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The Final thesis

Severe Asthma and Autoreactivity

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Summary

Objective: This narrative review aims to provide a thematic overview of the recent findings and theories on the topic of severe asthma and autoreactivity. The hypothesis was that autoimmunity and autoantibodies play a pathomechanistic role in asthma and contribute to its severity.

Background: There has been an increasing interest in the pathogenesis of asthma. A special focus has been placed on the formation of autoreactive processes, their role in the pathogenesis, diagnostics and therapy of asthma.

Methods: A literature review has been performed. A systematic search within the database PubMed and Google Scholar was done. Additionally, articles with the snowball method and articles from University resources were included.

Results: 75 articles, ranging from clinical studies and animal models to literature reviews, were included. Principles of autoimmunity in asthma appear as probable contributors to its pathogenesis. Autoimmunity in other diseases provides implications for investigations in future asthma research. Autoantibodies, such as antinuclear and anti-eosinophil, were discussed partly inconclusively, yet they appear as contributary factors to asthma development. Established diagnostic methods were presented as questionable and usefulness of localized tests was indicated. Benralizumab might have a good therapeutic effect, including its action on autoimmune regulation.

Conclusions: The current state of research indicates a possible correlation of autoreactive processes and autoantibodies with the pathogenesis and severity of asthma. Possible use of localized sputum and basophil activation tests to measure the contribution of autoantigens to disease severity and for monitoring therapy has been observed. More research is generally needed for the discussed topics.

Keywords

Asthma, autoreactivity, autoimmunity, autoallergy, autoantibody, immunoglobulin E mediated autoimmunity, sputum autoantibodies, antinuclear antibody, basophil activation test.

Introduction

In recent years growing interest has been focused on asthma pathogenesis. More specifically, evidence is accumulating about the types and subtypes of immune reactions, which were historically thought to be either allergic or non-allergic. Allergic asthma is known to be T helper 2 (Th-2) type prevailing with an early atopic onset often in childhood and was opposed to non-allergic asthma, which typically presents later in life in obese and female patients. The current state of knowledge tends to maneuver more towards a classification into type 2 (T2) high/ultra-high, which presents with eosinophilia and Th-2 cytokines, and type 2 low. T2 low characteristics are less clear, but it is mainly non-eosinophilic, possibly neutrophilic, involves Interleukin 1 beta (IL-1β) and IL-6, has less clear biomarkers and tends to be more corticosteroid-unresponsive (1) (see Figure 1 and 2 in Appendix). Another non-eosinophilic type that also has no increase in neutrophils was termed paucigranulocytic asthma, in which anti-inflammatory therapies are ineffective in the presence of airway obstruction (2).

Thus, the classification of asthma became more diverse and includes not only Th-2, but also T helper 1 (Th-1) and T helper 17 (Th-17) types of immune reactions, which subsequently means a likely autoimmune type reaction involvement, especially in T2 low. This is also known as autoreactivity or in terms of Immunoglobulin E (IgE) class autoantibody involvement it is called autoallergy (3). Over the last years the autoreactivity phenomena have also been studied in the pathogenesis of other diseases for example atopic dermatitis (AD), chronic spontaneous urticaria (CSU), systemic lupus erythematosus (SLE) or chronic obstructive pulmonary disease (COPD). Studies regarding autoreactivity in asthma and its subtypes were performed as well, yet the results are neither unanimously nor clearly towards a common direction. There have been numerous findings and proposed theories regarding what types of antigens (exempli gratia (e.g.) alveolar epithelial cells, nuclear antigens or beta adrenergic receptors) and autoantibodies (e.g. IgE or Immunoglobulin G (IgG) type autoantibodies respective to the mentioned antigens or antinuclear antibodies (ANA)) are involved and how they affect the severity of asthma and response to treatment in patients (4)(5).

The goal of this narrative review is to further investigate the hypothesis that autoimmunity and autoantibodies play a pathomechanistic role in certain asthma variants and lead to more severe outcomes. This is of importance for the understanding of asthma pathogenesis and its severity assessment in order to make prognostic decisions and treatment adjustments. The objectives of this paper are first to review the recent literature about the formation and role of autoreactivity

processes in the pathogenesis of asthma. Secondly, similar autoimmune phenomena in other partly related diseases are reviewed to find common aspects with implications for asthma pathogenesis. Thirdly, the kinds of autoantibodies which can form in asthma are thematized, with a special focus on antinuclear antibodies. Fourthly, it concentrates on possible diagnostics of these processes in patients, which includes autologous serum skin testing (ASST), autologous plasma skin testing (APST), serology, sputum sampling with subsequent laboratory analysis for autoantibodies, basophil activation tests (BAT) and also potential treatment options. Finally, the impact of autoimmune responses on asthma and its severity, their prognostic ability for asthma patients and future indications will be discussed.

Methods

Literature search strategy

An electronic search in the database PubMed and also Google Scholar was performed covering up until March 2022 by using the keywords mentioned below in all possible combinations. Inclusion criteria were to use only suitable papers published as full articles in the English language. Exclusion criterium was articles beyond the time limit of the last 5 years from the main PubMed search. The computerized search was supplemented with a manual one of the reference lists of the retrieved articles with the snowball method. Additionally, articles, which fulfilled the inclusion criteria, from the University supervisor and her colleagues were included for a more comprehensive approach. The titles and secondly the abstracts of all retrieved articles were assessed to identify reports related to the topic. Afterwards full articles were examined for their thematic relevance to this narrative review. The keywords used were asthma, autoreactivity, autoimmunity, autoallergy, autoantibody, immunoglobulin E mediated autoimmunity, sputum autoantibodies, antinuclear antibody, basophil activation test.

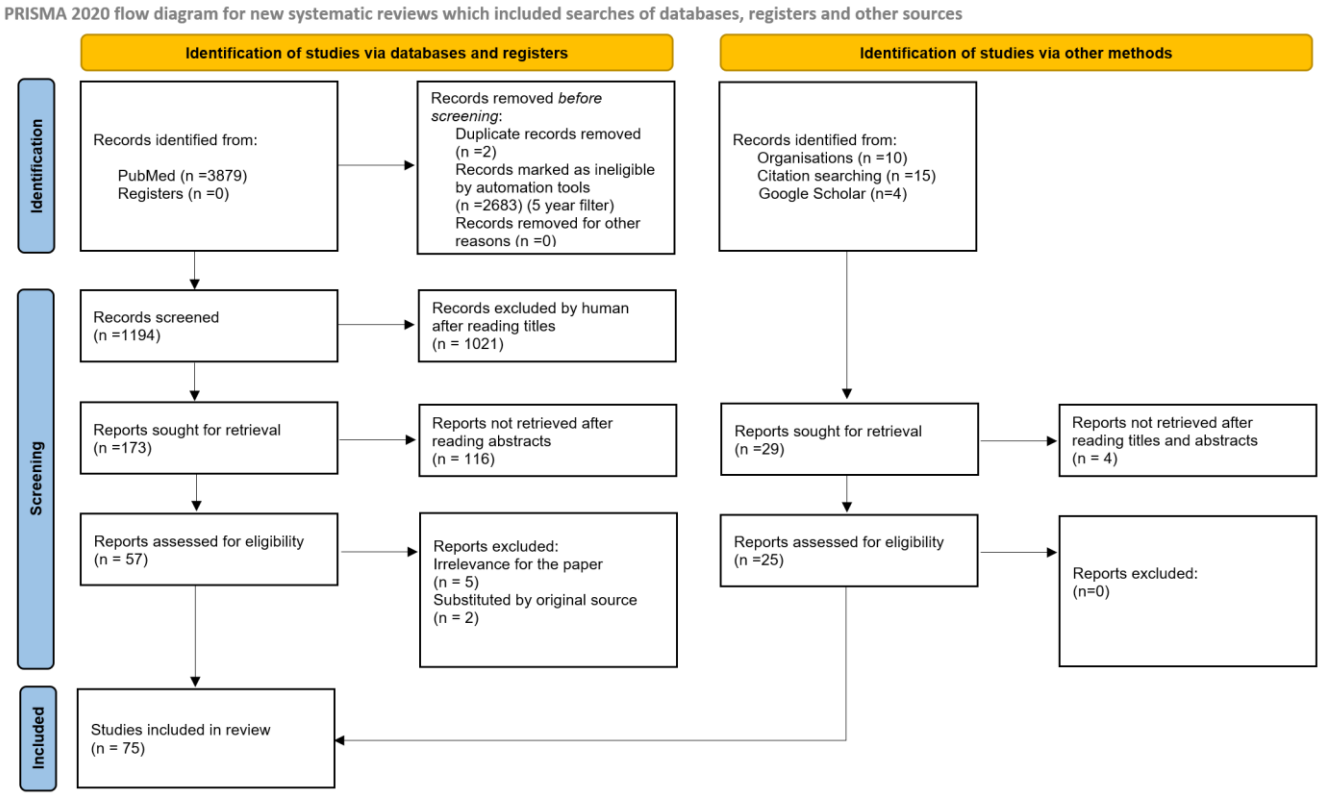

Figure 3, adapted from PRISMA 2020 flow diagram (6)

Results

Mechanisms of action

Autoimmunity and asthma

Over the last decades, it was possible to record a steady rise in cases of allergic and autoimmune diseases in concordance with each other (7). Recently, it was also shown that there is a clear correlation between atopic diseases, including allergic asthma, and several autoimmune diseases e.g. systemic lupus erythematosus, Sjogren's syndrome and Myasthenia gravis (8)(9). Allergic asthma is considered a primarily Th-2 type driven immune system disorder. This characteristically involves Th2 cells, eosinophils, basophils, mast cells, IgE and cytokines like IL-4, IL-5 and IL-13 and was opposed to Th-1 type immune response with involvement of interferon gamma and IL-12 (10). Growing evidence might change this view, since the development of Th-1 type responses were also reported as an addition to the pathomechanism for at least some allergic asthma types, representing autoallergy (3). This is also reflected by new evidence in the genetic research of allergic and autoimmune diseases. A meta-analysis with a large patient pool (62330 patients) identified a 10 % genetic overlap of autoimmune and asthma loci and nearly 50 % of these genes were involved in encoding for

functionally similar products. Additionally, they also reported common single nucleotide polymorphisms (SNPs) located in immune cell regions that were not found in diseases besides allergic or autoimmune ones (11). These results were confirmed by another substantial meta-analysis, which also reported an approximately 10% overlap of asthma and autoimmune disease SNPs, however with a twice as big sample size (480 SNPs from genome-wide association study (GWAS) catalog). Via enhancer marks an association between immune cells and numerous genetic variants of asthma was established and therefore these variants probably play a role in immune system regulation alterations (12).

Molecular mimicry

One possibility for the mixed kind of immune reaction in asthma is that autoreactive processes can develop after previous sensitization to a foreign antigen in an allergic kind of reaction. Included are subsequent cross-reactivity and an immune attack against self-antigens (13) (see Figure 4 in Appendix). This kind of molecular mimicry is seen as different from autoimmune reactions, during which solely autoantibodies get produced against self-antigens (14). It was found that allergic asthma patients have an almost doubled tendency (25% vs. 12%) for Ig-E autoreactivity compared to non-allergic asthmatics, while the majority of asthma patients generally did not show autoreactive processes towards the tested lung and also skin proteins. It was also shown that the allergic asthma group patients had a clear increase in IgE autoreactivity together with a worsening asthma control level (15). Previously it was thought that the cross-reactive binding of self-antigens compared to the related allergens is never complete due to factors like less IgE-binding epitopes on the self-antigens or a reduced binding affinity of B cell epitopes on them (14). Recently, different types of molecular mimicry were summarized. These types show different possibilities that can lead to cross-reactivity and include complete identical protein structures between host and foreign antigen (e.g. microorganisms or allergens), homology at the protein level, similar amino acids, similar epitopes or other structural overlap of proteins or peptides (16).

Immune system regulation

Expanding on this idea, it is worth mentioning that molecular mimicry alone might not be sufficient for inducing autoimmune processes. The responsible T- and B-cells also need to be able to escape immune tolerance checkpoints so that they can become autoreactive upon confrontation with possible autoantigens (16). Physiologically normal would be that upon contact between autoreactive lymphocytes and a self-antigen in the periphery the cells would be eliminated or inactivated, which is known as anergy. Up until today there are about 50

possible gene variations that can be connected to insufficient regulation of autoreactive lymphocytes like impaired apoptosis (17).

It was also stated that regulatory T cells (Tregs) are increasingly lacking in numbers and performance. Thus, autoantibodies can form, which was associated with decreased exposure to microorganisms in higher socioeconomic groups and this is commonly known as the hygiene hypothesis (4). A more specific reason for Treg changes is epigenetic modifications like deoxyribonucleic acid (DNA) methylation, which have been associated with allergic and autoimmune diseases, including asthma (18). This approach is an addition to the well-known concept of Tregs being responsible for keeping a balance of Th2 responses, which, when impaired, can lead to a strong likelihood of developing allergic diseases (19). In relation to that, it was concluded that Mucosa-associated lymphoid tissue lymphoma translocation protein 1 (MALT1) alterations, and therefore a decrease in their immune signaling regulative function, are associated with a decrease in Tregs and thus an increased performance of effector T-cells (20). To continue, the dysregulated function of MALT1 has been linked to immunodeficiency and autoimmunity in mice and humans as well as Th-2 skewing and increased serum IgE in atopic-like dermatitis mice (21). Following foreign antigen sensitization and disturbed mucosal barrier environment in MALT1 protease deficient mice, an autoreactive process with polyclonal T cells against self-antigens characterized the outcome (22). These MALT1 deficiency findings are collectively worth considering as a part of the pathogenesis of autoreactive processes in asthma.

Chronic inflammatory environment

Another way of increasing autoimmune reactions is thought to be constant ongoing inflammation as in chronic allergic states. That likely causes exposure of previously hidden antigens, which are consequently able to be recognized by the immune system as unknown in the proinflammatory environment (13). This concept of locally generated autoimmune responses with autoantibody production in chronically inflamed tissues as a source for their development is also backed up by a lack of connections to a family history of similar autoimmune events. In a study with severely affected eosinophilic asthmatics, a history of autoimmunity in the family or autoimmune symptoms could not be associated with a tendency towards autoimmune reactions in the airways. Consequently, it was concluded that autoimmune phenomena in asthma are rather an acquired condition due to the local dysregulated immune system (23).

Epitope spreading is the concept of broadening the immune reaction towards a given antigen and is generally divided into two possible ways, namely intramolecular spreading (within the same antigen) and intermolecular spreading (involving another antigen) (24). It was concluded that epitope spreading after exposure of hidden self-antigens is also involved in the formation of autoimmune responses. They also mentioned in their article that bystander activation of nonspecific lymphocytes due to the inflammatory environment contributes to autoimmunity as well (25). Accordingly, autoreactivity can be understood as an amplifying factor on top of the allergic processes.

Barrier changes

Furthermore, it was suspected that basic differences in the epithelial structural integrity itself could result in a higher occurrence of immune-based diseases. Having this in mind, it was shown that a decrease in epidermal growth factor (EGF) concentrations in children correlates positively with increased occurrence of IgE sensitization (26). The disturbed epithelial structural integrity on its own is also part of a major theory for increased numbers of allergic diseases, which is known as the barrier hypothesis and mainly regarding type 2 inflammatory disorders. It has been concluded that, depending on the environment, different products are associated with a damaged barrier and sensitization to allergens in asthma development. Especially occupational asthma is associated with contact to detergents and similar products like bleach, different enzymes, cleaning products or disinfectants. It was further described that the atopic march phenomenon could be attributed to leaky barriers. Here, the constant exposure of T cells causes their activation towards allergens in places where the T cells migrate, which results in e.g. AD, food allergy, asthma or allergic rhinitis. However, they mentioned that not only type 2, but also type 1 response can be connected to epithelial leakage and this is followed by the development of e.g. interferon-gamma and subsequent epithelial cell apoptosis (27). Thus, this theory with involvement of type 1 reactions and the constantly disturbed epithelial environment seems likely as a contributing factor for the emergence of autoimmune responses.

Other concepts

When considering the role of autoreactive IgE in asthmatic disease, it is also worth having a look at what IgE is accounted for in autoimmune diseases without a meaningful allergic potential. It was found that self-reacting IgEs were increased in autoimmune diseases like bullous pemphigoid or chronic spontaneous urticaria and also correlate positively with disease severity. Besides causing reactions in basophils and mast cells these autoantibodies also lead to activating dendritic cells to secrete interferons and thus induce plasma cells as well into an

autoimmune reaction (28). It was theorized for SLE that dendritic cells (DCs) play an important role in the development of autoimmunity with auto-reactive B and T-cells, which in turn leads to the production of autoantibodies. More specifically, the imbalance of stimulatory and tolerogenic DCs (29) and defective phagocytosis of autoantigens can subsequently lead to activation of the autoreactive lymphoid cells (30). This concept about DCs contribution could likely also play a role in the auto-reactive processes of asthma.

Another mechanism to induce cytokines, both Th1 and Th2, and therefore likely increase the inflammation in asthma patients, is invariant natural killer T (iNKT) cells, which have been linked to the severity in therapy-resistant asthmatic children. The exact role of how they act in asthma has not been securely established yet, hence more research would be needed on this matter (31).

There have also been investigations concerning the role of neutrophils and their induced neutrophil extracellular traps (NETs), which provides new insights into autoreactivity especially for the neutrophilic asthma type. Notably in severe asthma forms, NETs are thought to facilitate the development of autoantigens and therefore a resulting increase in autoantibodies as well as epithelial damage and inflammation (32).

Autoimmune phenomena in other diseases

To widen the perspective, it is worth looking at the following partly related diseases with similar autoreactive traits as found in asthma patients. From these findings possible implications for the pathogenesis of asthma could be drawn.

Atopic dermatitis

IgE autoreactivity was found initially in atopic dermatitis patients, where it was documented that patients with more extreme features had a high prevalence of IgE autoantibodies against e.g. keratinocytes or even enzymes like human protein manganese superoxide dismutase (33). Moreover, it has been concluded that ANA is present in AD as well, but so far it was not possible to attribute them to disease severity as opposed to IgE autoantibodies (34). Besides that, autologous serum skin tests and sweat injections have been performed with positive results, yet it is still not clear if autoreactive AD could be considered a separate AD type or if it is just an additional feature in more severe courses (35).

Eosinophilic granulomatosis with polyangiitis and asthma

As asthma is frequently part of an early manifestation of eosinophilic granulomatosis with polyangiitis (EGPA) it can be considered in terms of shared pathogenetic mechanisms. The most obvious part would be significant eosinophilia driven by IL-5, but EGPA is also known to be a Th-2-driven inflammatory disorder in which the production of antineutrophil cytoplasmic antibodies (ANCA) plays a major role. It was summarized that in EGPA not only ANCA but also anti-EPX autoantibodies take part in airway inflammation, while EPX can also be found in solely asthma patients. It was here connected to a reduced tolerance of the immune system with decreased IL-10 and the already discussed reduction of Treg cells, which leaves more room for the production of autoreactive lymphocytes (36). Researchers also highlighted the correlation of eosinophil extracellular traps (EETs) with severe eosinophilic asthmatic patients, where the EETs could lead to a heightened type 2 response (37). In another murine study an increase in epithelial cytokines (IL-1α, IL-1β, CXCL-1, CCL24, IL-33, and TSLP) and also increased ILC2 cell counts (producing IL-5 and IL-13) were measured after injecting EETs, which underlines their relevance for inducing the described immune response e.g. also towards autoantibodies and a worsening in the level of asthma (38).

Systemic lupus erythematosus

IgE autoantibodies were also reported in more than half of SLE patients against nuclear autoantigens. It was concluded that these autoantibodies would be suitable as a prognostic sign for the activity of SLE (39). Furthermore, it was noted that a disorder of self-tolerance related to complement deficiency experimentally resulted in autoantibody (e.g. ANA) production as well as inefficient removal of apoptotic cells, which subsequently evolved to SLE (40).

Chronic spontaneous urticaria

There have also been reports of autoreactivity in chronic spontaneous urticaria (CSU). Not only ASST positivity was connected to CSU, but autoantibodies of IgG and IgE type against various autoantigens e.g. high-affinity IgE receptor (FcεRI) or thyroid peroxidase (TPO) were also found. This IgE-mediated autoimmunity is thought to have a pathomechanistic contribution to CSU (41). One explanation for this would be the effects of autoantibody binding to IgE receptors on mast cells or basophils with cross-linkage of the respective receptors and resulting histamine release as speculated in a popular study in the past (42). A related smaller scale study was performed in which they also found IgG autoantibodies against FcεRIα as a likely reason for histamine release in a similar matter (43). Although some researchers came to contradictory conclusions this concept of histamine release is generally considered valid and presents both

the autoimmune type with IgG and the autoallergy type with IgE as possibilities for a pathomechanism in CSU and likely other diseases with mast cells or basophils involvement (44).

Chronic obstructive pulmonary disease

A disease on a similar spectrum as asthma would be COPD and autoreactive processes have been investigated here as well. It was recently theorized that, under the influence of IL-33, autoantibodies against pulmonary self-antigens formed, e.g. IgG antibodies against epithelial cells and smooth muscle cells were documented. These processes might also be facilitated by a damaged inflammatory environment as discussed before and then likely cause more inflammation and cytotoxicity as an example. It was further suspected that the autoantibodies in COPD could lead to small airway remodeling, destruction of epithelial alveolar type II cells due to autoantibody binding and subsequently induced cytotoxicity and/or phagocytosis, as well as complement activation (45). These factors could be considered for the pathomechanism of autoantibody action in asthma as well since the potential action was against cells relevant to asthma development. Indeed, investigations on the related IL-33 in asthma concluded that it was close to four times (3,84) more prevalent in atopic asthma than in healthy controls and that IL-33 was associated with severity aspects like inflammation and fibrotic tissue damage. Moreover, to assess its role in the disease exacerbations IL-33 blockage testing with antibodies was performed and it showed improvement in tissue remodeling and inflammation. The paper also showed that IL-31 in asthma research came to similar results as for IL-33 elevated prevalence, here with a Th 2 relationship, and was also associated with disease severity (46). Referring back to IL-33 in COPD there could also be a tendency to form autoantibodies in asthma in a similar kind of way.

COPD autoantigens were summarized in a review and numerous different ones were found to be increased in comparison to controls, e.g. against epithelial and endothelial cells, cytokeratin or nuclear antigens. Yet, the association of different autoantibodies with disease severity markers ranged from no association up to a clear correlation with severity as with GOLD staging or lung function tests themselves. There were also contradicting results from other studies, which were not able to correlate the same previously positively matched autoantibodies with COPD or its severity (47). These varying results could be attributed to the different quality of the reported studies and there seems to be a tendency towards acknowledging a role of autoantibodies in COPD patients with similar autoantigens as reported for asthma.

Autoantibodies in asthma

Numerous investigations were performed for autoantibodies in asthma. Although there have been promising results, the role of the formed autoantibodies in asthma patients partly still lacks conclusive evidence and different researchers produced varying results. As one example it was stated that anti-beta2 adrenergic receptor (β2AR) autoantibodies are probably the reason for beta-adrenergic hyporesponsiveness in patients with asthma or allergic rhinitis. Yet, the study also mentioned antinuclear antibodies, which were not found to positively correlate with a direct pathogenic role. They were rather accounted for a sign of a dysregulated immune system with autoimmune appearances, especially a reduction in Treg efficiency (4). Intriguingly, different conclusions about the role of ANAs were reported by other researchers, which will be discussed later in more detail. The following table (Table 1) provides a summary of the autoantibodies and their proposed impact, which will be investigated in this part.

Table 1 Autoantibodies in asthma

Autoantibody	Proposed effects and functions
β2AR	Beta-adrenergic hyporesponsiveness, Downregulation of the receptors and their anti-inflammatory and regulating properties
Anti-PPL	Chronic damage of epithelium, Inhibit epithelial repair
Anti-elastin	Biomarker to differentiate asthma from COPD, Increase in autoimmune inflammation and airway remodeling
Anti-alpha enolase	Biomarker for potential severity
ANA	Pathomechanistic role, Biomarker for potential severity and glucocorticoid insensitivity
Anti-EPX	Induce eosinophil degranulation, Glucocorticoid insensitivity
Anti-Epithelial cell	Cytotoxicity against airway epithelial cells

A list of the autoantibodies in asthma and their proposed impact, which are included in this paper.

Anti-beta2 adrenergic receptor autoantibodies

Coming back to autoantibodies targeting β2AR, in one of the earliest papers to report functional autoantibodies for a small group of asthma and rhinitis patients it was shown experimentally

that the autoantibodies can prevent the binding of high-affinity antagonists of the receptors (48), which is partly in line with the theory of beta-adrenergic hyporesponsiveness in asthma. Yet, it remained to investigate how the binding of other ligands like epinephrine or norepinephrine would be affected (49). It was correspondingly recorded that autoantibodies against β2AR also cause downregulation of the receptors in general as well as significantly downregulate the receptor function of producing cyclic adenosine monophosphate (cAMP) and so inhibit anti-inflammatory and regulating properties (50).

Anti-PPL autoantibodies

Another feature of the autoimmune responses in asthma is autoantibody-mediated epithelial injury. A newly found potential self-antigen is Periplakin (PPL, a desmosome component). The IgG/IgE anti-PPL autoantibodies probably contribute to chronic damage of epithelium as they provenly inhibit epithelial repair (51).

Anti-elastin autoantibodies

In terms of biomarkers there have been investigations concerning the use of the autoantibody anti-elastin to differentiate between asthma and COPD in patients. It was found that anti-elastin autoantibodies occurred markedly more frequently in sputum and serum from asthma patients than in COPD or controls and also correlated positively with smoking. It was speculated that this autoantibody leads to an increase in autoimmune inflammation associated with airway remodeling, yet a clear correlation between sputum anti-elastin autoantibodies and disease severity could not be found (52).

Anti-alpha enolase autoantibodies

Alpha enolase as an autoantigen was found to correlate with severe asthma disease courses. IgG autoantibodies towards alpha-enolase were significantly elevated in severe cases (41%) in comparison with mild to moderate cases (11%) and control subjects (3%). These autoantibodies were detected on a similar level in atopic and non-atopic patients and appeared more frequently in aspirin hypersensitivity asthmatic patients. Interestingly, a long-term follow-up of a small group of 6 patients was performed and showed a persistent presence of autoantibodies against alpha-enolase for up to the four years of data collection time (53). Although anti-alpha enolase autoantibodies are not specific to asthma and were also reported in various chronic or autoimmune diseases they could act as a biomarker for a more severe disease course probability.

Antinuclear antibodies

As mentioned, of particular interest in this paper are ANAs and their contribution to asthma severity and here the current state of the literature on ANAs in connection with asthma is investigated. Some studies demonstrated evidence for a pathomechanistic role of ANA in asthma. In a large retrospective cohort study, the association of ANA and asthma as well as the prevalence of infectious diseases was investigated in the light of the hygiene hypothesis. Interestingly, there has been a clear rise in asthma together with ANA occurrence reports when comparing results of patients from around 30 to 10 years ago. ANA itself was reported to rise from 5% to 12.8% in adolescents. Of those subjects with ANA the rise of reported asthma cases increased drastically from 2 to 27%. These numbers indicate a possible relation between ANA and asthma pathogenesis in younger patients. However, the authors were not able to link increased ANA numbers to asthma in general as the possible reason (54).

Another result was that ANAs in asthma appear to be an accountable risk factor for lethal outcomes or high severity courses with a need for hospitalization as well as an increased need for corticosteroid use. The most common pattern of ANAs in this study was of the speckled type, next to homogenous and nucleolar and there were no differences in relative ANA occurrence between atopic and non-atopic asthma cases. Besides that, especially alpha-enolase autoantibodies had a high prevalence in severe cases (5).

Similarly, it was concluded that sputum ANA immune phenomena, in connection with NETs, make up a hallmark of asthma severity like a decrease in lung function does. Furthermore, they could not find sputum or serum ANA level differences between eosinophilic or non-eosinophilic severe asthma patients. These patients were also associated with increased levels of matrix metalloproteinase (MMP-9) and tissue inhibitor of metalloproteinase-1 (TIMP-1), which is likely due to increased airway remodeling in severe asthma (55).

Extracellular DNA (eDNA) production in neutrophilic asthma has been reported by a further study, indicating its significance and correlation with disease severity. It was found that severe patients had markedly higher concentrations of extracellular DNA than mild to moderate patients or controls. Besides that, high counts of eDNA were associated with impaired lung function parameters and an almost tripled risk for severe exacerbations (56). Having in mind the existence of ANA against various forms of DNA, it can be suspected that the higher prevalence of extracellular DNA is potentially able to induce antinuclear autoantibodies and

may be a part of why these patients impress with more severe courses, yet this was not investigated in the study and could be part of future research.

Beyond the reports who account ANAs as a pathomechanistic part of asthma development there are also counterarguments from other researchers. In one recent study the results did not suggest ANA or rheumatoid factor (RF) to relate with asthma severity, though their results match the theory of some pathogenetic role for autoimmunity, e.g. with ANA, in non-allergic asthma. Yet, the authors noted that the quality of their results might not be completely accurate due to their sample size as a limiting factor (57). Furthermore, a report showed almost equal occurrences of ANA in severe asthmatics and controls when measured in serum. However, there was clear evidence for increased ANAs in the sputum of the respective patients, indicating a localized immune event instead (58). These non-congruent results from different parties suggest further evaluation of the role of ANAs in asthma.

ANA and anti-eosinophil peroxidase autoantibodies

Another study demonstrated increased levels of ANA IgG and anti-eosinophil peroxidase (EPX) IgG in severely affected eosinophilic asthmatics with prednisone dependency. It was also found that these immunoglobulin autoantibody complexes were able to induce eosinophil degranulation, which could not be suppressed with glucocorticoids, therefore implying their significance for severity. Interestingly, a distinct result was also that these mixed autoimmune events were rather localized with sputum anti-EPX and ANAs, often against dsDNA, where serum autoantibodies were not significantly elevated compared to controls (58). Severe eosinophilic asthma patients were examined and the role of smoking as a risk factor in their disease course was investigated. The study showed that patients with longer tobacco exposure (>10 pack-years) had a higher rate of airway inflammation with eosinophils as well as increased counts of anti-EPX and anti-macrophage receptor with collagenous structure (MARCO) autoantibodies in their sputum samples. These patients were also associated with insensitivity to glucocorticoids (59). (see Figure 5 in Appendix)

Epithelial cell autoantibodies

In a small-scale clinical study cytotoxicity against airway epithelial cells was found to be mediated by IgG autoantibodies in nonallergic asthma patients, which was observed to happen without the addition of complement (60).

Other concepts

Besides the capabilities of autoantibodies like blocking receptors or directly harming cells, the autoimmune responses in the airways are also proposed to lead to altered signaling like in the TL1A/DR3 axis, which subsequently leads to increased type-2 inflammation with upregulated amounts of ILC2. That is followed by rising numbers of IL-5 and consequently eosinophilia as well as a speculative result in steroid insensitivity (61).

Fields of application in clinical medicine

Methods to measure autoimmunity and its clinical significance

In terms of how to measure autoantibody occurrences in the body there are several possible methods such as autologous serum or plasma skin testing to visualize autoreactive processes and serum analysis. More recently sputum analysis and basophil activation tests have shown useful results, which will be discussed.

ASST

ASST positivity for autoantibodies in asthma patients for example is known to correlate positively with increased airway hyperresponsiveness (62). On the other hand, there are theories that autoantibody-related histamine release is not the only factor that can result in a positive ASST outcome, since it was shown that in vivo and in vitro testing results did not correlate well. The idea was developed that other vasoactive factors and coagulation cascade activation can lead to ASST positivity as well (4).

APST

Autologous plasma skin tests (APST) have also been used in studies, as it was shown that they demonstrate a positive result more often than ASSTs while testing for chronic urticaria. This was suspected to correlate with thrombin generation via the extrinsic coagulation pathway and positive D-dimers in plasma. These coagulation events in chronic urticaria were also associated with disease severity (63). Accordingly, it was also found that APSTs were positive in non-allergic asthma patients in 90% (19 out of 21), which was associated with circulating vasoactive factors in their plasma similar to the CU patients. Additionally, they found VEGF in plasma and they suspected that eosinophils could be the source of the tissue factor and VEGF as was shown for CU. Furthermore, it was believed that the coagulation cascade could be part of asthmas´ clinical picture, though there have not been reports of human studies concerning that

(64). Hence, ASST and APST tests are not very specific towards autoreactivity and their positivity can be influenced by additional factors as mentioned above.

Serum testing

As already described in detail within the autoantibodies section, serum autoantibodies are a common measurement to test for their presence in asthmatic patients and associated disease severity. To summarize, serum autoantibodies were associated with significance for inflammation or disease severity in asthma patients, yet there were also conflicting results which were not able to find clear data on their existence or importance (52)(55)(58).

Sputum testing

Besides that, it was demonstrated that sputum autoantibodies (Sp-Abs) probably correlate better with the clinical presentation and degree of severity than serum autoantibodies (Se-Abs). It was mainly found that sputum autoantibodies like autoantibodies against Smith antigen (Sp-anti-Sm), U1 small nuclear ribonucleoprotein (Sp-anti-U1-SnRNP), and thyroid peroxidase (Sp-anti-TPO) occurred more often in severely affected asthma patients. Compared with this, solely the serum thyroid peroxidase (Se-anti-TPO) autoantibodies were significantly elevated too in severe cases compared to controls. Yet, the connection between anti-TPO and asthma is not clear so far (65). As explained, similar results were documented where sputum ANA and anti-EPX autoantibodies were significantly present and active in eosinophilic asthmatics with pronounced symptoms, while serum autoantibodies could not deliver a similar presence (58).

Basophil activation testing

To detect the clinical relevance of an allergen there is the option to test it via a cumulative allergen inhalation challenge. This test is a very direct method and has a risk of serious complications like clinical deterioration during a resulting asthma attack. The less invasive skin prick test can also show the sensitivity towards a given allergen, yet it is known to not correlate well with the actual clinical response in the airways upon allergen contact. As a more safe and less invasive alternative the basophil threshold sensitivity, a form of basophil activation testing (BAT) which can be expressed as CD-sens, can be measured during laboratory analysis of a blood sample. It was shown that in patients with intermittent asthma, e.g. sensitive to cat/dog dander or tree pollen, analysis correlated the inhalation challenge allergen PD(20) results positively with CD-sens reactions. Notably, there was no correlation found between allergen PD(20) challenge and basophil allergen reactivity. In this study with 26 participants an interesting finding was also that the IgE antibody serum measurements did not parallel these

results, showing that CD-sens probably correlates better with the local immune response in the airways (66). Another study found relating results which specifically monitored how asthma severity correlates with the CD-sens results, here in children with cat allergy. They showed that the level of asthma intensity can be associated with CD-sens results and as such this BAT is suitable as a marker for severity (67).

These results for allergic asthma indicate basophil activation testing like the basophil threshold sensitivity test as a potential option to evaluate the clinical relevance of autoantigens/autoantibodies in asthmatic patients in a similar way. Indeed, via a triple-staining flow cytometry-based basophil activation test of intrinsic asthma patients´ serum the presence of anti-FcεRI autoantibodies was measured. For the asthma patients a ca. tripled amount (29/78) had positive results compared to the controls (4/32), which indicates their significance and role of an autoimmune response in intrinsic asthma (68). As another study (preprint research paper) suggesting the use of BAT, IgE autoantibodies aimed towards eosinophil proteins were indicated in research via BAT as potential biomarkers for severity of autoreactivity. First the authors proved the presence of IgE and IgG autoantibodies towards EPX and eosinophil cationic protein (ECP) in serum markedly in more severe forms of asthma, which they subsequently followed up with BATs. The BATs also showed the potential of these autoantibodies to take part in asthma clinical severity. It should be noted that controls with IgE autoantibodies upon stimulation with EPX or ECP additionally showed a response with basophils of approx.. 11%/13%, though this was significantly lower than in the respective asthma group with approx.. 37%/28% and could be attributed to an unspecific activation response. To further indicate their potential, EPX and ECP autoantibodies are known to cause a rise in inflammation in asthmatic patients which enhances the disease severity (69).

Going further, the BAT also has the potential to be used as a treatment response monitoring tool as shown for allergen specific immunotherapy (ASIT) (70). Additionally, basophil activation testing has been useful in determining the effects of ASIT in more detail, showing e.g. that formed IgG antibodies play a major role in inhibiting basophils and their effects in allergic diseases. It was concluded that these IgG antibodies could either compete for allergen binding or could also act as anti-IgE specific IgGs (71).

Potential treatment options

Unresponsiveness to glucocorticoids in these asthma patients led to new therapeutic approaches. There have been clinical trials with anti-interleukin (IL)-5 monoclonal antibodies

like Mepolizumab and Reslizumab, where the purpose was to reduce exacerbations of asthma in severe courses of the disease. In one trial it was shown that for almost half of the patients, who had moderate to severe levels of severity, met the treatment indications for the antibody therapy and often had a high need for prednisone, no optimal response was recorded. This was associated with the presence of autoimmune processes like autoantibodies. The antibodies recorded for correlation with inadequate response were sputum anti-eosinophil peroxidase IgGs. A small proportion of approx. 14% even worsened under therapy, which was associated with formation of immune complexes followed by complement activation (72). Another investigation was done for Benralizumab (a monoclonal antibody that targets the alpha subunit of the interleukin-5 receptor) that leads to eosinophil depletion via cytotoxic effects (73). Besides reducing the need for glucocorticoids, the advantage of this therapy is that it should also work for autoimmune-influenced asthma cases (74).

Modulation of Tregs as a treatment option was done before e.g. in areas of transplantation and autoimmune diseases. Recent studies also show promising possibilities to use specific Tregs with T-cell receptors (TCR) or chimeric antigen receptors (CAR) to precisely manipulate processes of the disease e.g. support in epithelium repair (75). Similarly, other concepts of asthma treatment have been taken under investigation as altering cells like alveolar macrophages to positively enhance their response and its outcomes. It was found that increasing macrophages abilities, which includes phagocytosis, production of retinoic acid to induce Tregs and decreasing their cytokine production would benefit house-dust-mite allergic asthma (76). This would likely also benefit the autoreactive processes.

Discussion

It can be stated that there have been numerous findings recently, especially concerning asthma pathogenesis and the contribution of autoreactive features to disease severity.

Genetic analysis has shown a significant overlapping of loci, which accounted for structural and functional important features, yet it is not possible to conclude about the actual clinical picture from these findings, but rather give an idea of a potential relation between asthma and autoimmune disease.

As found in several papers, molecular mimicry seems to be an important factor for the development of autoantigens. The more identical allergens are with potential autoantigens the higher the chances should be for their problematic cross-recognition by the immune system.

Depending on what autoantigens are involved the outcomes are characterized by how they can be affected or what the consequence is for the immune system as a whole.

It has also been shown clearly that molecular mimicry on its own is only one factor of the altered immune responses in autoreactive processes (16). The opinions of researchers that other factors also maneuver towards autoreactivity and more severe outcomes of asthma are of importance as well, and have been proposed previously in a similar fashion (13). Especially regulatory functions of the immune system like impaired Treg cells or failure of immune checkpoints are to be considered in future research. The findings on the topic of immune regulatory factors in this paper seem to connect already existing theories, yet, some results are only a start of analyzing the processes in vivo and further studies are needed to provide accurate research concerning the pathogenesis in humans. Furthermore, a chronic heightened inflammatory state also presents itself as a likely cause of dysregulated immunity with inadequate production of autoantibodies towards self-antigens. The barrier hypothesis appeared to be meaningful, as impaired epithelial barriers have several downsides and it was implicated in the emergence of autoimmune responses. Another part with potential in the pathogenesis, the role of dendritic cells, was shown for SLE, though asthma research is indicated to make further conclusions on their role in autoreactivity in asthma.

It is worth looking at other partly related diseases as these could show what mechanisms might be happening in asthma patients as well. Thus, these concepts could be tested or further deepened in asthma research to get more insights, e.g. the role of IL-33 in the development of autoantibodies.

As for autoantibodies, the synthesis of research about anti-beta2 adrenergic receptor (β2AR) autoantibodies indicated them as a rather certain part of autoreactivity in asthma, though there also remain aspects that need further examination e.g. how exactly the binding of ligands becomes affected. Besides that, potential autoantigens like periplakin or anti-elastase still need further research to make weightful statements. Interestingly, alpha-enolase seems to account for a potential biomarker for severe cases, which can be evaluated in future research.

The topic of autoallergy in asthma in this paper resulted in similar considerations as found previously, yet new results showed e.g. IgE autoantibodies in asthma against eosinophil proteins, which correlated with disease severity (69).

The results concerning ANA in asthma as a part of autoreactivity and severity indicate them as a considerable contributor, although some contradicting results came up too. Yet, those

contradicting results might be due to quality problems like relatively small study groups. Besides their role as a potential biomarker they might also be a factor in causing glucocorticoid insensitivity, where anti-EPX autoantibodies could additionally be part of the local immune mechanisms.

As shown, ASST and APST can be positive in autoimmune responsive patients, yet their accuracy is likely inhibited by other factors which can lead to positive results (4).

It also appears clear that testing for local effects in the lungs with sputum is more precise than general serum testing and it can be recommended to further implicate this approach in patients with severe courses and non-responders to treatment. As mentioned, this can be combined with BATs as an accurate and non-risky tool to evaluate the level of severity or response to therapy.

These patients could then be prescribed rather new medications like Benralizumab, which probably benefits them in several ways including dampening the autoimmune responses.

Conclusions

Under consideration of the recent findings, it can be stated with more certainty that autoimmunity and autoantibodies do play a role in the pathogenesis of asthma and its severity. However, broader studies with more accurate mirroring of the asthmatic population are needed to confirm the existing findings and to investigate the established theories.

Among the mechanisms of autoreactivity induction are molecular mimicry, a dysregulated immune system and its components like T-regulatory cells as well as a chronic inflammatory environment. These could be seen as the contemporary major theories.

Similar autoimmune phenomena in other diseases were able to suggest possible mechanisms for future research in asthma.

Autoantibodies such as antinuclear or anti-eosinophil peroxidase are indicated as likely contributors in a dysregulated pulmonary immune environment. For antinuclear antibody research being partly diverse and rather non-conclusive further research is needed, which could be aided by the use of sputum samples and basophil activation tests to detect occurrences and significance of these autoantibodies in asthma. A promising new medication appears to be Benralizumab.

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Appendix

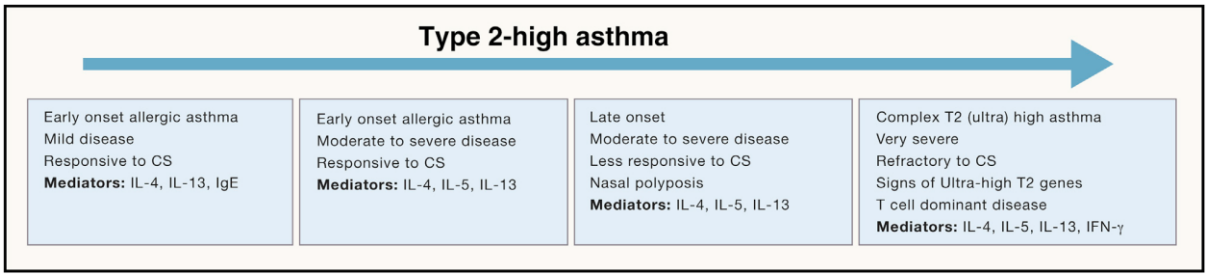

Figure 1 Type-2-high asthma (1)

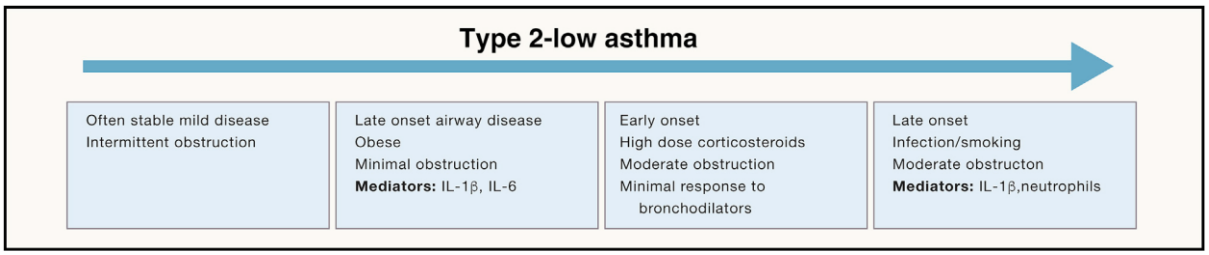

Figure 2 Type-2-low asthma (1)

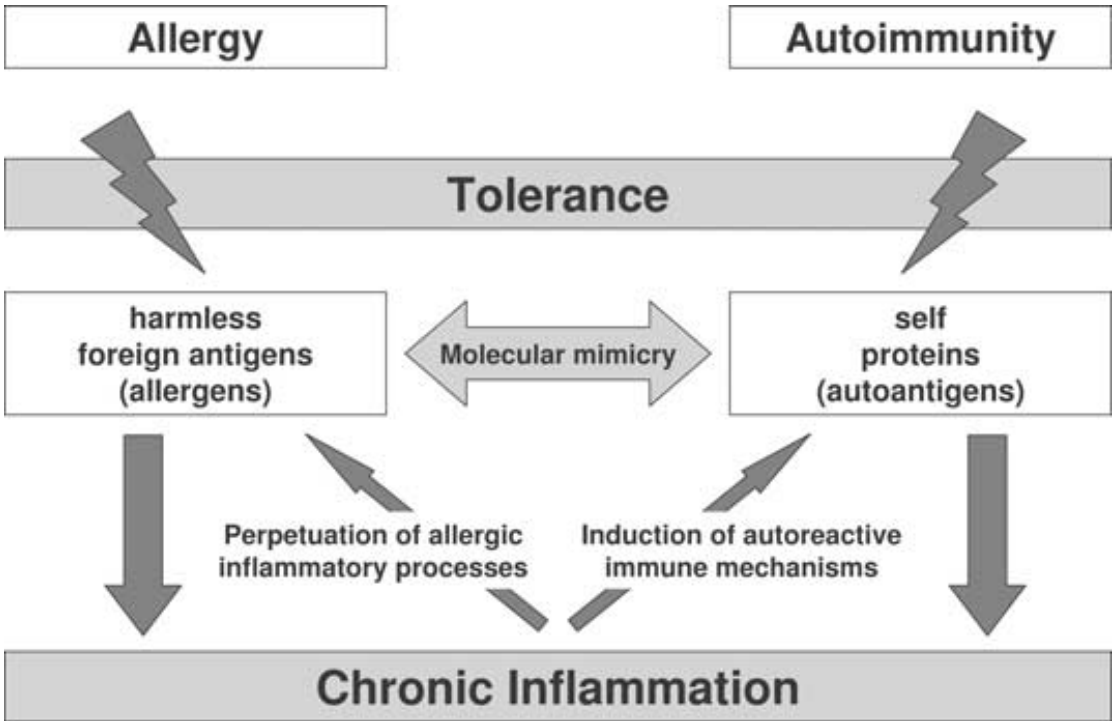

Figure 4 Relationship between allergic inflammatory disorders and autoimmune diseases (13)

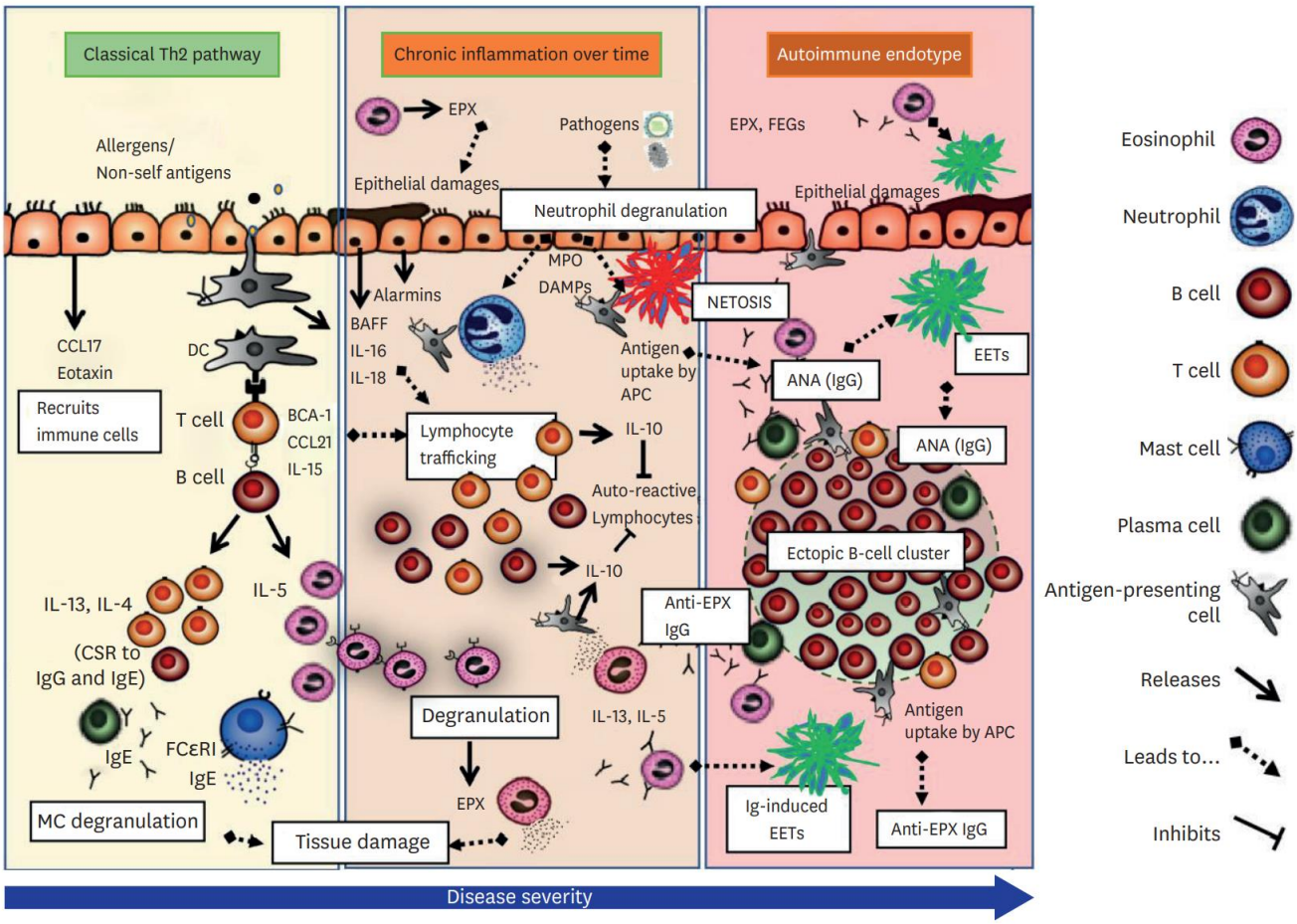

Fig. 1. Working hypothesis for localized autoimmune phenomenon in asthmatic lung. Classical Th2 pathway leads to IL-5, IL-4, IL-13 release, recruitment of eosinophils (eotaxin) and lymphocytes (CCL17), and favors class-switch to IgE. Eosinophil activation and degranulation releases EPX and mediators of tissue damage. With disease progression and chronic inflammation over time, increased localized expression of BCA-1, BAFF, IL-15, IL-16 and CCL17 will allow homing of lymphocytes into the submucosa. Reduced number of regulatory lymphocytes with possible lower IL-10 production will allow activation of the autoreactive lymphocytes (present as a small percentage of the total lymphocyte pool) in the vicinity of their cognate antigens (products of degranulation and tissue damage). Over time, B cell clusters with interspersed APCs and T cells in near proximity are formed. BAFF, CXCL13, CCL21, IL-15 and IL-16 released by different sources including B cells themselves support ectopic B cell clusters, its organization and autoantibody formation. Low levels of anti-EPX IgG and ANAs (polyclonal IgG autoantibodies) formed initially during earlier episodes of degranulation, trigger Ig-induced cytolysis (EETs) on recruited eosinophils (increased eotaxin), thereby increasing self-antigen exposure. The extracellular traps allow efficient antigen priming by APCs and B cells that further leads to increase in *in situ* ANA and anti-EPX IgG production. In some patients, pulmonary infection triggers release of pro-inflammatory mediators like IL-18 which along with neutrophil degranulation 'NETosis' (with possible NET formation) supports further tissue damage, accumulation of self-autoantigens and autoantibody production. Drawing is not to scale.
APC, antigen presenting cell; ANA, anti-nuclear antibodies; BAFF, B cell activating factor; BCA-1, B cell attracting chemokine; CSR, class switch recombination; DAMP, danger-associated molecular pattern; EET, eosinophil extracellular trap; EPX, eosinophil peroxidase; Ig, immunoglobulin; IL, interleukin; NET, neutrophil extracellular trap; MC, mast cell; MPO, myeloperoxidase.

Figure 5 Hypothesis for localized autoimmune phenomenon in asthmatic lung (10)