attractive target for the visualization of inflammation [41,42]. Biffi et al. utilized a dendritic polyglycerol sulfate (dPGS) near infrared fluorescent dye conjugate known to bind to L and P-selectin and C3/C5 complement factors to monitor the inflammation sites in an allergic asthma mouse model [43]. They found that the fluorescence intensity ratio between the lung areas of asthmatic and healthy mice was four times higher for the dPGS in comparison to the free dye *in vivo* after intravenous administration.

Compared with the classical protease probe, selectins have the advantage of rapid uptake *in vivo* [43]. For example, a significant fluorescence difference is exhibited between the asthmatic lung and healthy groups a few hours following probe injection. However, the MMPSense and ProSense probes, which are activated in the presence of inflammation-associated enzymes, such as cathepsin and MMP present in the lungs, demonstrate a maximal fluorescence intensity within the lungs approximately 24 h after probe injection. Moreover, the activated MMPSense and ProSense probes do not remain at the site of interest for a long time. Instead, they produce a strong liver signal owing to their excretion pathway. On the other hand, L-selectin/P-selectin-targeting imaging is not specific for allergic airway inflammation since that it can also visualize the extent of inflammation in arthritis [44].

4. FR- β expression on lung macrophages

Type 2 helper T cells (Th2) secrete cytokines that regulate the synthesis of allergen-specific IgE cause airway hyper-reactivity, stimulate mast cell infiltration, promote pulmonary eosinophilia, and induce the accumulation of alternatively activated macrophages (AAMs) in the lungs during the inflammatory process in asthma [45]. A wealth of data indicates that AAMs are involved in

the pathogenesis of asthma. In particular, arginase-1 (Arg1) and acidic mammalian Chitinase (AMCase), molecular markers of AAMs, are both markers for asthma [46,47]. Treatment with either an inhibitor of Arg1 or a neutralizing antibody to AMCase has been found to ameliorate the symptoms of asthmatic mice [47,48]. The chemokines specifically released by AAMs also attract Th2 polarized T cells, eosinophils, and basophils into asthmatic lung tissue. Studies have found that the adoptive transfer of AAMs into the lungs of sensitized mice increases the influx of myeloid dendritic cells and thereby exacerbates airway inflammation [49].

FRs are cell surface receptors that bind and transcribe folate folic acid derivatives and exist as three main subtypes *in vivo*. FR- β expression is restricted primarily to the placenta and white blood cells of myeloid lineage, including AAMs [50]. Although it can be detected on both CD34 + bone marrow cells and normal human neutrophils, the receptor on these cells is functionally inactive and unable to bind folate or folate-linked drugs [51,52]. The upregulation of a functional FR- β on AAMs is assumed to be intrinsically involved in the development of asthma. Thus, folate-linked imaging agents can be specifically targeted to these FRs to depict the lung inflammation in asthma. Shen et al. [53] successfully detected the allergic inflammatory process underlying asthma by targeting FR- β . However, the molecular imaging of lung diseases particularly asthma, using FR- β has inherent limitations. For example, folate conjugate uptake can also be observed in the liver as a consequence of FR- β expression on AAMs or Kupffer cells [54]. Functional FR- β is identified on AAMs that accumulate in autoimmune diseases, such as rheumatoid arthritis [55] and bacterial infections [56]. Hence, folate conjugate uptake is not specific to asthma inflammation.

5. Nanoparticles

NIRF imaging agents can be divided into two groups: 1) small-molecule agents and 2) nanoparticle-based probes. Nanoparticles are novel fluorescent probes that can enhance tissue contrast or can identify specific biological changes. They are defined as a class of colloidal materials having physical dimensions in the sub-100 nm range [57]. This field of study has advanced rapidly in recent years, due to the capabilities to target tissues or different cell types *in vivo*. Compared with conventional molecular probes, including small organic dyes and fluorescent proteins, fluorescent nanoparticles based on aggregation-induced emission fluorogens have shown significant advantages in tunable emission and brightness, good biocompatibility, superb photo and physical stability, potential biodegradability and facile surface functionalization [58]. A combination of fluorescence imaging and nanoparticles can overcome many of the individual limitations and have a high sensitivity and resolution.

Alveolar macrophages (AMs) are the most prominent sentinel immune cells in the defense of the respiratory tract from airborne pathogens and have been implicated in the development and progression of asthma [59]. AMs are recruited to the airways during asthma, suggesting an important role for these cells in asthma pathogenesis. New investigations indicate that AMs may play central roles in promoting airway inflammation, particularly in asthma that is resistant to steroid therapy [60]. Asthma is a T-helper (Th)2-oriented disease, in which Th2 cytokines skew macrophage differentiation into AMs with an M2 phenotype [56]. The enhanced presence of M2 macrophages in asthma are not surprising given the Th2-driven lung environment during asthma [61]. However, it is not yet clear whether they actively contribute to asthma pathogenesis [62]. Since nanoparticles can be recognized by phagocytic cells and consequently accumulate in macrophages [63], a recent study firstly used NIRF-loaded in combination with fluorescence reflectance imaging for successfully distinguishing healthy mice

and mice with allergic lung inflammation *in vivo*. Additionally, verified Itrybe nanoparticles were phagocytosed by alveolar M2 macrophages in the peribronchial and alveolar areas [64]. However, nanoparticles are foreign substances and, for human application, factors such as toxicity or biocompatibility must be broadly considered for long-term effects. Further studies are needed to undergo rigorous toxicological examination before incorporation into clinical practice.

6. Siglec-F expression on eosinophils

Siglecs are a family of single-pass transmembrane cell surface proteins that are predominantly found on leukocytes [65]. To date, 15 and 9 Siglecs have been discovered in humans and mice, respectively [66]. Previous studies have shown that Siglec-8 is exclusively expressed on eosinophils, and its ligation with monoclonal antibodies could specifically reduce eosinophil viability through the induction of apoptosis [67]. Siglec-F is a functional homolog of the human Siglec-8. Both proteins preferentially recognize a sulfated glycan ligand closely related to sialyl Lewis X. Tateno et al. found that mouse Siglec-F also preferentially recognized by 6'-sulfo-sLex as a glycan ligand identical to Siglec-8 [68]. Zimmermann et al. [69] discovered that the administration of a single dose of Siglec-F antibody resulted in a rapid reduction in eosinophils in the blood and peripheral tissues. The induction of allergic lung inflammation in mice also caused the upregulation of Siglec-F on blood and bone marrow eosinophils, as well as the quantitative upregulation of endogenous Siglec-F ligands in the lung tissue and airways [70]. Furthermore, one study found that the application of an NIRF-labeled anti-Siglec-F antibody in fluorescence imaging could significantly differentiate asthma from healthy lung tissues and assess the treatment response of dexamethasone [71].

The direct binding of Siglec-F to eosinophils overcomes the deficiencies that the above mentioned biological probes may present by binding to other sites of inflammation not specific to the lung. However, Siglec-F expression is not exclusive to eosinophils, but also binds to AMs [72]. Moreover, recent reports have demonstrated that Siglec-F functions differently in animals and humans. For example, Mao et al. showed that Siglec-F-mediated apoptosis differed in magnitude and underlying mechanism in mice from Siglec-8-mediated human eosinophil apoptosis [73]. However, there are other biomarkers, such as CCR3, EMBP, CD23, CD48, and CD147, which are considered useful as potential *in vivo* targeting probes. They also bind to other immune cells, such as mast cells or Th2 cells, and might affect the course of the disease. Thus, their application is limited.

7. Summary

Fluorescence imaging has the potential to increase the sensitivity and specificity of the airway to guide diagnostics. A crucial part of this review consists of in the development of novel molecular imaging probes that can highlight more specific biomarkers of allergic airway inflammation. These biomarkers can be targeted probes for allergic airway inflammation for mainly two reasons: 1) the most important are that the *in vivo* administration of NIRF imaging agents is efficient delivery and/or activation of the agent in the tissue of lung; 2) and have no toxic threat to living animals. All the abovementioned specific probes, such as MMPs, cathepsins, selectins, FR- β , Siglec-F and Itrybe nanoparticles can detect and help evaluate airway inflammation, but none of these biomarkers are without problems and limitations. Among them, Siglec-F serves as the most specific biomarker for allergic lung inflammation. Unfortunately, they have not yet been

used in humans until now, although they work well and were effective in preclinical models. There are some limitations in the translation from animals into the clinic. For example, these biomarkers have been reported to monitor allergic lung inflammation successfully in animal models, but no animal model completely recapitulates all features of the human disease [74]. Animal models of allergic airway disease based on natural or active sensitization to allergens have been reported in mice [75], rats [76], guinea pigs [77], cats [78], dogs [79], pigs [6], sheep [80], rabbits [81], horses [82], and non-human primates [83]. It must be recognized that most of the animal models do not develop "asthma" (only cats and horses can spontaneously develop a condition physiologically similar to asthma [74]), they exhibit an asthma-like syndrome that is similar to the disease in humans. This fails to model the etiology and natural history of human asthma, which develops over time through multistep processes [84]. Therefore, some species differences including anatomical, physiological and immunological responses of human asthma and animal models must be considered. Problems arise due to the different way that lung function is monitored in animals and humans, which might produce different results. Moreover, validating the toxicity associated with small-molecule and nanomaterial-based probes is also critical for the translation of these materials to the clinic [15]. Further studies are needed to safety and dose finding before human studies, especially for nanoparticles. Despite this, optical imaging provides relatively inexpensive and non-harmful methods and is preferred over other imaging methods used in preclinical research and drug development. Furthermore, NIRF imaging is already used daily in the ophthalmology clinic, and there is no doubt that pulmonary clinical use will result from the ongoing advances in near-infrared fluorophore chemistry and optical engineering [85]. Biomarkers of fluorescence imaging hold considerable promise for advancing personalized medicine. In the future, further research is necessary to establish the specific contributions of different biomarkers in differentiating asthma phenotypes and in monitoring airway inflammation and treatment responses. Taken together, fluorescence imaging of biomarkers provides a novel, noninvasive, specific, quantitative, and real-time approach to monitoring airway inflammation in asthma. Although it is still in the preclinical phase, this imaging technique has the potential to expand the horizons of asthma research.

References

- [1] The Global Asthma Report 2014, Global Asthma Network, Auckland, New Zealand, 2014.
- [2] J. Borak, R.Y. Lefkowitz, Bronchial Hyperresponsiveness, *Occup Med (Lond)*, 2015 kvq158.
- [3] R.J. Martin, Therapeutic significance of distal airway inflammation in asthma, *J. Allergy Clin. Immunol.* 109 (2002) S447–S460.
- [4] H.Y. Kim, R.H. DeKruyff, D.T. Umetsu, The many paths to asthma: phenotype shaped by innate and adaptive immunity, *Nat. Immunol.* 11 (2002) 577–584.
- [5] P.M. O'Byrne, G.M. Gauvreau, J.D. Brannan, Provoked models of asthma: what have we learnt? *Clin. Exp. Allergy* 32 (2009) 181–192.
- [6] J.E. Allen, R.J. Bischof, H.Y.S. Chang, et al., Animal models of airway inflammation and airway smooth muscle remodelling in asthma, *Pulm. Pharmacol. Ther.* 22 (2009) 455–465.
- [7] D.R. Taylor, Biomarkers of inflammation in asthma: a clinical perspective, *Semin. Respir. Crit. Care Med.* 33 (2012) 620–629.
- [8] H.L. Patsky, C.J. Cates, A. Li, et al., Tailored Interventions Based on Exhaled Nitric Oxide versus Clinical Symptoms for Asthma in Children and Adults, *Coche. Lib.*, 2009.
- [9] S.J. Szefler, S. Wenzel, R. Brown, et al., Asthma outcomes: biomarkers, *J. Allergy Clin. Immunol.* 129 (2012) S9–S23.
- [10] F. Fatemi, E. Sadroddiny, A. Gheibi, et al., Biomolecular markers in assessment and treatment of asthma, *Respirology* 19 (2014) 514–523.
- [11] E.H. Walters, D.W. Reid, D.P. Johns, C. Ward, Nonpharmacological and pharmacological interventions to prevent or reduce airway remodeling, *Eur. Respir. J.* 30 (2007) 574–588.
- [12] A.M. Holmes, R. Solari, S.T. Holgate, Animal models of asthma: value,

- limitations and opportunities for alternative approaches, *Drug Discov. Today* 16 (2011) 659–670.
- [13] C.Y. Yick, J.H. von der Thüsen, E.H. Bel, et al., In vivo imaging of the airway wall in asthma: fibered confocal fluorescence microscopy in relation to histology and lung function, *Respir. Res.* 12 (2011) 85.
 - [14] V. Cortez-Retamozo, F.K. Swirski, P. Waterman, et al., Real-time assessment of inflammation and treatment response in a mouse model of allergic airway inflammation, *J. Clin. Invest.* 118 (2008) 4058.
 - [15] S.A. Hilderbrand, R. Weissleder, Near-infrared fluorescence: application to in vivo molecular imaging, *Curr. Opin. Chem. Biol.* 14 (2010) 71–79.
 - [16] S.M. Leonardo, M.M. Harnett, A.F. Lerch-Gaggl, et al., Fluorescence-based assays as tools for understanding immunologic processes, *Ann. Allergy Asthma Immunol.* 102 (2009) 84–90.
 - [17] M.A. Markus, C. Dullin, M. Mitkovski, et al., Non-invasive optical imaging of eosinophilia during the course of an experimental allergic airways disease model and in response to therapy, *PLoS One* 9 (2014) e90017.
 - [18] J. Rao, A. Dragulescu-Andrasi, H. Yao, Fluorescence imaging in vivo: recent advances, *Curr. Opin. Biotechnol.* 18 (2007) 17–25.
 - [19] W. Mattos, S. Lim, R. Russell, et al., Matrix metalloproteinase-9 expression in asthma: effect of asthma severity, allergen challenge, and inhaled corticosteroids, *Chest* 122 (2002) 1543–1552.
 - [20] E.A. Kelly, N.N. Jarjour, Role of matrix metalloproteinases in asthma, *Curr. Opin. Pulm. Med.* 9 (2003) 28–33.
 - [21] D.D. Cataldo, K.G. Tournoy, K. Vermaelen, et al., Matrix metalloproteinase-9 deficiency impairs cellular infiltration and bronchial hyperresponsiveness during allergen-induced airway inflammation, *Am. J. Pathol.* 161 (2002) 491–498.
 - [22] K.Y. Vermaelen, D. Cataldo, K. Tournoy, et al., Matrix metalloproteinase-9-mediated dendritic cell recruitment into the airways is a critical step in a mouse model of asthma, *J. Immunol.* 171 (2003) 016–1022.
 - [23] E.A. Becky Kelly, W.W. Busse, N.N. Jarjour, Increased matrix metalloproteinase-9 in the airway after allergen challenge, *Am. J. Respir. Crit. Care Med.* 162 (2000) 1157–1161.
 - [24] D.D. Cataldo, J. Bettiol, A. Noël, et al., Matrix metalloproteinase-9, but not tissue inhibitor of matrix metalloproteinase-1, increases in the sputum from allergic asthmatic patients after allergen challenge, *Chest* 122 (2002) 1553–1559.
 - [25] K.S. Lee, S.M. Jin, H. Lee, et al., Imbalance between matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 in toluene diisocyanate-induced asthma, *Clin. Exp. Allergy* 34 (2004) 276–284.
 - [26] K.J. Greenlee, Z. Werb, F. Kheradmand, Matrix metalloproteinases in lung: multiple, multifarious, and multifaceted, *Physiol. Rev.* 87 (2007) 69–98.
 - [27] S.J. McMillan, J. Kearley, J.D. Campbell, et al., Matrix metalloproteinase-9 deficiency results in enhanced allergen-induced airway inflammation, *J. Immunol.* 172 (2004) 2586–2594.
 - [28] C.N. Felsen, E.N. Savari, M. Whitney, et al., Detection and monitoring of localized matrix metalloproteinase upregulation in a murine model of asthma, *Am. J. Physiol. Lung Cell Mol. Physiol.* 306 (2014) L764–L774.
 - [29] K. Kumagai, I. Ohno, S. Okada, et al., Inhibition of matrix metalloproteinases prevents allergen-induced airway inflammation in a murine model of asthma, *J. Immunol.* 162 (1999) 4212–4219.
 - [30] D.Y. Kim, S.Y. Ryu, J.E. Lim, et al., Anti-inflammatory mechanism of simvastatin in mouse allergic asthma model, *Eur. J. Pharmacol.* 557 (2007) 76–86.
 - [31] F. Lecaille, J. Kaleta, D. Brömme, Human and parasitic papain-like cysteine proteases: their role in physiology and pathology and recent developments in inhibitor design, *Chem. Rev.* 102 (2002) 4459–4488.
 - [32] S. Conus, H.U. Simon, Cathespins and their involvement in immune responses, *Swiss Med. Wkly.* 140 (2010) w13042.
 - [33] H. Kordeck, J.D. Peterson, Noninvasive quantitative tomography of the therapeutic response to dexamethasone in ovalbumin-induced murine asthma, *J. Pharmacol. Exp. Ther.* 329 (2009) 882–889.
 - [34] E.T.M. Keelan, S.T. Licence, A.M. Peters, et al., Characterization of E-selectin expression in vivo with use of a radiolabeled monoclonal antibody, *Am. J. Physiol.* 266 (1994) H278–H290.
 - [35] K. Ley, The role of selectins in inflammation and disease, *Trends Mol. Med.* 9 (2003) 263–268.
 - [36] S.G. Royce, M. Lee, M.L.K. Tang, The contribution of L-selectin to airway hyperresponsiveness in chronic allergic airways disease, *J. Asthma Allergy* 3 (2010) 9–17.
 - [37] S.J. Romano, D.H. Slee, Targeting selectins for the treatment of respiratory diseases, *Curr. Opin. Investig. Drugs* 2 (2001) 907–913.
 - [38] J. Monteseirin, E. Llamas, H. Sánchez-Monteseirin, et al., IgE-mediated downregulation of L-selectin (CD62L) on lymphocytes from asthmatic patients, *Allergy* 56 (2001) 164–168.
 - [39] S.C. Pitchford, S. Momi, S. Giannini, et al., Platelet P-selectin is required for pulmonary eosinophil and lymphocyte recruitment in a murine model of allergic inflammation, *Blood* 105 (2005) 2074–2081.
 - [40] T. Momose, Y. Okubo, S. Horie, et al., Interferon-gamma increases CD62L expression on human eosinophils, *Int. Arch. Allergy Immunol.* 120 (1998) 30–33.
 - [41] S.G. Royce, M. Lee, M.L.K. Tang, The contribution of L-selectin to airway hyperresponsiveness in chronic allergic airways disease, *J. Asthma Allergy* 3 (2010) 9.
 - [42] E.R. Banerjee, Triple selectin knockout (ELP-/-) mice fail to develop OVA-induced acute asthma phenotype, *J. Inflamm. (Lond.)* 8 (2011) 19.
 - [43] S. Biffi, S. Dal Monego, C. Dullin, et al., Dendritic polyglycerolsulfate near infrared fluorescent (NIRF) dye conjugate for non-invasively monitoring of inflammation in an allergic asthma mouse model, *PLoS One* 8 (2013) e57150.
 - [44] N. Beziere, C. Schacky, Y. Kosanke, et al., Optoacoustic imaging and staging of inflammation in a murine model of arthritis, *Arthritis Rheumatol.* 66 (2014) 2071–2078.
 - [45] M. Peters-Golden, The alveolar macrophage: the forgotten cell in asthma, *Am. J. Respir. Cell Mol. Biol.* 31 (2004) 3–7.
 - [46] N. Zimmermann, N.E. King, J. Laporte, et al., Dissection of experimental asthma with DNA microarray analysis identifies arginase in asthma pathogenesis, *J. Clin. Invest.* 111 (2003) 1863.
 - [47] Z. Zhu, T. Zheng, R.J. Homer, et al., Acidic mammalian chitinase in asthmatic Th2 inflammation and IL-13 pathway activation, *Science* 304 (2004) 1678–1682.
 - [48] H. Maarsingh, A.B. Zuidhof, I.S.T. Bos, et al., Arginase inhibition protects against allergen-induced airway obstruction, hyperresponsiveness, and inflammation, *Am. J. Respir. Crit. Care Med.* 178 (2008) 565–573.
 - [49] B.N. Melgert, T.B. Oriss, Z. Qi, et al., Macrophages: regulators of sex differences in asthma? *Am. J. Respir. Cell Mol. Biol.* 42 (2010) 595–603.
 - [50] Y.G. Assaraf, C.P. Leamon, J.A. Reddy, The folate receptor as a rational therapeutic target for personalized cancer treatment, *Drug Resist. Updat.* 17 (2014) 89–95.
 - [51] J.A. Reddy, L.S. Haneline, E.F. Srouf, et al., Expression and functional characterization of the β -isoform of the folate receptor on CD34+ cells, *Blood* 93 (1999) 3940–3948.
 - [52] X.Q. Pan, X. Zheng, G. Shi, et al., Strategy for the treatment of acute myelogenous leukemia based on folate receptor β -targeted liposomal doxorubicin combined with receptor induction using all-trans retinoic acid, *Blood* 100 (2002) 594–602.
 - [53] J. Shen, V. Chelvam, G. Cresswell, et al., Use of folate-conjugated imaging agents to target alternatively activated macrophages in a murine model of asthma, *Mol. Pharm.* 10 (2013) 1918–1927.
 - [54] W. Xia, A.R. Hilgenbrink, E.L. Matteson, et al., A functional folate receptor is induced during macrophage activation and can be used to target drugs to activated macrophages, *Blood* 113 (2009) 438–446.
 - [55] M.J. Turk, G.J. Breur, W.R. Widmer, et al., Folate-targeted imaging of activated macrophages in rats with adjuvant-induced arthritis, *Arthritis Rheum.* 46 (2002) 1947–1955.
 - [56] W.A. Henne, R. Rothenbuhler, W. Ayala-Lopez, et al., Imaging sites of infection using a ^{99m}Tc -labeled folate conjugate targeted to folate receptor positive macrophages, *Mol. Pharm.* 9 (2012) 1435–1440.
 - [57] Y. Sheng, L. De Liao, N.V. Thakor, et al., Nanoparticles for molecular imaging, *J. Biomed. Nanotechnol.* 10 (2014) 2641–2676.
 - [58] L. Yan, Y. Zhang, B. Xu, et al., Fluorescent Nanoparticles Based on AIE Fluorogens for Bioimaging, *Nanoscale*, 2015.
 - [59] J. Balhara, A.S. Gounni, The alveolar macrophages in asthma: a double-edged sword, *Mucosal Immunol.* 5 (2012) 605–609.
 - [60] M. Yang, R.K. Kumar, P.M. Hansbro, et al., Emerging roles of pulmonary macrophages in driving the development of severe asthma, *J. Leukoc. Biol.* 91 (2012) 557–569.
 - [61] A.P. Moreira, C.M. Hogaboam, Macrophages in allergic asthma: fine-tuning their pro-and anti-inflammatory actions for disease resolution, *J. Interferon Cytokine Res.* 31 (2011) 485–491.
 - [62] P. Robbe, C. Draijer, T.R. Borg, et al., Distinct macrophage phenotypes in allergic and nonallergic lung inflammation, *Am. J. Physiol. Lung Cell Mol. Physiol.* 308 (2015) L358–L367.
 - [63] R.F. Minchin, D.J. Martin, Minireview: nanoparticles for molecular imaging—an overview, *Endocrinol* 151 (2010) 474–481.
 - [64] M.A. Markus, J. Napp, T. Behnke, et al., Tracking of inhaled near-infrared fluorescent nanoparticles in lungs of SKH-1 mice with allergic airway inflammation, *ACS nano* 12 (2015) 11642–11657.
 - [65] B.S. Bochner, Siglec-8 on human eosinophils and mast cells, and Siglec-F on murine eosinophils, are functionally related inhibitory receptors, *Clin. Exp. Allergy* 39 (2009) 317–324.
 - [66] P.R. Crocker, J.C. Paulson, A. Varki, Siglecs and their roles in the immune system, *Nat. Rev. Immunol.* 7 (2007) 255–266.
 - [67] E. Nutku, H. Aizawa, S.A. Hudson, et al., Ligation of Siglec-8: a selective mechanism for induction of human eosinophil apoptosis, *Blood* 101 (2003) 5014–5020.
 - [68] H. Tateno, P.R. Crocker, J.C. Paulson, Mouse Siglec-F and human Siglec-8 are functionally convergent paralogs that are selectively expressed on eosinophils and recognize 6'-sulfo-sialyl Lewis X as a preferred glycan ligand, *Glycobiology* 15 (2005) 1125–1135.
 - [69] N. Zimmermann, M.L. McBride, Y. Yamada, et al., Siglec-F antibody administration to mice selectively reduces blood and tissue eosinophils, *Allergy* 63 (2008) 1156–1163.
 - [70] M. Zhang, T. Angata, J.Y. Cho, et al., Defining the in vivo function of Siglec-F, a CD33-related Siglec expressed on mouse eosinophils, *Blood* 109 (2007) 4280–4287.
 - [71] M.A. Markus, C. Dullin, M. Mitkovski, et al., Non-Invasive Optical Imaging of Eosinophilia during the Course of an Experimental Allergic Airways Disease Model and in Response to Therapy, *PLoS One* 9 (2014) e90017.
 - [72] Y. Feng, H. Mao, Expression and preliminary functional analysis of Siglec-F on mouse macrophages, *J. Zhejiang Univ. Sci. B* 13 (2012) 386–394.
 - [73] H. Mao, G. Kano, S.A. Hudson, et al., Mechanisms of Siglec-F-induced

- eosinophil apoptosis: a role for caspases but not for SHP-1, Src kinases, NADPH oxidase or reactive oxygen, *PLoS One* 8 (2013) e68143.
- [74] J.H.T. Bates, M. Rincon, C.G. Irvin, Animal models of asthma, *Am. J. Physiol. Lung Cell Mol. Physiol.* 297 (2009) L401–L410.
- [75] R.A. Martin, S.R. Hodgkins, A.E. Dixon, et al., Aligning mouse models of asthma to human endotypes of disease, *Respirology* 19 (2014) 823–833.
- [76] T. Tschernig, D. Neumann, A. Pich, et al., Experimental bronchial asthma—the strength of the species rat, *Curr. Drug Targets* 9 (2008) 466–469.
- [77] F.L.M. Ricciardolo, F. Nijkamp, V.D. Rose, et al., The guinea pig as an animal model for asthma, *Curr. Drug Targets* 9 (2008) 452–465.
- [78] K. Mullane, M. Williams, Animal models of asthma: Reprise or reboot? *Biochem. Pharmacol.* 87 (2014) 131–139.
- [79] R.W. Chapman, Canine models of asthma and COPD, *Pulm. Pharmacol. Ther.* 21 (2008) 731–742.
- [80] W.M. Abraham, Modeling of asthma, COPD and cystic fibrosis in sheep, *Pulm. Pharmacol. Ther.* 21 (2008) 743–754.
- [81] S. Keir, C. Page, The rabbit as a model to study asthma and other lung diseases, *Pulm. Pharmacol. Ther.* 21 (2008) 721–730.
- [82] B. Herszberg, D. Ramos-Barbón, M. Tamaoka, et al., Heaves, an asthma-like equine disease, involves airway smooth muscle remodeling, *J. Allergy Clin. Immunol.* 118 (2006) 382–388.
- [83] M.R. Van Scott, J.L. Hooker, D. Ehrmann, et al., Dust mite-induced asthma in cynomolgus monkeys, *J. Appl. Physiol.* 96 (2004) 1433–1444.
- [84] A.M. Holmes, R. Solari, S.T. Holgate, Animal models of asthma: value, limitations and opportunities for alternative approaches, *Drug Discov. Today* 16 (2011) 659–670.
- [85] J.V. Frangioni, In vivo near-infrared fluorescence imaging, *Curr. Opin. Chem. Biol.* 7 (2003) 626–634.