

Angiogenesis and bronchial vascular remodelling in asthma and COPD

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Summary

In the last years, growing attention has been focused on the vascular component of airway remodelling in chronic bronchial inflammation, such as asthma and COPD, and on its role in the progression of disease. In particular, in asthmatic subjects, the bronchial mucosa shows a larger number of vessels with higher dimension and a greater vascular area. Recently, insights have been obtained on the pivotal role of vascular endothelial growth factor (VEGF) in promoting vascular remodelling and angiogenesis. In fact, many studies conducted on biopsies, induced sputum or BAL, have shown the involvement of VEGF and of its receptors in the vascular remodelling processes. However, multiple mechanisms act in concert in chronic airway diseases, resulting in bronchial vascular remodelling, with a variety of growth factors and cytokines involved. Presumably, the vascular component of airway remodelling is a complex multi-step phenomenon engaging several mediators. The aim of this review was to analyze the morphological aspects of the vascular component of airway remodelling and the possible mechanisms involved in asthma and COPD.

KEY WORDS: bronchial asthma, COPD, angiogenesis, pulmonary vessels, VEGF, airway remodelling, bronchial microcirculation.

Introduction

It is well known that the functional consequences of inflammation on asthma and COPD is the airflow limitation, which can be easily reverted in asthma but not in COPD. In both diseases, the inflammation is associated with cellular and structural changes called "remodelling", that may lead to thickening of the airway wall, thus promoting airway narrowing and airflow limitation (1, 2). The structural changes characterizing airway remodelling include alterations in the composition, size, mass, number and organization of the cellular and molecular components of the airway wall. The airways in the main categories of diseases differ in the nature and extent of inflammatory cell infiltrate (eosinophils or neutrophils), degree of gland cell enlargement and goblet cell hyperplasia, increased smooth muscle mass, reticular basement membrane thickening, loss of alveolar attachments, and destructive airspace enlargement (3-5). In the case of chronic airway diseases, the remodelling accompanies with vascular alteration, caused by multiple mechanisms acting in concert, as growth factors and cytokines released from various sites of airway and vascular walls (6, 7). Most of the literature regarding angiogenesis and bronchial vascular remodelling in chronic airway inflammation arises from studies on asthmatic patients (8-12). Interestingly, it has been recently shown that bronchial vascular changes may also occur in COPD (13-15). It is reported that bronchial microcirculation can develop qualitative and quantitative alterations such as vasodilatation, increased permeability and angiogenesis. Currently, it is not fully known whether or not these changes are always present and have a precise temporal sequence. However, microvascular changes in asthma and COPD may contribute to an increase in airway wall thickness which may be associated with disease progression (7). This review focuses on the morphological aspects of the vascular component in airway wall remodelling in asthma and COPD.

Bronchial microcirculation of asthmatic and COPD patients can develop alterations such as vasodilatation, increased permeability and angiogenesis.

Angiogenesis and bronchial vascular remodelling in asthma

Bronchial microvasculature plays several functions that are essential for maintaining homeostasis. Provi-

Enhanced vascularity of the medium airways contribute to airflow limitation in patients with asthma.

tion of oxygen and nutrients, temperature regulation, humidification of inspired air (16) are only some examples. For this reason, researchers are focusing a lot of attention on the vascular component of airway remodelling in asthma. There is evidence that structural vascular changes may significantly occur in the mucosa of the asthmatic airways. In asthma, the increase of vessel caliber through vasodilation, together with angiogenesis and interstitial edema caused by microvascular leakage may lead to a change in airway microvasculature (13, 17, 18). Furthermore, in asthmatic patients the airway microvasculature changes are associated with an increase in airway blood flow (Q_{aw}) and blunted β_2 -adrenergic vasodilator responsiveness (19), suggesting the presence of endothelial dysfunction whose role in the pathogenesis of bronchial asthma is not clear yet (20, 21). In asthmatic airways, a link between airway inflammation and changes in microvasculature has been suggested (22, 23). Many mediators and growth factors which are directly related to airway inflammation in asthma, such as histamine, the major preformed mast cell mediator, prostaglandins, leukotrienes, and cytokines, seem to be also involved in the induction of vascular responses, such as angiogenesis, vasodilation and microvascular leakage (24, 25). However, this interpretation mostly derives from *in vitro* studies or from studies on model animals. The only growth factor whose role has been established through *in vivo* and asthmatic airway studies is the Vascular Endothelial Growth Factor (VEGF). VEGF is a potent multifunctional cytokine with several important effects on vascularity such as acting on angiogenic sprouting as well as on vascular leakage and permeability (26). Hoshino et al. (18) showed that the levels of three angiogenic factors, VEGF, basic fibroblast growth factor (bFGF), and angiogenin were significantly increased in the airways of asthmatic subjects compared to controls. Moreover, the authors found a significant correlation between microvasculature and the amount of angiogenic factors. Another interesting finding of this *in vivo* study was that in the submucosa the cells resulting positive to angiogenic factors were CD34+, eosinophils, and macrophages (27). These results were successively confirmed by Chetta et al. (28). In their study authors showed that biopsy specimens of bronchial mucosa of patients with mild-to-moderate asthma had an upregulation of VEGF expression of compared to controls (28). They also found that VEGF was related to the number of vessels and to the thickness of the basement membrane (28). Using colocalization analysis, they showed that main cellular source of VEGF were mast cells, thus supporting the idea these cells, though release of VEGF, contribute to the angiogenesis process in asthma. Mast cells are a heterogeneous group of cells and can be divided into two groups depending on the protease enzymes that express: only tryptase or only chymase, and tryptase plus chymase. In asthmatic subjects, it has demonstrated

that about the 25% of mast cells expresses only chymase (29). Another interesting finding obtained with colocalization analysis on the bronchial mucosa showed a close relationship between chymase positive mast cells and VEGF (29), suggesting that chymase positive mast cells may play a role in the vascular component of airway remodelling in asthma. It has been suggested that remodelling process might be beneficial in airway disease and that airway wall-thickening protects against airway narrowing and air trapping by making the airway wall stiffer (30). However, the detrimental effects seem to be more important than the protective ones. It is well known that remodelling may increase the irreversible component of airway obstruction, accelerate the decline in pulmonary function, and facilitate the persistence of airway hyperresponsiveness, the loss of smooth muscle stretch relaxation, the increase in contractile response, and the loss of elastic recoil (3). The clinical relevance of airway vascular remodelling remains to be determined. The results concerning the effects of airway microvasculature on clinical features and pulmonary function in asthma are still scarce. In literature, some studies (10, 13, 25) showed a direct relationship between an increase in microvasculature, expressed as the density of vessels, and the severity of asthma. In this sense, the airway wall of severe asthmatics was more vascularized than that of mild-to-moderate patients. Orsida et al. (31), in a study including patients both having and not having steroid treatment, showed that the vessels density was weakly related to bronchial hyperresponsiveness to methacholine, and that vessel density was greater in those patients with the most marked hyperresponsiveness. In the same study he also showed a significant positive correlation between percentage change in forced expiratory volume in the 1st second (FEV_1) after bronchodilator and number of vessels/ mm^2 for the asthma patients overall. Taken together these findings suggest that the increased microvasculature may have pathophysiological relevance in asthma. Another notable result derives from the study by Hashimoto et al. (13), in which the relative impact of the increased microvasculature of medium and small airways on airflow limitation was assessed in nine asthmatic patients who underwent lobectomy or pneumonectomy for a solitary peripheral carcinoma. In this study it was found that the FEV_1 , expressed as a percentage of predicted value, had a positive correlation with the vascularity in the inner area of the medium airways, but not of the small ones. Even though correlation does not imply causality, however the results of the Hashimoto study seem to support the idea that the enhanced vascularity of the medium airways contributes to airflow limitation in patients with asthma. Ricciardolo et al. (32) recently remarked the role of VEGF, investigating the expression of bradykinin receptors in bronchial biopsies of asthmatic and COPD patients, the association with angiogenic factors, and the capability of bradykinin to release VEGF. The expression of bradykinin receptors was found to be positively correlated to vascular remodelling in asthma, suggesting a role for bronchial asthmatic fibroblasts in releasing vascular growth fac-

tors (VEGF and angiogenin) upon activation of bradykinin receptors (32).

Angiogenesis and bronchial vascular remodelling in COPD

The structural changes that characterize COPD remodelling are epithelial damage, mucus gland hyperplasia, subepithelial collagen deposition, increased smooth muscle, and vascular alterations (3). Differently to asthma, in COPD the vascular airway remodelling seems to be less represented as well as angiogenesis. The *in vivo* studies concerning vascular changes of the bronchial wall in COPD airways show dissimilar results. Kuwano et al. (33) in their work did not find any significant difference in vascular area between mild COPD patients and controls. On the other hand, more recent studies by Hashimoto et al. (13), Calabrese et al. (14), and Zanini et al. (15) have shown an increase of the vascular area of the airway wall in COPD patients.

The association between the vascular component of airway remodelling and the bronchial expression of VEGF and TGF- β may contribute to the development of airway obstruction in COPD.

In particular, Hashimoto et al. (13) showed that in COPD patients the vascular area was increased in the small airways, but not in the medium airways and no correlation was found between the degree of vascularity and airflow limitation. In his study, Calabrese et al. (14) examined mucosal microvasculature in large airways of current COPD smokers finding a significant difference in their vascular area compared to controls. Even in this study no correlation between microvasculature data and clinical and functional data was observed, as well as between symptomatic smokers with normal function and symptomatic smokers with moderate COPD. Overall, the authors showed that the increase of the bronchial vascularity was associated with higher cellular expression of VEGF and vascular expression of $\alpha v\beta 3$ integrin (14). $\alpha v\beta 3$ integrin is an adhesion molecule that is upregulated in new vessel proliferation in response to angiogenic stimuli, while it is downregulated or absent on resting endothelium (34). Zanini et al. (15) assessed bronchial vascular remodelling in the central airways of COPD patients and showed that in these subjects the bronchial vascular area and vessel size were increased. Furthermore, they observed an increased expression of VEGF, bFGF, and transforming growth factor-beta (TGF- β) in COPD patients, as compared to controls, providing the evidence of the strong relation between the vascular component of airway remodelling and the bronchial expression of VEGF and TGF- β . Supporting these research, Soltani et al. (35) have shown that airway remodelling in smokers and in patients with mild-to-moderate COPD was associated with fragmentation of the reticular basement membrane and altered distribution of vessels in the airway wall. The COPD patients, both current smokers and

ex-smokers, had a major fragmentation and splitting of the reticular membrane compared to healthy nonsmokers. Also the number of vessels staining for VEGF in the reticular basement membrane was higher in both groups of COPD patients, compared to healthy nonsmokers and these morphological features may have potential physiological consequences in COPD. In that study (35), the authors found that in current smokers with COPD, VEGF vessel staining positively correlated with FEV₁, expressed as percent of predicted value. Similarly to what was found in asthmatic airways, VEGF also seems to be a protein crucially involved in the vascular remodelling of COPD. In a more recent work, the same group observed that also mast cells (36) and TGF- $\beta 1$ activity (37) are involved in the increased angiogenesis in the reticular basement membrane in COPD patients. Kranenburg et al. (38) showed that COPD airways had a higher expression of VEGF in several cell populations, such as in the bronchial, bronchiolar, and alveolar epithelium and in bronchiolar macrophages, as well as airway smooth muscle and vascular smooth muscle cells in both the bronchiolar and alveolar regions. They also found that the expression of VEGF in bronchial mucosal microvessels and airway smooth muscle cells, bronchiolar epithelium, and medial vascular smooth muscle cells of larger pulmonary arteries associated with bronchiolar airways was inversely related to FEV₁. Although association does not establish causality, taken together, these findings strongly suggest a role for VEGF in airway and vascular remodelling, and thereby in the development of airway obstruction in COPD. Vascular structural changes in airway mucosa of COPD patients do not seem to imply any quantitative change in *Qaw*, even if qualitative changes may occur. Also in this case there is a difference between asthma and COPD: while in asthmatic patients *Qaw* levels were increased (19), patients with COPD may show normal *Qaw* levels. *Qaw* was measured in age-matched healthy current smokers, healthy ex-smokers, ex-smokers with COPD, and healthy lifetime nonsmokers and it did not significantly differ among groups (39). The authors also found that Salbutamol inhalation increased *Qaw* significantly in lifetime nonsmokers and healthy ex-smokers, but not in healthy current smokers and ex-smokers with COPD (39). Similar results were found by Paredi et al. (40), who showed that *Qaw* was similar in patients with COPD compared to controls, and that, after inhalation of 200 mcg of Salbutamol, it was significantly higher in controls. Both these studies (39, 40) demonstrate that cigarette smoking as well as COPD are associated with a lower vasodilator response to inhaled Salbutamol, and that this may be interpreted as an expression of endothelial dysfunction. More recently, our group hypothesized that the increased vascularity and angiogenic factors in the central airways of COPD patients may impact on the maturation of dendritic cells (DCs) (41). In bronchial biopsies from large airways of COPD patients, we showed a reduced maturation of DCs, that was strictly associated with the number of vessels and the expression of angiogenic factors such as VEGF and TGF- β , suggest-

ing that angiogenesis in the airway wall might be associated with altered trafficking of DCs in the lungs (41).

Significance of the vascular component of airway remodelling

It is well known that changes in bronchial microvasculature may result in airway wall thickening and in the reduction of the luminal area.

The functional effects of vascular remodelling may be amplified by pre-existing airway wall architecture modifications, both in asthma and COPD.

Several studies in asthma and COPD showed a significant correlations between the morphological or biological data (number of vessels, vascular area, expression of angiogenic factors) and lung function parameters, such as FEV₁ (13, 27, 31, 38, 42, 43), and airway hyperresponsiveness (31, 42). The functional effects of vascular remodelling may be amplified by pre-existing airway wall architecture modifications, such as bronchial mucosal thickening, cellular infiltration, collagen deposition and bronchial smooth muscle changes (16, 44). The thickening of each of the three layers composing the airway wall can have a different effect on its functionality (44). The thickening of the inner airway wall layer (epithelium, lamina reticularis, and loose connective tissue between the lamina reticularis and the airway smooth muscle (ASM) layer) can lead to the amplification of the effect of ASM shortening; the thickening of the outer (or adventitial) layer could decrease the static and dynamic loads on the ASM; and an increase in the ASM layer thickness can increase the strength of the muscle. Also the remodelling of the connective tissue in the smooth muscle compartment could vary the amount of radial constraint provided to the ASM, and the thickening and fibrous connective tissue deposition in all layers could decrease airway distensibility and allow ASM adaptation in shorter lengths (44). Hypertrophy and hyperplasia of mucous glandular structures and loss of alveolar attachments are

Microvascular changes in the bronchial airway mucosa are probably the consequence of the action of many angiogenic factors, but VEGF seems to have a pivotal role.

considered to be the main structural changes of airway remodelling in COPD and they may play a key role in the functional effects of airway remodelling (45). Lastly, we can here only point out the important role of small airways. Despite a considerable amount of interest in small airways disease since the 1960s, these airways remain a difficult area to study because of their relative inaccessibility and lack of a readily available, reproducible, and noninvasive technique to assess their function (5, 46). However, in COPD patients the exact explanation for the link between structural airway changes, with particular reference to the vascular component, and the functional and clinical consequences are not yet clearly defined and further studies are needed.

Conclusions

In the last two decades, researchers have put a lot of effort in the investigation of the microvascular changes happening in bronchial airway mucosa during chronic inflammation, such as asthma and COPD. Up to now, most of the studies have been focused on asthma, while less work has been done in COPD. For this reason it is necessary to better differentiate the effects of smoking on bronchial microvasculature from the specific changes due to airflow obstruction. Although there are evidences suggesting that vessel changes in the bronchial wall happen both in asthma and COPD, these two diseases have distinct alterations. In fact, while angiogenesis seems to be a typical feature of asthma, in COPD little evidence supports the view that vasodilatation is prevalent. Microvascular changes in the bronchial airway mucosa are probably the consequence of the action of many angiogenic factors, and VEGF seems to be most involved in asthma and COPD, and different types of cells can play a role as the source of VEGF. The clinical and functional effects of the vascular remodelling still need to be determined both in asthma and COPD, even if some reports suggest that the vascular component of airway remodelling may contribute to worsening of airway function. There are few data regarding the effects of current therapies on bronchial vascular remodelling. Some longitudinal studies conducted on asthma, using biopsy quantification of vascular changes, showed that inhaled corticosteroids could effectively act on vascular remodelling in asthmatic airways, partially reversing microvascular changes. In COPD, evidence from a cross-sectional study suggests also that long-term treatment with inhaled corticosteroids is associated with a decrease in airway microvasculature. A more specific knowledge about angiogenic processes and their consequences in the airway wall both in asthma and especially COPD are urgently needed, as well as new therapeutic strategies for these conditions.

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