Severe asthma: inflammation

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SUMMARY. Severe asthma is a heterogeneous disease. Identification of specific sub-phenotypes of asthma may further improve our understanding of its pathophysiology. The genetic and environmental elements that may be important in the development of the disease are poorly understood, but it appears that the pathophysiological background of severe asthma includes both allergic and non-allergic elements. Inflammatory cells are usually present and activated in the airways of patients with severe asthma, and persist despite treatment, but their relevance to lack of asthma control and disease severity is largely unknown. Histopathological studies of severe asthma suggest that 1/2 to 2/3 of patients with severe asthma have persistent large airway tissue eosinophils, despite continued administration of high-dose systemic and inhaled steroids. Severe asthma has also been associated with neutrophilic inflammation, but the precise role of neutrophils is not yet clear. Up to 50% of patients with severe asthma, however, show no evidence of increased airway inflammation. "Steroid resistance" may occur at several levels, not all of which are related to lack of effect of steroids on inflammation. It appears that the factors leading to the development of severe asthma are complex and the disease is probably a mixture of various syndromes that have different elements, but also share similarities at the pathophysiological level. A better understanding of the immunological and histopathological phenotypes of severe asthma should enhance our ability both to understand the pathogenesis of these syndromes and to improve our therapeutic approach, leading to better targeting of both current and novel forms of treatment. ***Pneumon 2011, 24(3):306-313.***

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INTRODUCTION

Asthma is a chronic inflammatory airways disease with variable but, in most patients, fully reversible airways obstruction. Persistent airflow limitation, however, can develop in a subgroup of patients with asthma who have no significant history of smoking, in spite of optimal treatment.[1-6] The aetiology of persistent airflow limitation in asthma is still unknown, although

most investigators assume that such loss of lung function is causally related to persistent inflammatory processes in the airway wall.[7,8] It is postulated that the persistence of airway inflammation in severe asthma may result from deficiencies in the endogenous homeostatic processes that promote resolution of inflammation. A lower capacity for the biosynthesis of lipoxins, which are physiological endogenously derived protective signals, has been linked to severe asthma.[9,10] The studies from the Severe Asthma Research Program (SARP) confirm the presence of lipoxin underproduction and greater oxidant stress in severe asthma.[11,12] In addition, alveolar macrophages from individuals with severe asthma show decreased production of other potentially protective mediators, including prostaglandin (PG)E2 and 15-hydroxyeicosatetraenoic acid (15-HETE), and also defective apoptotic inflammatory cell uptake.[13]

Markers of apoptosis, such as Bcl-2, are high in individuals with asthma, while markers of proliferation and activation, such as proliferating cell nuclear antigens, nuclear factor-kB and CD40-L, show increased expression in corticosteroid-dependent asthma.[14] Neurogenic mechanisms contribute to inflammation in asthma[15] but their role in severe asthma needs to be further explored.

Inflammatory cells are usually present and activated in the airways in severe asthma and persist despite treatment, but their relevance to lack of control and disease severity is largely unknown. These cells include not only eosinophils and neutrophils, but also T-lymphocytes, mast cells, macrophages and airway structural cells, which are also crucially involved in the inflammatory reaction and remodelling that take place in asthma (Figure 1). Although it is accepted that asthma is characterized by eosinophilic infiltration, inflammatory phenotypes of severe asthma can be characterized by the persistence of eosinophilic or neutrophilic infiltration, or by the absence of inflammatory infiltration (paucigranulocytic).[16,17]

Physiological and histopathological data suggest that the inflammatory changes involve the lung periphery to the same extent as the more proximal airways. Although the exact relationship with disease severity is not yet completely understood, autopsy studies have provided evidence that both increased inflammation and wall thickening in patients who died of asthma, may have contributed to instability of the disease, poor control and resistance to treatment.[18,19] Studies of living patients with asthma suggest that the contribution to disease severity of distal lung inflammation may be equally or even more important than that of proximal inflammation.[20,21]

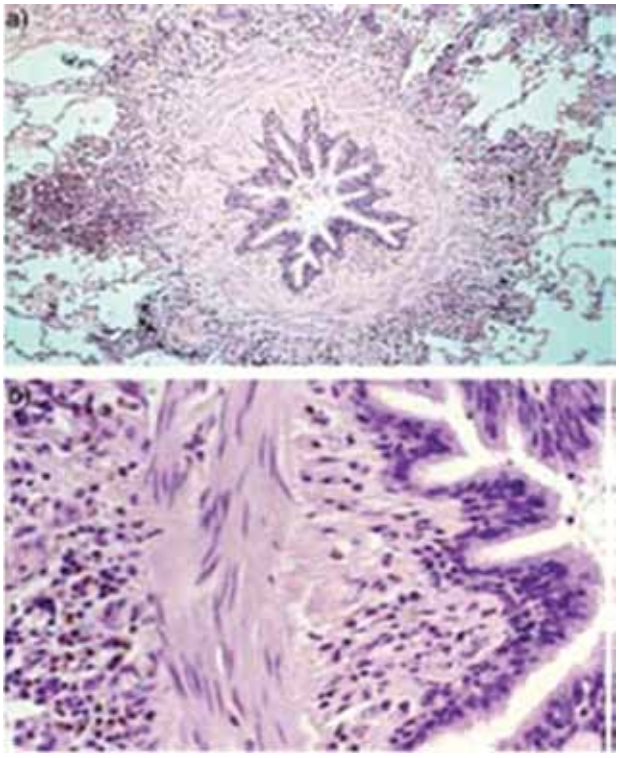

FIGURE 1. Photomicrographs showing a bronchiole from a subject who died during an asthma attack. a) Luminal occlusion caused by muscle constriction, thickening of the airway wall, increased smooth muscle mass and marked inflammatory process in the airway wall, characterized mainly by eosinophils. b) Detail from a). The distribution of the inflammatory process is more obvious: there is a greater density of eosinophils in the area outside the smooth muscle ("outer" region) than in that inside ("inner"region). (Haematoxylin and eosin staining). (Adapted from ref. 17).

Evidence of increased inflammatory cell recruitment to the small airway compartment compared to the medium or large airways has been shown in patients with severe asthma. The distribution of inflammatory cells may also be different in the distal lung, with an increase in mast cells, and specifically chymase-positive mast cells, in the small airway outer wall and alveolar attachments.[19,22]

Various possible ways of classifying phenotypes of severe asthma have been proposed, including eosinophilic versus non-eosinophilic asthma, intrinsic versus extrinsic asthma, brittle versus stable airflow limitation, early onset versus late onset asthma, and aspirin-sensitive asthma. There is reasonable supporting evidence for the presence of at least four general severe asthma phenotypes.

I. Eosinophilic phenotype

Histopathological studies of severe asthma suggest that 1/2 to 2/3 of patients with severe asthma have persistent large airway tissue eosinophils, despite administration of continued high-dose systemic and inhaled steroids. These patients have an increase in the numbers of CD3+, CD4+ and CD8+Tcells, and clinically they have a higher number of exacerbations and near fatal events. Synek et al[23] reported increased numbers of eosinophils and epithelial CD3+cells in the large, but not in the small airways in fatal asthma, compared with mild-to-moderate asthma cases that died from non-respiratory causes. The presence of eosinophils, as measured by examination of sputum, bronchoalveolar lavage (BAL), bronchial biopsy or exhaled nitric oxide, (NO) may represent a specific subtype of severe asthma characterized by a higher level of active symptoms, lower FEV_1 and a greater likelihood of exacerbations and near-fatal events, than a subtype without eosinophils.[16,24-26]

The factors controlling eosinophilic inflammation in severe asthma are not clear. While interleukin (IL)-5, eotaxin and other mediators may promote eosinophilic infiltration, there is no greater up-regulation of these specific chemo-attractants in severe eosinophilic asthma compared with the milder forms of the disease.[27] For example, IL-4 or IL-13 appear to be elevated in both atopic and non-atopic forms of mild asthma,[28] while neither IL-4 nor IL-13 has been definitively shown to increase in relation to disease severity or eosinophilic disease. Conversely, in an analysis of BAL cells or tissue-derived IL-4 or IL-13 mRNA and protein, lower levels were found in patients with severe (i.e., steroid-treated) asthma than in control subjects with milder asthma.[29]

Nevertheless, eosinophilic inflammation may persist and have an impact on structural changes of the airways.[30] For instance, patients with severe asthma and persistent eosinophilia have been shown to exhibit higher levels of transforming growth factor (TGF) (specifically, TGF-β2)[16,22,31] and a thicker subepithelial basement membrane (SBM), than those without eosinophilia.[16]

The differentiation between severe asthma subtypes by the presence or absence of eosinophils has been applied to early- and late-onset severe asthma.[32] Persistent eosinophilia appears to be more prevalent in late-onset than in early-onset disease, despite similar high-dose corticosteroid use. In early-onset disease, increases in eosinophils are associated with increases in T lymphocytes and mast cells, whereas late-onset disease with eosinophils has little evidence of involvement of other inflammatory cell types (Figure 2).[33] Late-onset disease is characterized additionally by higher levels of cysteinyl-leukotrienes than found in early disease, even when controlling for the numbers of eosinophils present.

II. Neutrophilic phenotype

Severe asthma may be associated with neutrophilic inflammation, but the precise role of neutrophils is not clear. The increase in neutrophils does not always exclude the absence of eosinophils, and the two cell types may be present in tissue concomitantly.[16,34] This increase in neutrophils has been observed in sputum, BAL and biopsy studies from patients with severe/difficult-to-control asthma on high doses of inhaled/oral steroids.[35] It is possible that the neutrophilia in steroid dependent asthma is due to the corticosteroid therapy itself, which has been shown to reduce eosinophil and to increase neutrophil numbers, by inhibition of neutrophil apoptosis.[36]

The neutrophilia may represent a continuous influx of cells from the bloodstream due to continuous antigenic stimulation of the bronchi.[37] Several mediators linked to neutrophils, including leukotriene (LT)B4, IL-8, macrophage inflammatory protein-1a and tumour

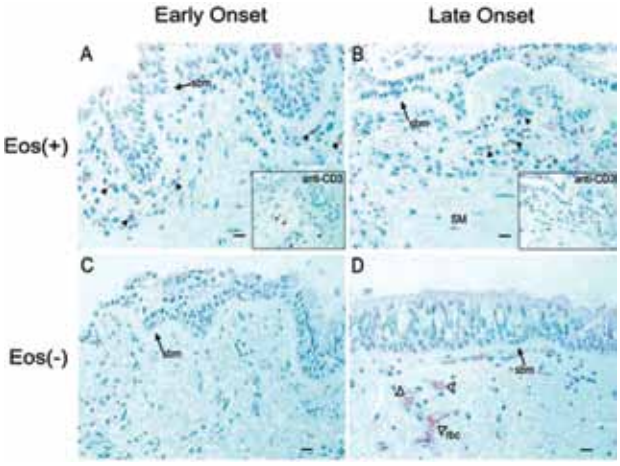

FIGURE 2. Pathology of severe asthma phenotypes. (*A*) Early onset, eosinophil (+). Inset shows concurrent presence of CD3 lymphocytes). (*B*) Late onset, eosinophil (+). Note that the eosinophils are highly degranulated. Inset shows absence of CD3 lymphocytes. (*C*) Early onset, eosinophil (-). Lack of obvious inflammation. (*D*) Late onset, eosinophil (-). Lack of obvious inflammation, thin subepithelial basement membrane (SBM). Arrowheads indicate BMK(+) eosinophils. EOS: eosinophils; rbc: red blood cells; SM: smooth muscle. (Adapted from ref. 33).

necrosis factor-a (TNF-a), have been found increased in severe asthma.[38,39] They induce neutrophil chemotaxis, activation and survival, and they up-regulate endothelial adhesion molecules. Epidermal growth factor receptor (EGFR), a marker of epithelial stress/damage, is increased in proportion to disease severity. EGFR expression in the bronchial epithelium correlates with IL-8, indicating that EGFR can also contribute to this sustained neutrophilic inflammation.[40,41] The presence of neutrophils is also associated with an increase in matrix metalloproteinase 9 (MMP-9) in BAL fluid and tissue (specifically the SBM).[42] In severe asthma, expression of MMP-9 is poorly inhibited by corticosteroids both *in vivo* in BAL fluid and *in vitro* in BAL cell supernatants.

III. Pauci-inflammatory phenotype

Although there is little doubt that inflammation is increased in asthma and correlated with the clinical manifestations in many individuals, there is a subgroup of patients with severe asthma in whom virtually no inflammation (at least of the classic cell types) is evident on endobronchial biopsy. Very little is understood about the pathogenesis of disease in this group, but a hypothesis for this phenotype includes the presence of localized distal lung inflammation, or, as in patients with neutrophilic inflammation, a totally different, perhaps bronchiolitic disease. In support of this hypothesis is the observation that tissue samples from patients with late-onset severe asthma without "classic" inflammation show no evidence of the SBM thickening commonly seen in most biopsies from patients with asthma.[32] It is also possible that structural alterations of the lungs have resulted in persistent clinical symptoms, but inflammation, in the classic sense, is no longer present at the time of examination. In support of this hypothesis, the studies by Benayoun and colleagues[43] report that the primary differentiating factor between tissue from patients with mild and severe asthma was related not to airway inflammation, but to an increase in the amount of smooth muscle. Finally, there may be a type of inflammation involving "non classic" inflammatory/asthma cells. In a study of severe oral steroid–treated patients with asthma, an increase was observed in monocyte/macrophage activation[44] and cells of the innate immunity process, such as macrophages are increasingly being recognised to be important players in the different asthma phenotypes.

IV. Phenotype of poor steroid responsiveness

It is likely that absolute steroid resistance rarely occurs, even in severe asthma[45]. Studies of the phenotype of poorly steroid responsive (i.e., resistant) asthma have traditionally focussed on the lymphocyte as the target cell for the resistant pathways. Phenotypes of severe asthma associated with persistent eosinophilic inflammation in either early or late-onset disease may represent the more classic version of poor steroid responsiveness, where the inflammation is not responsive to steroids. In phenotypes without persistent eosinophil/lymphocytic inflammation, the mechanisms are less clear.

The phenotype of poor steroid responsiveness probably encompasses many different underlying causes (Table 1). It is possible that in some cases the inflammation is responsive to steroids, but the steroids do not have an impact on the structural elements driving the severity. Alternatively, a different type of inflammation, as might be seen in neutrophilic-associated disease, may not be as responsive to steroids as an eosinophil-associated process.[46]

Possibilities for poor steroid responsiveness in this group include sequestration of the glucocorticoid receptor by high levels of pro-inflammatory mediators, diminished binding of the glucocorticoid receptor to the genome, or increased levels of an alternatively spliced glucocorticoid receptor, which has no direct transcriptional-related effects.[47,48] Abnormalities in the balance of the histone acetylation and deacetylation pathways in asthma, which contribute to regulation of inflammatory gene transcription, may also play a role.[49]

A recent study suggested that peripheral blood cells from patients with severe asthma had less histone deacetylation activity in response to steroids than those of patients with milder asthma, thereby preventing some of the anti-inflammatory response to steroids.[50]

TABLE 1. Causes of steroid resistance

1. Eosinophilic inflammation unresponsive to steroids
   a. Lymphocytic process unresponsive to steroids
      i. Altered transcription factor binding
      ii. Increased glucocorticoid receptor β
      iii. Decreased histone deacetylation
   b. Eosinophilic processes unresponsive to steroids
      i. "Hypereosinophilic" syndrome
      ii. Aspirin-sensitive asthma
2. Non typical inflammation
   a. Neutrophil predominance
   b. Inflammation in small airways
3. Structural changes to airways

LUNG STRUCTURAL CHANGES

Airway remodelling may determine the nature and extent of airway narrowing and, therefore, the degree of the response and the likelihood of a fatal or near-fatal event. One of the strongest risk factors for a near-fatal or fatal asthma attack is a previous near-fatal event, which supports the concept that underlying inflammatory or structural changes in the airways, and perhaps, in the lung parenchyma, may contribute to perpetuating the risk. It is yet to be clarified what the precise changes are, however, and what is their role in disease severity. Numerous lung structures have been implicated, including the SBM, epithelium, smooth muscle, nerves, and blood vessels.

Epithelial cell and goblet cell abnormalities, reticular basement membrane (RBM) thickening and smooth muscle abnormalities are observed in the majority of asthma fatalities and in patients with severe asthma and all of these changes probably contribute to airway narrowing. Increased thickness of the RBM is found in the bronchi of individuals who died of status asthmaticus and in endobronchial biopsies from living patients with severe asthma. Thickening of the RBM in the large airways is the structural change which has been most extensively studied in asthma, because of the relative accessibility of endobronchial biopsy, but its relationship to other structural changes deeper in the airway wall or to remodelling of more distal airways is presently unclear. It has been shown that patients with severe asthma have thicker RBM than normal control subjects and subjects with milder asthma,[16,32] which has been associated with persistent eosinophilia and higher expression of TGF-β in the submucosa.[16,51,52]

There is increasing interest in the mechanisms involved in the differentiation and repair of the airway epithelium, especially as it applies to severe asthma. TGF-β from the epithelial cells could stimulate adjacent fibroblasts to increase collagen production, thereby increasing the thickness of the RBM.[53] When an airway fibroblast is stimulated by the combination of TGF-β and a Th2 cytokine, such as IL-4 or IL-13, a profound increase in the production of eotaxin-1, a potent eosinophil chemoattractant, occurs, with an additive effect on the production of pro-collagen I.[53] Thus, in eosinophilic forms of severe asthma associated with increased TGF-β (even in the presence of a modest degree of Th2 inflammation), the fibroblast could not only contribute to the fibrotic response but also perpetuate tissue eosinophilia. Figure 3 depicts possible interactions between eosinophils and other cell types in severe

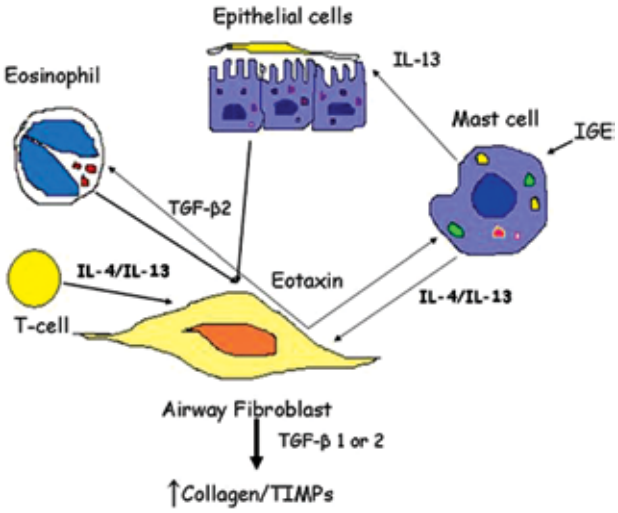

FIGURE 3. Interactions between eosinophils and fibroblasts involving transforming growth factor β (TGF β 1 or 2), which perpetuate eosinophilia and result in increased deposition of collagen. IL: interleukin, TIMPs: tissue inhibitors of matrix metalloproteinases.

asthma that may contribute to fibrosis.[54] As discussed above, however, not all forms of severe asthma imply eosinophilic inflammation. For instance in the study by Benayoun and co-workers[43] mucosal eosinophilia was not found to be related to disease severity, and nor were neutrophilia, epithelial damage, or SBM thickness. In contrast, the presence of higher numbers of fibroblasts and increased airway smooth muscle mass was able to distinguish patients with severe persistent asthma from patients with milder disease. The amount of smooth muscle in the airways of patients with severe asthma appears to play a key role, particularly in the context of fatal asthma. Patients dying of status asthmaticus were reported to have increased smooth muscle mass throughout the airways from the largest airways to nearly the smallest.[55] In addition, smooth muscle cells can also contribute to the recruitment of inflammatory cells into the bronchi where they produce pro-inflammatory cytokines and chemokines and expression of leukocyte adhesion molecules.[56] Sobonia and coworkers[57] studied 6 cases of severe allergic asthma with persistent airflow obstruction where death had occurred from non-respiratory causes. In contrast to studies of fatal cases, increased thickness of the airway smooth muscle area was not observed, which suggests that this factor may differentiate fatal from severe asthma, but further studies comparing airway dimensions in cases of clinically severe (but not fatal) asthma with cases of fatal

asthma would provide better evidence.

Finally, changes in the elastic properties of both the airway wall and its parenchymal attachments, such as might result from pathological remodelling processes, may have dramatic effects on airway narrowing as important modulators of airway wall stiffness and smooth muscle responsiveness. In this context, elastin has been shown to be abnormal (decreased or disordered) in patients who died of asthma in both the large and small airways.[58-60] Proteolytic enzymes (MMP-2, MMP-9) that alter elastin composition have been reported increased in several instances in asthma, particularly in cases of status asthmaticus.[61,62] Disruption of the alveolar attachments, and therefore of the airway–parenchymal tethering, has been noted in the small airways of individuals who died of status asthmaticus, providing a pathological correlate for the physiological findings.[58]

CONCLUSION

Severe asthma remains a disease which is poorly understood and frustrating to care for, mainly because of its heterogeneity. It appears that the factors leading to the development of severe asthma are complex and the disease is probably a mixture of various syndromes that have differentiating elements, but also share similarities at the pathophysiological level. A better understanding of the immunological and histopathological phenotypes of severe asthma should enhance our ability both to understand the pathogenesis of these syndromes and to improve the approach to their treatment.

Adequate tissue sampling of the distal lung for the evaluation of the outer airway wall, small airways and their surrounding parenchyma is still needed in order to widen our knowledge about severe asthma phenotypes. In addition, imaging studies, performed on patients with well phenotyped, genotyped and physiologically characterized severe asthma are required for us to better link structure with function and genetic susceptibility.

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