



Unravelling the complexity of tissue inflammation in uncontrolled and severe asthma

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Purpose of review

The clinical and inflammatory heterogeneity in asthma constitutes a major challenge for improved treatment. This review describes the nature of the inflammatory complexity and how it can be decoded to yield improved disease understanding and personalized treatment. The focus is on the difficult task of revealing the immunological complexity as it occurs inside diseased patient tissues.

Recent findings

The inflammatory heterogeneity in asthma stretches beyond the classical division into allergic Th₂ eosinophilic versus Th₁ and/or Th₁₇ neutrophilic (or paucigranulocytic) phenotypes. Rather than having one distinct type of inflammation, many patients display a patchwork of overlapping immune signatures. The patient diversity is further increased by differences in regard of distal lung involvement. Faced with this staggering complexity, calls have been made for a pragmatic biomarker-guided identification of treatable traits. In parallel, novel high-dimensional analyses and multiplex imaging aid the long-term goal of decoding the underlying molecular endotypes.

Summary

Asthma is vastly heterogeneous with multiple and superimposed inflammatory and anatomical phenotypes. Despite the intensive research and introduction of highly immune-selective drugs, basic questions remain; especially as still too many of today's uncontrolled patients remain poorly understood. Here, pragmatic biomarker strategies, combined with novel methodological approaches that ultimately reveal the complete immunological complexity, will pave the way for improved differential diagnosis and personalized medication.

Keywords

asthma, endotypes, histopathology, phenotypes, precision medicine, tissue inflammation

INTRODUCTION

Asthma represents a global health burden and is currently affecting more than 300 million patients worldwide [1]. The disease is characterized by variable respiratory symptoms and variable airflow limitation. Asthma is also regarded an inflammatory disease in which the driving mechanisms are mostly, but not always, linked to chronic airway inflammation and tissue remodelling. Importantly, the type of inflammation may vary greatly between patients [1,2]. Asthma should therefore be viewed as a broad umbrella term. Indeed, the disease is vastly heterogeneous in its clinical presentation, underlying pathobiology and response to treatment. This heterogeneity constitutes a major challenge and is currently under intense investigation.

The purpose of this review is to provide an updated overview on asthma heterogeneity from a tissue inflammation perspective. Focus will be on clinical observations and how complex inflammation patterns within the diseased tissue are linked to

clinically relevant patient categories. Strategies for implementation of the latest research into improved personalized medication and future challenges are also discussed.

TRADITIONAL CONCEPTS OF ASTHMA

The traditional picture of asthma has been that of a disease characterized by an allergen-driven eosinophilic inflammation [1,3]. Consequently, 'allergic asthma' animal models were developed. These,

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KEY POINTS

- Most asthma patients display a patchwork of overlapping immune signatures and may thus simultaneously have multiple types of immune responses that in turn may vary over time.
- The heterogeneity in asthma is further expanded by patient differences in regard of distal lung involvement.
- Faced with the staggering asthma heterogeneity and complex endotypes, a pragmatic approach for improved personalized medication is biomarker-guided identification of treatable traits.
- New methodology for decoding the immunological and structural complexity opens for the long-term goal of revealing the true nature of the endotypes underlying today's hard-to treat patient categories.

together with clinical observations, suggested that CD4⁺ Th2 T lymphocytes (Th2 helper cells) act as main immune orchestrators by releasing Th2 affiliated cytokines like IL-5, IL-4 and IL-13 (Fig. 1) [4–6]. Associated with the asthma inflammation is tissue

remodelling with key features being smooth muscle enlargement, thickening of the epithelial basement membrane (RBM) and goblet cell hyperplasia [7,8].

Although the archetype view of asthma as an allergic and steroid-responsive disease is relevant to a large category of the patients, many patients do not fit into this model. This is evidenced by the fact that patients respond differently to standard treatment. Roughly, 40% of the patients report persisting symptoms despite inhaled corticosteroids (ICSs) and long acting β_2 agonists [1,9]. Although some of the unsatisfying response is because of poor adherence or wrong inhalation technique, it is clear that a large group of the nonresponders simply have other types of atypical and ICS-resistant phenotypes.

NOVEL CONCEPTS

Emerging inflammatory/immunological phenotypes and endotypes

New data have provided a picture of asthma as a diagnosis that encompasses an almost overwhelming diversity of inflammatory patterns. Current efforts aim to identify clinically useful phenotypes and reveal

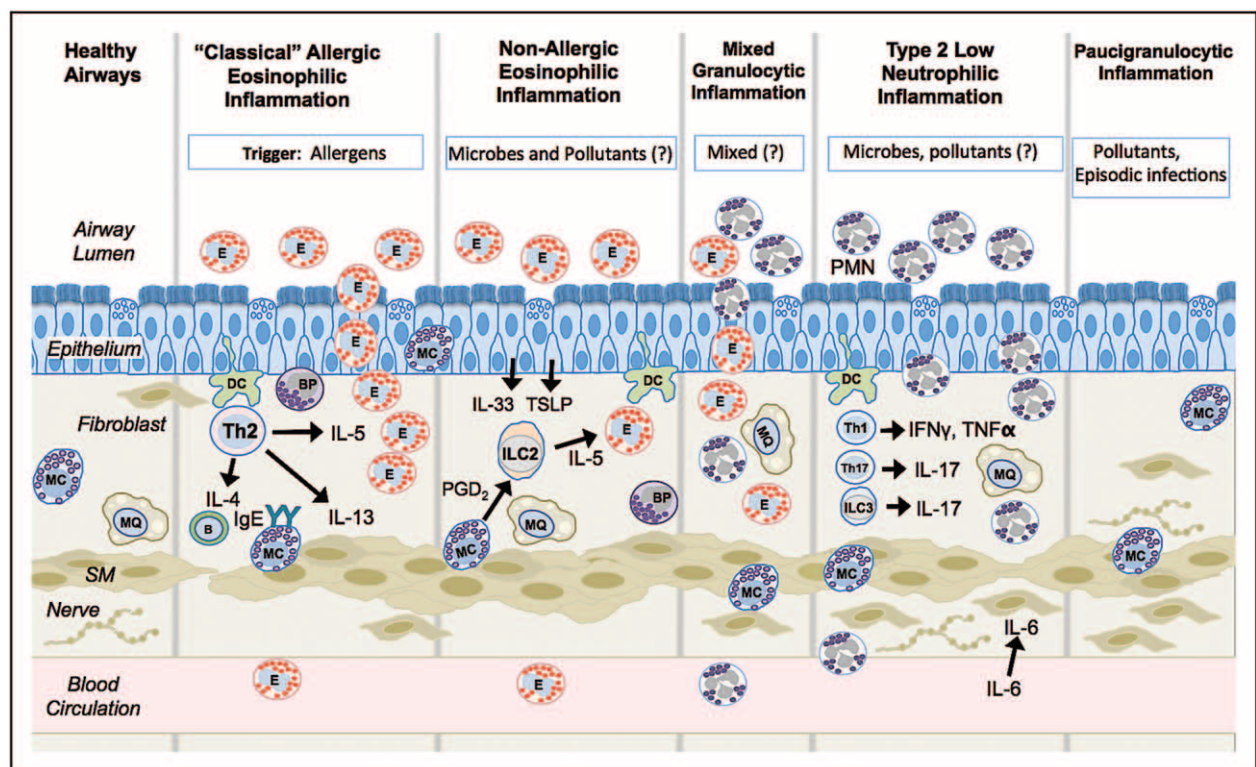


FIGURE 1. Schematic presentation of granulocyte-based inflammatory phenotypes in asthma. Focus is on the proposed and currently most highlighted mechanisms underlying the granulocyte profiles. B, B lymphocyte; BP, basophil; DC, dendritic cell; E, eosinophils; IFN, interferon; IL, interleukin; ILCs, innate lymphoid cells; MCs, mast cells; MQ, macrophage; PGD₂, prostaglandin D₂; PMNs, neutrophils; SM, smooth muscle; Th, T helper lymphocyte; TNF, tumor necrosis factor; TSLP, thymic stromal lymphopoietin.

the underlying endotypes. An endotype is here defined as the actual cellular and molecular mechanisms underlying the symptoms, whereas an inflammatory phenotype is merely the clinical and immunological presentation as measured by the tools at hand [10,11]. With these definitions, it is not surprising that significant progress has been made to describe inflammatory phenotypes, whereas little is still known about the underlying endotypes.

An early attempt of categorizing inflammatory heterogeneity was to analyse sputum granulocytes, an approach that identified eosinophilic, neutrophilic and paucigranulocytic patient groups [12] (Fig. 1). Not surprisingly, with the archetype picture of asthma as an allergic disease, the eosinophil high category has drawn most attention with a focus on Th2 lymphocytes, Th2 cytokines, mast cells and IgE. Importantly, more recent research has now revealed that in certain patients the eosinophilia may not be allergy-driven but rather a result of innate immunity responses involving TSLP, IL-33 and IL-5-secreting type 2 innate lymphoid cells (ILC2s) [4,13–15] (Fig. 1). Consequently, it has been suggested that the expression ‘Th2 high asthma’ should be broadened to ‘Type 2 high asthma’.

In terms of noneosinophilic asthma phenotypes, a high neutrophilic signature has been suggested to result from upstream type 1 (Th1) [16] or Th17 [17,18] responses caused by infections and/or inhaled pollutants. Another recently suggested contributor of Type 2 low asthma is metabolic disturbances and systemic inflammation [19], 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 [20,21].

The inflammation profile in paucigranulocytic asthma (PGA) is largely unknown. Biopsy data suggest that the airways in PGA have similar leukocyte densities compared to healthy individuals, not only for granulocytes, but also for most immune cells [22,23]. Hence, PGA can in some cases be regarded as ‘paucileukocytic’ asthma [22]. Notably, despite a paucity of immune cells, the airways in PGA are still subjected to remodelling, in particular airway smooth muscle enlargement, RBM thickening and, in severe cases, epithelial metaplasia [22,24]. These associated histopathologies and obstructivity in PGA have been suggested to be a result of dysregulated neuronal muscular control, pollutants and/or oxidative stress [24,25]. Another suggested possibility is that the remodelling in PGA is a result of a burnt-out previous inflammation or intermittent bursts of aggravated inflammation [22,24].

Eosinophilic and neutrophilic responses may in some cases occur in concert leading to mixed

granulocytic asthma [12] (Figs. 1 and 2). This phenomenon is conceptually important because it provides a good example of inflammatory heterogeneity within individual patients. This added complexity, which actually occurs in all inflammatory phenotypes, represents a major future challenge. That said, the crude granulocyte-based asthma categorization has proved itself to be clinically useful and, especially eosinophilia, correlate with important clinical features such as treatment response, onset of disease and symptom profiles [1,26^{***}].

Expanded complexity and mixed inflammatory phenotypes

The full complexity of inflammation in asthma cannot be explained merely by a granulocyte or type 2-based classification. This should not be a surprise, considering the well-established clinical heterogeneity and differences among real-life patients in environmental exposure, cause, onset, comorbidities and so on [1,26^{***}]. Furthermore, it should be remembered that, although rarely stated in textbook descriptions of asthma immunology, the theoretical models for the mechanisms that evoke eosinophilia or neutrophilia are mainly generated from studying refined ‘clean’ immune responses in experimental *in vitro* or animal models. The situation is different in real-life patients where new data suggest that a variable degree of overlapping immunological traits should be expected in virtually all patients (Fig. 2).

One example of overlapping immune traits in asthma regards Th17, Th1 and Th2 responses. Thus, although the basic nature of these responses seems to be mutually exclusive [27], other recent observations found mixed Th2-Th17 or Th2-Th1 signatures [28,29]. The observations of mixed inflammatory responses should actually be expected due to the fact that patients are commonly exposed to multiple environmental triggers (consider, e.g. the common scenario of an allergic asthmatic being subjected to tobacco smoke exposure, or a subclinical infection and so on). As in most chronic inflammatory diseases, the asthma inflammation evokes intrinsic damage-repair responses [30,31] and increases the risk of autoimmune responses. The latter aspect is supported by several reports suggesting that parallel autoimmune mechanisms may be yet another component of the asthma immunopathology [32,33^{***},34^{***}].

The mixed inflammation in asthma can be caused in two principle ways. One alternative is through a spatial heterogeneity where different immunological signatures are present within distinct tissue microenvironments or anatomical lung regions (as discussed below). Another possibility is through the recently exposed vast plasticity of

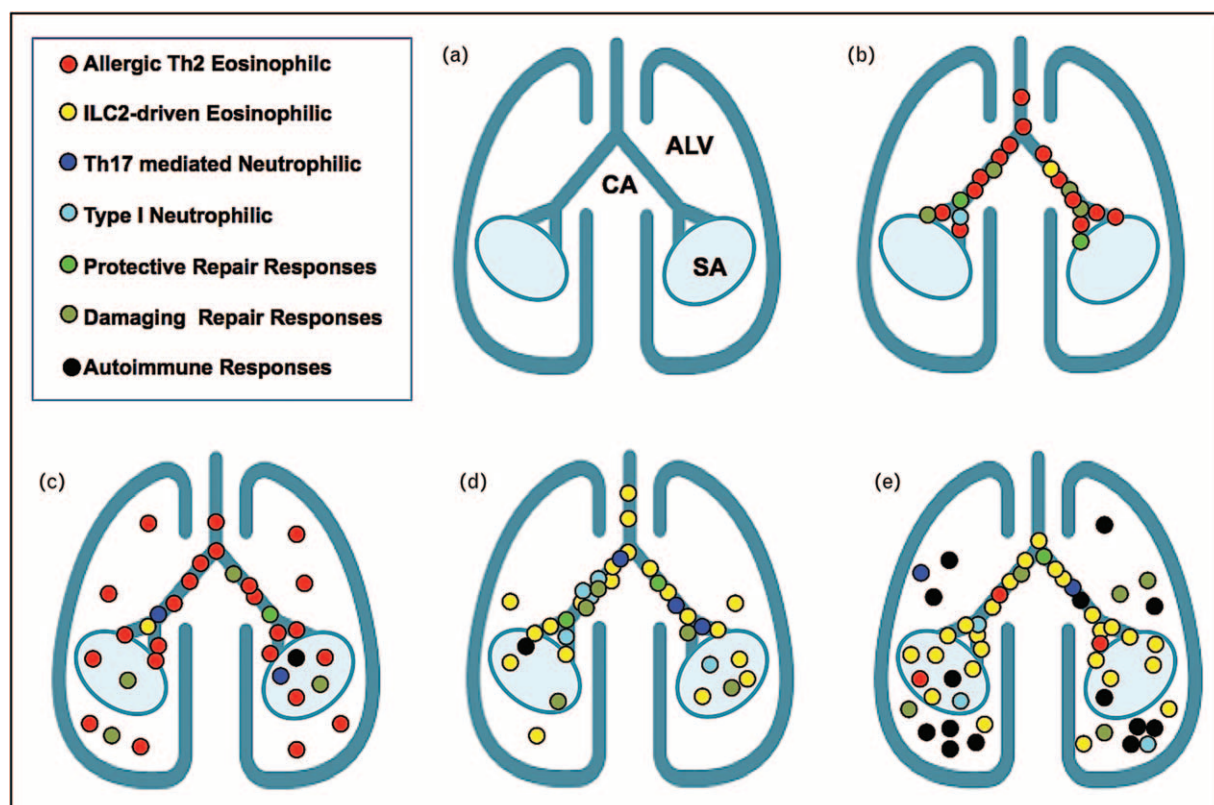


FIGURE 2. A simplified illustration of how integrated anatomical and inflammatory phenotypes may create complex spatio-inflammatory phenotypes among patients diagnosed with asthma. Anatomical regions are outlined in A. Note that small airways are symbolically represented by ovals. Proposed immune responses (upper left) are illustrated as circles. Panels B–E exemplify some of the many tentative phenotypes to be expected in real-life patients. Panel B illustrates a patient with a mixed but foremost allergic inflammation that is restricted to the central airways. Panel C exemplifies a case with similar inflammatory signature but also distal lung involvement. A foremost nonallergic eosinophil inflammation with moderate distal involvement is depicted in D. Panel E illustrates a tentative case with a significant autoimmune distal component (E). ALV, the alveolar parenchyma; CAs, central airways; ILC, innate lymphoid cell; Th, T helper.

immune cells. For example, modern single cell analyses show that CD4⁺ T-helper subset (like Th1, Th2 and Th17) may transdifferentiate or occur as Th1-Th2 or Th2-Th17 hybrids [5,35,36]. A similar heterogeneity and transdifferentiation is also present within ILC populations [37–39]. The possibility of individual T helper or ILC cells simultaneously releasing a mixture of, for example, IL-17 and Th2 cytokines may thus be one additional mechanism explaining mixed inflammatory phenotypes. Further, other plastic immune cells present in asthmatic airways, like mast cells, also have the capacity to release a variety of type 2 and type 1 cytokines [40]. Altogether, it is reasonable to assume that, rather than having one distinct type of inflammation, the immunological endotype in most patients is defined by a complex network of overlapping immune responses and where the relative proportion of these give rise to the crude granulocytic signatures, clinical phenotypes and response to

treatment (Fig. 2). It is of note that although some studies have found granulocyte stability over time, there is also clear evidence that patients may display temporal changes in their immune signature.

Distal lung inflammation and anatomical phenotypes

Another important dimension of asthma heterogeneity is the difference among patients in regard to anatomical distribution of the inflammation (Fig. 2). This phenomenon is often overlooked but has important implications for both symptom profiles and treatment.

Traditionally, asthma has been described as a disease of the central airways [41]. As a result, distal lung regions have remained less explored (which has also been a consequence of the risk and invasiveness of obtaining distal tissues). However, studies of fatal asthma and transbronchial biopsies from

stable disease provide ample evidence of both small airway and alveolar inflammation in asthma.

Direct proof of a classical eosinophilic inflammation in small airways comes from histological reports of increased bronchiolar eosinophilia and IL-5-producing CD4 lymphocytes [42,43]. Increased and activated mast cells have also been reported, both in bronchioles and within the alveolar parenchyma [44–46]. Supporting a functional role of these findings, distal lung mast cell alterations and an alveolar Th2 signature may be present foremost in patients who remain uncontrolled despite standard inhalation therapy [47]. Another indication of clinical relevance comes from a study of nocturnal asthma, where symptomatic night episodes were associated with peripheral airway resistance and increased alveolar, but not bronchial, eosinophilia [48]. Moreover, new findings suggest that the autoimmune traits and distal eosinophilic granulomatous inflammation are present in a significant subset of patients with severe refractory asthma [34[■]].

Not surprising, the inflammation in distal lungs gives rise to peripheral structural alterations. Among the bronchiolar histopathologies are smooth muscle hypertrophy, expanded extracellular matrix and wall thickening. The alveolar region is subjected to matrix alterations and an increase of myofibroblasts [49,50]. Interestingly, in a recent study, a loss of peripheral pulmonary vessels was found to be common in asthma and associated with increased disease activity and odds of asthma exacerbation [51[■]].

Taken together, from the direct histological observations, bronchoalveolar lavage (BAL) studies and noninvasive parameters of distal inflammation [52,53,54[■]], it seems clear that distal lung inflammation and remodelling is present in a significant proportion of the asthma patients. The clinical relevance of this is supported by the many reports that have linked a peripheral involvement in asthma to a reduced response to standard inhalation therapy and worsening of disease [47,53,55–57]. Hence, in broader discussions of asthma heterogeneity, the issue of anatomical and site-specific phenotypes should be taken under consideration.

Future Directions

When combined, the immunological and anatomical heterogeneity in asthma expands the amount of possible phenotypes, or endotypes immensely (Fig. 2). If one also considers that to date most clinical studies have only involved highly riched patient group, the number of ‘real life’ asthma phenotypes becomes almost overwhelming. This

complexity calls for focused and parallel research strategies. Therefore, while continuing the long-term goal of decoding the endotypes, a pragmatic parallel approach is to focus on identifying biomarkers that can identify traits treatable by already available or emerging medications [11,16,26[■]] (Fig. 3).

Identification of treatable traits; the pragmatic path to improved personalized medication

After decades of stagnation, the pharmaceutical industry has recently provided a plethora of new targeted treatment options [1,58[■]]. Many more are under clinical development. Most new drugs are highly target specific antibody-based drugs and this paves the way for improved and personalized medication.

So far, focus has been on targeting mast cell-associated events or the steroid-resistant component of type 2 eosinophilic inflammation. Newly registered, or late clinical stage, drugs in these categories include anti-IL5 (Mepolizumab and Reslizumab), anti-IL-5R (Benralizumab), anti-IL-4Rα (Dupilumab; note that both IL-4 and IL-13 signal through IL4Rα), anti-TSLP (Tezepelumab), anti-IgE (Omalizumab) and CRTH2 antagonists (e.g. Fevipiprant and Timapiprant). As expected, none of these drugs work in the broad patient population and treatment is guided by eosinophil counts and/or type 2 biomarkers [1,58[■],59,60]. To provide a broader type 2 neutralization, attempts are also made to, for example, block the type 2-promoting transcription factor GATA3 [61], or develop bispecific biologics, for example, an anti-IL-4Rα/IL-5 [62[■]].

Approaches to target neutrophilic and type 2-low asthma are lagging behind, mostly because of the poor knowledge regarding the underlying mechanisms. Some attempts have been made with, for example, anti-IL17 (Brodalumab) and anti-TNF (Infliximab), but the results in asthma have been poor or nonconclusive because of lack of biomarkers for potential responders.

A broadened biomarker use is also warranted to sharpen the stereotypic use of glucocorticosteroids and to minimize overmedication and adverse effects in steroid-resistant phenotypes [26[■]]. A good start is to use eosinophilia as a crude marker of steroid-sensitivity [26[■],63]. It will also be important to identify phenotypes with distal inflammation as bronchioles and the alveolar parenchyma are poorly targeted by conventional inhalation therapy. Accordingly, in patients with treatment-responsive but distal inflammation, fine particle inhalers may improve the symptoms [53]. With their systemic

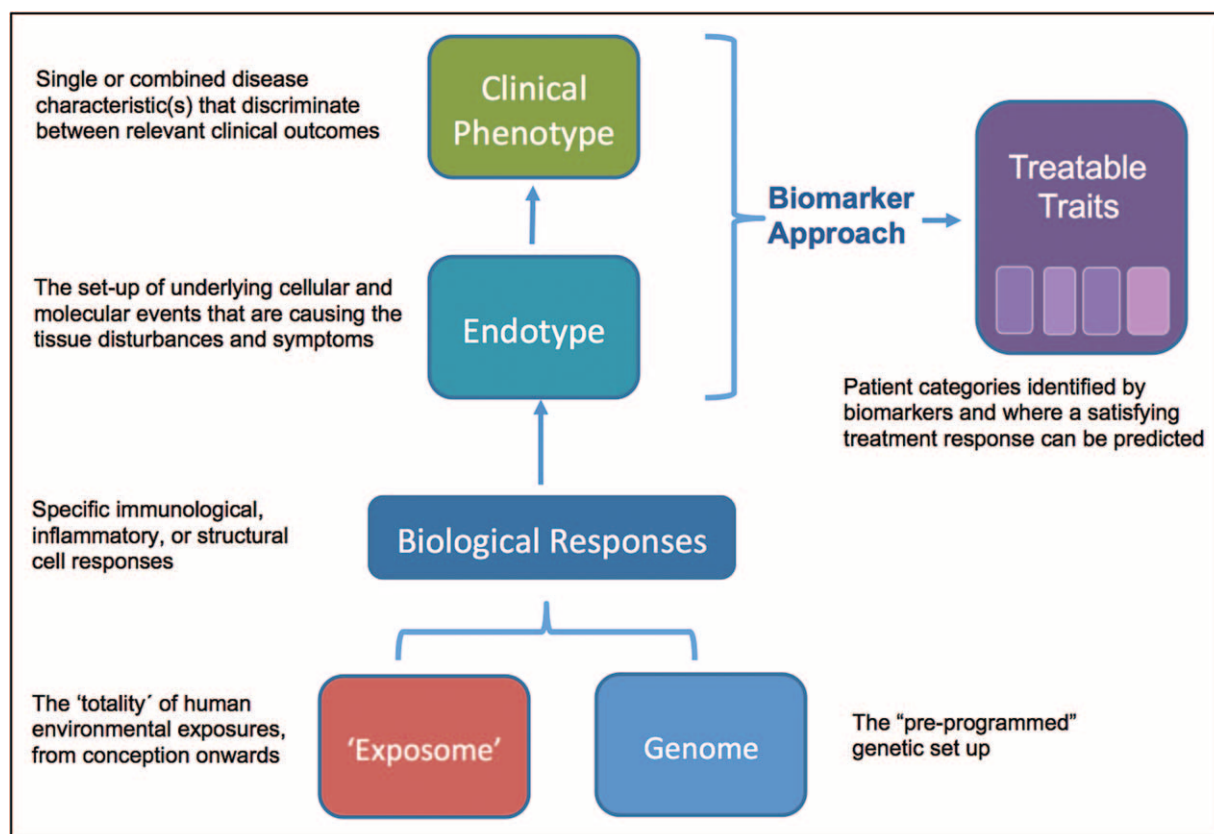


FIGURE 3. The emerging biomarker approach for improved personalized medication is shown in the context of the relationship and definitions of the hierarchical building blocks behind the biological heterogeneity in asthma.

delivery route, biologicals also target the distal airways.

Further decoding of the immunological heterogeneity: can we reveal the 'complete' picture?

The rationale for further dissecting the complicated asthma endotypes is several-fold; to identify novel treatable mechanisms for today's poorly controlled patient categories, to guide the development of adequate experimental 'asthma models' and to reveal novel biomarkers for personalized medication.

Modern omics methodologies offer highly multiplex gene and protein signature profiling. Recent large and sophisticated studies that have integrated multivariate clinical and biological data have identified multiple new asthma clusters and proposed several new phenotypes [28,29,64–66]. Equally important, these impressive studies have also highlighted the magnitude of the disease complexity and the need for a humble and focused approach for a clinically meaningful phenotyping.

It is generally believed that the root causes of asthma symptoms stem from immunological and

structural events inside the diseased tissue. If one accepts this notion, a discussion about sampling and direct versus indirect parameters becomes inevitable. For practical and ethical reasons, most large clinical studies that have generated sophisticated molecular fingerprints have mainly relied on luminal (BAL and sputum) or blood samples. However, many key immune cells are tissue dwellers and are therefore not represented in luminal samples (as are the many immunologically active structural cells). Moreover, the subsets of immune cells that have entered the lumen are likely to be phenotypically distinct from their tissue counterpart [67–69]. Promising approaches have recently been made to apply omics techniques on asthma tissue samples [64,70], but the data interpretation is challenging because of difficulties in normalizing the results against the, often unknown, degree of structural and cellular changes in the analysed samples. Accordingly, contribution of indirect non-tissue samples, animal studies and surrogate biomarkers (e.g. fractionated exhaled nitric oxide), it should be acknowledged that the seemingly advanced schematic graphs of immunological pathways in asthma (exemplified in Fig. 1 and reviews elsewhere) are primarily based on predictions rather than direct

tissue observations. Indeed, some very basic questions remain such as: who are the actual immune orchestrating and cytokine-secreting cells in different phenotypes, what is the true extent of mixed inflammatory pathways, the relative contribution of proximal versus distal inflammation, occurrence of T cell and ILC hybrids and other effector cell plasticities? Related to these questions is also the poorly explored issue of longitudinal stability of key immunological signatures. Moreover, the actual causal links between currently highlighted immune pathways and tissue dysfunctions and symptoms remain vague. The latter aspect is important as cytokines and mere presence of immune cells are not pathogenic per se. Here, it seems warranted to merge the new immunological data with more sophisticated analyses of the 'old' but still underexplored classical hallmark diseases of asthma [41,71] like effector cell degranulation, plasma extravasation [41,72], neuronal dysfunction and luminal plugging [73].

When it comes to the unravelling of activation status and spatial arrangement of immune cells within diseased patient tissues, novel multiplex imaging techniques will likely be very informative with their capacity for simultaneous visualization of multiple immune cells, molecular markers and structural tissue alterations, as indicated by recent concept studies in the cancer field [74] (where tissue is readily available). Applied on focused asthma cohorts, this approach represents a powerful complement to the rapidly development of promising less invasive methodologies such as breathomics [75], advanced blood immune profiling and so on.

CONCLUSION

Asthma is a vastly heterogeneous disease in which the multitude of clinical phenotypes is created by multiple, often overlapping, inflammatory and anatomical phenotypes. The rapid progress in the field has led to a paradigm shift where the current unravelling of the complex tissue inflammation together with personalized biomarker-guided medication will cater for improved differential diagnosis and treatment.

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Conflicts of interest

J.E. is founder of Medetect AB, a CRO company specialized in tissue-based target validation and software for histomic data generation.

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