Respiratory Medicine 210 (2023) 107168

Contents lists available at ScienceDirect

Respiratory Medicine

journal homepage: www.elsevier.com/locate/rmed

Original Research

Anatomical and histopathological approaches to asthma phenotyping

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ARTICLE INFO

Keywords:
Asthma
Anatomy
Phenotypes
Histology
Immunology
Alveolar
Bronchiolar
Inflammation

ABSTRACT

Asthma is typically characterized by variable respiratory symptoms and airflow limitation. Along with the pathophysiology and symptoms are immunological and inflammatory processes. The last decades research has revealed that the immunology of asthma is highly heterogeneous. This has clinical consequences and identification of immunological phenotypes is currently used to guide biological treatment. The focus of this review is on another dimension of asthma diversity, namely anatomical heterogeneity. Immunopathological alterations may go beyond the central airways to also involve the distal airways, the alveolar parenchyma, and pulmonary vessels. Also, extrapulmonary tissues are affected. The anatomical distribution of inflammation in asthma has remained relatively poorly discussed despite its potential implication on both clinical presentation and response to treatment. There is today evidence that a significant proportion of the asthma patients has small airway disease with type 2 immunity, eosinophilia and smooth muscle infiltration of mast cells. The small airways in asthma are also subjected to remodelling, constriction, and luminal plugging, events that are likely to contribute to the elevated distal airway resistance seen in some patients. In cases when the inflammation extends into the alveolar parenchyma alveolar FCER1-high mast cells, eosinophilia, type 2 immunity and activated alveolar macrophages, together with modest interstitial remodelling, create a complex immunopathological picture. Importantly, the distal lung inflammation in asthma can be pharmacologically targeted by use of inhalers with more distal drug deposition. Biological treatments, which are readily distributed to the distal lung, may also be beneficial in eligible patients with more severe and anatomically widespread disease.

1. Introduction

With >350 million patients worldwide asthma constitutes a global health burden [1]. Typical for the disease is variable airflow limitation and inflammatory processes. The best studied form of immunopathology is the classic allergic type 2 eosinophilic inflammation. However, the inflammation type may differ between patients [1,2]. Likewise, patients vary in terms of the distribution of inflammation and associated tissue disturbances. The inflammation is not restricted to the central airways and may also involve the small airways, or even the alveolar parenchyma [3,4]. Asthma should therefore be viewed as a broad umbrella term where both immunological and anatomical heterogeneity constitute a challenging complexity.

The purpose of this review is to provide an updated overview on asthma heterogeneity from an immunopathological and anatomical perspective. Focus will be on the latter issue that has remained relatively poorly discussed.

2. Anatomical asthma phenotypes

Methods to get insight into what immunological and inflammatory events operate inside the diseased tissue can be divided into direct or indirect approaches. Example of indirect measurements are analyses of lumen content (BAL and sputum), exhaled air, or peripheral blood. Approaches for direct visualization of what is occurring inside the diseased tissue include examinations of autopsies (fatal asthma) and bronchial biopsies. Transbronchial biopsies from asthma have only been collected in a handful research studies. This explains why the tissue inflammation distal to the central airways remains poorly characterized. However, there is today evidence that also distal airways as well as the alveolar parenchyma and extrapulmonary tissues are subjected to immune alterations and remodelling. There are thus several anatomical arenas for the inflammation in asthma and patient differences in engagement of these creates anatomical phenotypes (Table 1 and Fig. 1). Although this concept is important for understanding asthma heterogeneity, due to the relative few patients involved in histological studies,

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https://doi.org/10.1016/j.rmed.2023.107168
Received 3 October 2022; Received in revised form 16 February 2023; Accepted 16 February 2023
Available online 21 February 2023

the link between anatomical and clinical phenotypes has yet to be defined. Nonetheless, the anatomical distribution of inflammation in asthma is likely to have a significant impact on clinical presentation and response to treatment and thus deserves a broader recognition.

3. Immunopathology of the central airways

The bronchi are subjected to a wide range of immunological and inflammatory processes [3,5]. These in turn contribute to classical pathophysiological features of asthma such as airway hyperreactivity, bronchoconstriction, and plasma extravasation with formation of exudate and leukocyte-rich luminal plugs [6,7]. Associated with the inflammation is bronchial remodelling with key features being smooth muscle enlargement, thickening of the epithelial basement membrane (RBM), goblet cell hyperplasia, and altered extracellular matrix [8–11]. Obese patients may also have infiltration of adipose tissue within the bronchial wall [12].

The traditional picture of the bronchial inflammation in asthma is that of an eosinophilic inflammation [1,13] where the eosinophilia and associated remodelling are fuelled by type 2 cytokines (IL-5, IL-4, and IL-13) released from $CD4^+$ Th_2 T-lymphocytes. Type 2 cytokines may also be derived from type 2 innate lymphoid cells (ILC2), type 2 CD8 lymphocytes, mast cells etc [3,5,14]. Thus, the eosinophilic inflammation in asthma is complex and of different types, that also respond differently to treatment. A large category of the patients has a classic allergic eosinophilic inflammation that generally respond well to inhaled corticosteroids. However, around 40% of the patients have persisting symptoms despite standard ICS and LABA therapy [1,15]. While one factor behind this is due to poor adherence or inadequate inhalation technique, non-responders may simply have an ICS resistant type of inflammation. This inflammation may be eosinophilic or non-eosinophilic. In the first case, a type 2-high and steroid-resistant eosinophilic inflammation is commonly observed in severe cases with concomitant nasal polyps. In these patients the eosinophilia may not primarily be allergy-driven but rather a result of innate immunity responses involving TSLP, IL-33 and IL-5-secreting ILC2 cells [5,16–19]. Autoimmune mechanisms have also been suggested as drivers in patients with eosinophilic asthma that respond poorly to ICS [20,21].

In terms of non-eosinophilic asthma phenotypes, a high neutrophilic signature has been suggested to result from upstream type 1 (Th1) or Th17 responses caused by infections and/or inhaled pollutants [22–24]. Another suggested contributor of type 2-low asthma is metabolic imbalance and systemic inflammation [25], a feature that has most relevance in obese and older patients. This possibility is further supported by recent cluster analyses and elevated systemic IL-6, a biomarker of systemic inflammation, in this patient category [26,27].

As shown by recent detailed analysis of proteomic, genomic, and gene expression patterns, individual asthma patients may also have a complex mixture of immune pathologies and inflammation types [3,6]. This enhanced complexity is outside the scope of this review.

4. Distal airway inflammation

The distal airways (bronchioles) are defined by having an inner diameter of <2 mm. In contrast to bronchi, they also lack cartilage support and are thus more prone to constriction-induced collapse. Studies that have involved autopsies, transbronchial biopsies, and surgical resections provide evidence for a distal airway immunopathology in asthma. Furthermore, physiological and imaging studies suggest that the bronchioles have a significant contribution to the lung function

Table 1
Anatomical compartments of functional relevance to asthma.

Anatomical Region	Prevalence	Immunopathology	Clinical Consequence	Targeted by Inhalation Therapy	Targeted by Biologics
RESPIRATORY TRACT					
Nasal Region	Asthma with CRwNP	Typically type 2 eosinophilic inflammation	Nasal symptoms, Link with severe eosinophilic asthma	No	Yes
Central Airways *i.e., bronchi*	All asthma patients	Type 2 eosinophilic* or non-type 2 inflammation + structural remodelling	Airflow limitation trough bronchial constriction, lumen plugging, oedema and remodelling etc) AHR	Yes	Yes
Distal Airways *i.e., bronchioles/small airways*	Most asthma patients	Type 2 or non-type 2 inflammation (like bronchi) + structural remodelling	Airflow limitation through bronchiolar constriction, lumen plugging, oedema and remodelling)	Partly: Improved with fine particle inhalers	Yes
Alveolar Parenchyma	Unknown but likely a significant proportion	Type 2 or non-type 2 inflammation + structural remodelling	Largely Unknown: Cytokine spill-over: increased systemic inflammation, Tentatively mild alterations in distal lung compliance due to increased myofibroblasts and modest remodelling	Poor: tentatively improved with fine particle inhalers	Yes#
Distal Pulmonary Vessels	Unknown but significant proportion of the asthmatics	Cellular Inflammation and Structural Remodelling, "vascular pruning"	Unknown: However, distal vascular pruning has been correlated with eosinophilia, worse lung function and in increased odds of asthma exacerbation	Poor	Yes
Lung-Draining Lymph nodes	Likely in most asthma type 2 patients	Lymph node eosinophilia, type 2 and adaptive immunopathological alterations	Partly unknown: local immunoregulation and systemic distribution of lymph-mediated cells/ factors	Poor	Yes
EXTRA-PULMONARY COMPARTMENTS					
Bone Marrow	All asthma patients	Increased eosinophilopoeisis and production of other leukocytes	Contributes to tissue inflammation	Poor	Yes
Blood	All asthma	Blood eosinophil and/or altered blood leukocyte profiles	Fuelling the inflamed respiratory tissue with immune cells and systemic cytokines	Poor	Yes
Adipose Tissue	Most obese asthmatics	Adipose inflammation and liberation of systemic cytokines	Systemic "inflammation", fuelling the lung with cytokines	Poor	Yes

Type 2 = Type 2 cytokine (IL4,IL5,IL13) linked inflammation; CRwNP = Chronic Rhinosinusitis with Nasal polyps.

* The eosinophilic inflammation in asthma can be further divided into classic allergic (generally steroid sensitive) or non-allergic eosinophil inflammation (more steroid resistant).

Depends on the permeability of the normally very tight pulmonary capillary endothelial tight junctions.

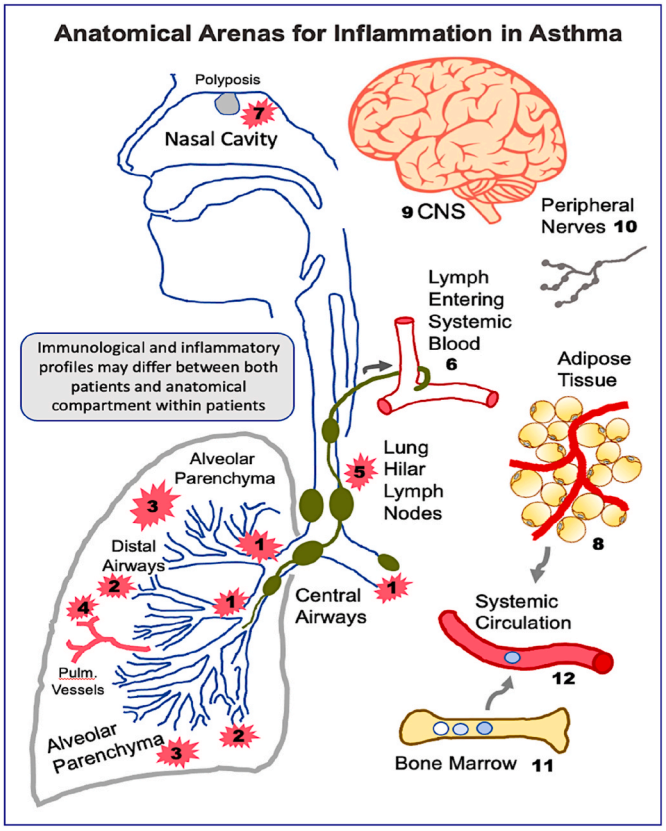

Fig. 1. A schematic overview of anatomical regions of relevance for the immunopathology and treatment of asthma. Bronchial inflammation (1) may be accompanied by a more peripheral inflammation involving the distal airways (bronchioles; 2), and even the alveolar parenchyma (2). New data suggest that also pulmonary arteries and veins may be subjected to inflammation (4). Due to the physiological lymph drainage the large extrapulmonary lymph nodes accumulate lung-derived inflammatory cells (5), many of which travel further with the lymph flow to eventually enter the systemic blood circulation at the confluence of the left subclavian and internal jugular vein (6). Depending on asthma phenotype the immunological processes in asthmatic lungs may also be influenced by extrapulmonary factors such as comorbidities e.g. chronic rhinosinusitis with nasal polyps (CRwNP) (7), or cytokine release from inflamed adipose tissue in obese patients (8). Further, both the perception of symptoms and lung immune processes are affected by CNS and peripheral neuronal input (9,10). As site for leukopoiesis, and important target site for systemic asthma drugs, the bone marrow (11)together with the systemic circulation (12) has a central role by fuelling the inflamed tissue with de novo recruited immune cells and systemic inflammatory mediators.

impairment in asthmatics [28–30]. For example, although bronchioles have only little contribution to airflow obstruction in healthy lungs, they may account for most of the total airflow resistance in asthmatics [31]. Information about the frequency of distal airway engagement in asthma is limited. However, the prevalence is significant since independent studies report a frequency range of 25–60% asthma [32–35]. Further, distal airway involvement seems to be present in all severity stages and in both adult and paediatric asthma [32–35].

Focus for the exploration of the distal airway inflammation in asthma has been on T lymphocytes, eosinophils, and mast cells. Direct proof of a classical eosinophilic type 2 inflammation comes from histological studies demonstrating eosinophilia and IL-5-producing $CD4^+$ lymphocytes in asthmatic bronchioles [36,37]. In support of a distal airway pro-eosinophilic milleu in asthma are observations of bronchiolar expression of IL-5 and the eosinophil chemoattractant chemokines MCP-1 and eotaxin [37,38].

Also mast cell numbers are increased in distal airways and chymase-positive MC_{TC} mast cells in the outer layer of the bronchiolar walls have been shown to correlate with lung function [39]. Other examples of the strategic position of mast cells are reports of their preferred localization to the bronchiolar smooth muscle [40,41]. In fatal asthma bronchiolar mast cells were also shown to be degranulated [40,41].

Importantly, the inflammation in distal airways of asthmatics is not only of an eosinophilic type 2 variant. For example, Wenzel et al. found increased neutrophils in both bronchi and distal airways in severe asthmatics [42].

The leukocyte-rich inflammation in distal airways is a likely contributor to the structural alterations that have been observed in asthmatic bronchioles. Among the histopathologies are distal airway smooth muscle hypertrophy, expanded extracellular matrix, wall thickening as well as distal luminal plugging [8,10,31,43](Fig. 2).

5. Alveolar parenchyma and peripheral lung involvement

Alveolar immune alterations in asthma are both multifaceted and linked with structural alterations. Naturally, these changes are less dramatic than in lung diseases like COPD and lung fibrosis and the functional consequences remain unknown.

In a study investigating nocturnal asthma patients symptomatic night episodes were associated with peripheral airway resistance and increased alveolar, but not bronchial, eosinophils [44]. Newer observations with video-assisted thoracoscopic surgery suggest that autoimmune traits and alveolar eosinophilic granulomatous inflammation are present in a significant subset of patients with severe refractory asthma [45]. Other studies of fatal asthma report increased $CD45^+$ cells (i.e., total leukocytes) and eosinophils at bronchiolar – alveolar attachments [46,47].

The increase of alveolar eosinophils in asthma agrees with a separate transbronchial study that found that asthmatics that were not clinically controlled by routine ICS therapy had a type 2-skewed T-helper profile in the alveolar compartment, a phenomenon that was not observed in similarly treated but clinically controlled asthmatics or healthy controls [48]. Interestingly, the uncontrolled patients did not display a type 2 profile in the bronchi [48], presumably due to the effective deposition of the inhaled steroid at this locale.

Healthy human lungs have a large pool of resident alveolar mast cells [49,50]. A series of studies show that this alveolar mast cell pool is phenotypically altered in asthma [51–53]. Specifically, mast cells in asthmatics upregulated the expression of high affinity IgE receptors and receptor bound IgE immunoglobin [51,52]. Mast cell responses in asthmatic lungs may tentatively also be activated by innate responses, for example during viral-induced asthma exacerbations. In support of this are observations that viral infections in human lungs rapidly expand the alveolar mast cell pool and mast cell expression of CD34 and VCAM-1 [54].

The alveolar region also contains large numbers of macrophages. They are classically divided into localization-based types; interstitial macrophages residing in the alveolar walls and phenotypically distinct luminal alveolar macrophages. Through multiple BAL studies it has been shown that alveolar macrophages are activated in asthma where they execute a complex mixture of pro-inflammatory as well as homeostatic functions [55–57]. The increased alveolar macrophage response in asthma may be linked monocytes since newly recruited $CD68^+$, $S100A8/A9^+$ monocytes have been observed in the alveolar tissue from children with fatal asthma [58]. In experimental models it has also been shown that type 2 cytokines can induce local proliferation of macrophages [59].

As expected, the multifaceted cellular inflammation in the alveolar region has consequences for the structural cells and the alveolar wall architecture. The alveolar region in asthma is for example subjected to matrix alterations and an increase of myofibroblasts [60–62], albeit not as severe as in classical interstitial lung diseases. Airway allergic responses and the alveolar inflammation in asthma may also be associated

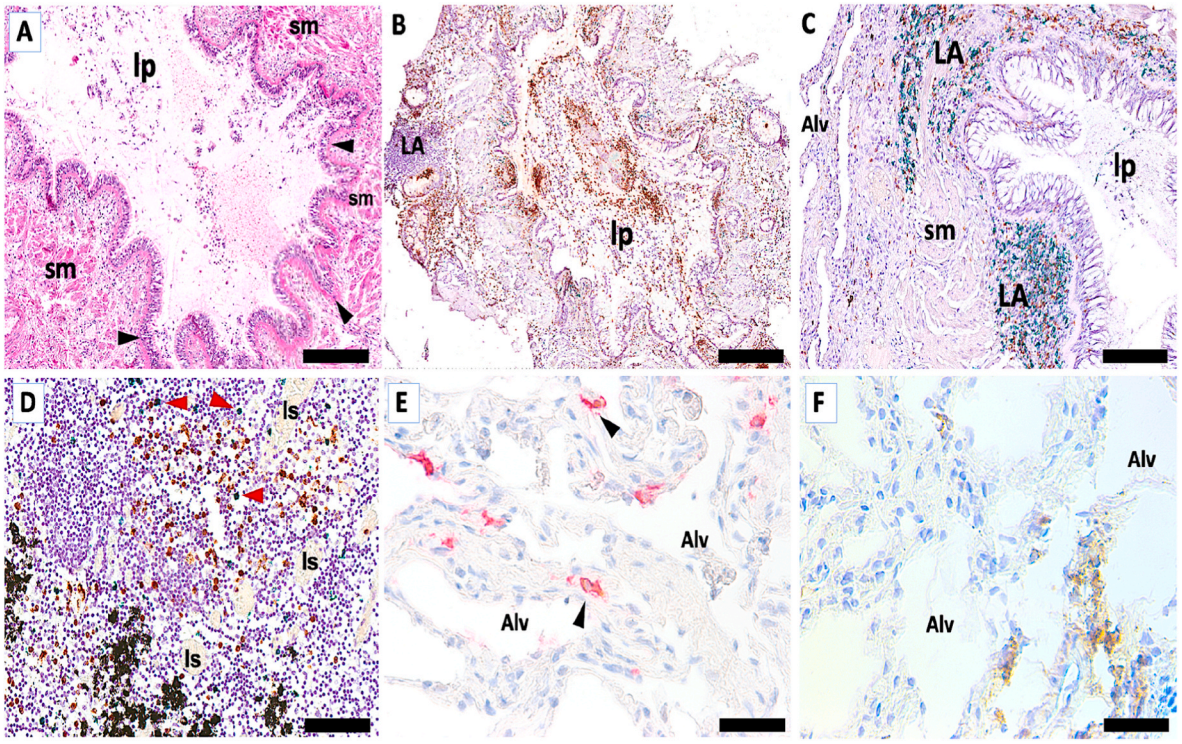

Fig. 2. Micrographs illustrating some histopathological features in asthmatic small airways (A-C), lymph nodes (D), and the alveolar parenchyma (E-F). (A) Haematoxylin-stained section exemplifying thickening of the bronchiolar epithelial basement membrane (arrowheads) in a fatal asthma case. (B) Immunohistochemical visualization of a marked eosinophilia (brown chromogen) and occluded distal airway. (C) Expanded ectopic lymphoid tissue with $CD20^{+}$ B-lymphocytes (green) and $CD4^{+}$ T helper cells (brown) in a fatal asthma case. (D) Lymph node infiltration of eosinophils (brown) and basophils (green and marked with red arrowheads; black color is endogenous anthracotic pigment in macrophages). (E-F) Immunohistochemical staining of alveolar mast cells (E) and clusters of eosinophil granule protein (F) in transbronchial biopsies from non-controlled asthmatics. Abbreviations: sm, smooth muscle; lp, luminal plug; LA, lymphoid aggregate; ls, lymph sinuses; Alv, alveolar parenchyma. Scale bars: A-B 250 μm, C 150 μm, D 100 μm, E-F 40 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

with remodelling of the medium-size pulmonary vessels [63–65]. In a recent HRCT study computerized 3D rendering revealed a significant loss of peripheral pulmonary vessels as a common finding in asthma [65]. This change was linked with eosinophilic inflammation and increased disease activity and odds of asthma exacerbation [65].

Thus, although it seems that both alveoli and pulmonary vessels are subjected to significant immunological and structural alterations in asthma, further studies are needed to define the extent and functional consequences of these in relevant clinical settings.

6. Extrapulmonary compartments

Extrapulmonary compartments may contribute to the inflammation in asthma. The large hilar and tracheal lymph nodes are in physical contact with the lungs via the inflow of lung lymph fluid [66]. Due to their inaccessible location, they have remained poorly studied in asthma patients. However, it has been shown that, apart from the expected influx of antigen-presenting dendritic cells and migratory T cells, extrapulmonary lymph nodes in asthmatics may also contain large numbers of eosinophils [67](see also Fig. 2 D). These eosinophils express MHC2 (HLA-DR) and the costimulatory molecules CD40 and CD80 [67], indicating a potential role in local antigen presentation. The lymph fluid, with its rich content of activated immune cells, eventually empty into the systemic blood circulation and this will likely enhance the systemic spread of the asthma-associated inflammation (Fig. 1).

Naturally, both the systemic blood pool and bone marrow are important arenas for the systemic component of asthma [5,68] and fuel the lung tissue with *de novo* recruited immune cells and pro-inflammatory molecules. Apart from plasma cytokines and chemokines other circulatory molecules affecting the asthmatic lung tissue include brain-derived factors [69,70]. This humoral CNS contribution, together with respiratory peripheral neurons [71–73] (Fig. 1), may have an underappreciated impact of the inflammatory picture in asthma.

Another extrapulmonary compartment of relevance to asthma is the adipose tissue. Obesity-associated asthma tends to be more of a non-eosinophilic type 2-low and neutrophilic phenotype. Although obesity is a risk factor for asthma and obesity-associated asthma has distinct symptom and inflammatory profiles, the link between obesity and asthma is still a matter of debate. The relationship seems to be complex and multifactorial with potential involvement of chronic systemic inflammation, abnormal ventilation, reduced physical activity, hormonal influences, and genetics [74–76]. In terms of functional relevance for lung inflammation it has been speculated that in obese asthma there is a spill over of proinflammatory cytokines e.g., IL6 from inflamed adipose tissue into the systemic circulation (Fig. 1), an event that would contribute to both the systemic and lung inflammatory profile.

Due to the established relationship between allergic rhinitis (AR) and asthma, the nasal mucosa is another compartment relevant to asthma. Both conditions commonly coexist, and rhinitis is a risk factor for asthma development [77]. Although this suggests a causal link, the immunological mechanisms involved in the spreading of inflammation to the lungs in AR patients that develop asthma remain unknown.

7. Treatment considerations in relation to the anatomical heterogeneity in asthma

The heterogeneous anatomical engagement among asthmatics has implications for the treatment. This is illustrated by multiple studies linking a peripheral lung involvement to an impaired response to standard inhalation therapy and worsening of disease [29,31,48,78–80].

There can be several reasons to the reduced treatment response in this category of patients. Some patients may have a type of distal immunopathology that is steroid-resistant, as for example indicated by distal inflammation in patients on oral steroids [45]. Alternatively, the distal inflammation could be steroid-sensitive but left poorly treated with standard ICS since common inhaler devices were designed for a foremost central deposition [80–83]. The rational approach to improve the treatment in the latter patient category is to use inhalers with more distal drug deposition [32,35]. Advances in drug formulation and inhaler engineering have generated a range of inhalation systems that provides a more distal lung distribution through smaller drug particle size and slower inhaled aerosol velocities [35,80,82]. The significance of a more distal treatment has been demonstrated already two decades ago [80,84]. More recently, the potential benefit of pharmacologically targeting the distal airways by ultrafine particle inhalation have also been shown in a series of real-life studies [80,85,86]. For more severe patients that are eligible for antibody-based treatment, the current intramuscular or subcutaneous administration effectively target the distal airways. An illustration of this is the improved small airway functions or reduced alveolar nitric oxide (NO) that have been achieved with mepolizumab, benralizumab, dupilumab, or omalizumab [87–90]. To what extent biologics modify any alveolar inflammation parameter is less studied.

8. Monitoring distal lung alterations in asthma

Since peripheral inflammation may be present in asthma, and there are available options for more distal treatment, monitoring of small airway function would be an important complement to identify those patients who benefit most from a more distal lung targeting with ultrafine inhaled medications (or biologics in eligible patients). The methodological approaches to study distal lung functions fall into several categories like spirometry [35,83,91,92], oscillometry [93,94], and multiple breath washout [95,96]. Radiological and non-invasive imaging techniques like HRCT, MRI and SPECT/PET have the potential to assess both central and distal lung inflammation and tissue remodelling. Importantly, radiological approaches may also be used to reveal alterations in the pulmonary vasculature [65]. Due to the rapid technical development the use of these techniques will likely grow. However, despite their obvious potential, the anatomically resolved physiological readouts generally do not inform about underlying immunological processes.

Although debated, it has been advocated that collection of bronchial biopsies, or even distal biopsies, may be justified in highly selected hard-to-treat patients where direct information on the type of tissue inflammation could guide the selection of further treatment [97]. For this purpose, collection of bronchial cryobiopsies, a procedure that recently was shown to be well tolerated [98], may offer advantages over standard forceps sampling due to larger biopsy size.

9. Conclusions and future aspects

The immunological and physiological alterations in asthma may, apart from the well-studied bronchi, involve the small airways, the alveolar parenchyma, pulmonary vessels, and extrapulmonary tissues. Patient heterogeneity regarding what regions that are stricken by inflammation likely represents a clinically important dimension of the asthma diverseness. However, several critical questions remain, especially in relation to the monitoring of distal inflammation and to what extent this can be used to improve treatment. A related challenge is to better define the heterogeneity of inflammatory signatures within individual patients, not only between anatomical compartments, but also within regions at the same airway level. Future research addressing these questions seems also critical for the understanding of the full biological complexity within the asthma umbrella diagnosis.

Funding

This work was supported by the Swedish Heart and Lung Foundation (20190508).

CRediT authorship contribution statement

Jonas S. Erjefält: responsible for the literature search, interpretation, Conceptualization, and, writing of this manuscript.

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