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Pulmonary vascular changes in asthma and COPD

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Abstract

In chronic lung disorders such as in asthma and chronic obstructive pulmonary disease (COPD) there is increased bronchial angiogenesis and remodelling of pulmonary vessels culminating to altered bronchial and pulmonary circulation. The involvement of residential cells such as endothelial cells, smooth muscle cells and pulmonary fibroblasts, all appear to have a crucial role in the progression of vascular inflammation and remodelling. The regulatory abnormalities, growth factors and mediators implicated in the pulmonary vascular changes of asthma and COPD subjects and potential therapeutic targets have been described in this review.

Keywords: Asthma; COPD; airway smooth muscle; pulmonary fibroblast; vascular endothelial growth factor; angiogenesis

Abbreviations:

ASM; airway smooth muscle
BM; basement membrane
BALf; Bronchoalveolar lavage fluid
CRP; C-reactive protein
COPD; chronic obstructive pulmonary disease
COX; Cyclooxygenase
ECM; Extracellular matrix
EPC; Endothelial progenitor cell
FGF; fibroblast growth factor
IL; interleukin
IPF; idiopathic pulmonary fibrosis
PDE; phosphodiesterase
PDGF; platelet derived growth factor
TGF; transforming growth factor
VEGF; vascular endothelial growth factor
$VEGF_{165}$; VEGF-A isoform 165
VSM; vascular smooth muscle

1. Introduction

Respiratory diseases are increasing cause of morbidity and mortality for all age groups and races in the changing global environment. The major chronic airway diseases include asthma and chronic obstructive pulmonary disease (COPD). Despite differences in the causal agents, both asthma and COPD exhibit various degrees of inflammatory changes, airway narrowing leading to airflow limitation and structural alterations of the pulmonary airways and vessels [1]. The existence of transient disease phenotypes which overlap both diseases and which progressively decline lung function has complicated the search for effective therapies. In both asthma and COPD there is increased angiogenesis and vascular changes in the bronchial circulation, particularly both in intimal (endothelial) and medial (smooth muscle) layers [2,3]. Asthma studies were the first to confirm that changes in the bronchial vasculature are associated with remodelling of the airway wall [4-8]. Thereafter, a number of studies reported the involvement of specific growth factors, such as fibroblast growth factor (FGF), transforming growth factor (TGF) β1 and vascular endothelial growth factor (VEGF), during vascular remodelling in asthma and COPD [9-12]. Vascular remodelling, including thickening of the intimal and medial layers due to vascular smooth muscle (VSM) cell hypertrophy, is reported to progressively decline lung function [10,13] and vascular alterations in the pulmonary circulation may impair gas exchange in the alveolar compartments that result in pulmonary hypertension in some COPD patients [14]. Previous research has focused on the contribution of recruited inflammatory cells and their secreted mediators to the development of vascular remodelling. More recently, the involvement of residential cells such as endothelial cells, airway smooth muscle (ASM) cells and pulmonary fibroblasts, all appear to have a crucial role in the progression of vascular inflammation and remodelling. In this review we addressed the issues on the regulatory mechanisms and the involved cells and their

derived mediators implicated in the pulmonary vascular changes in patients with asthma and COPD.

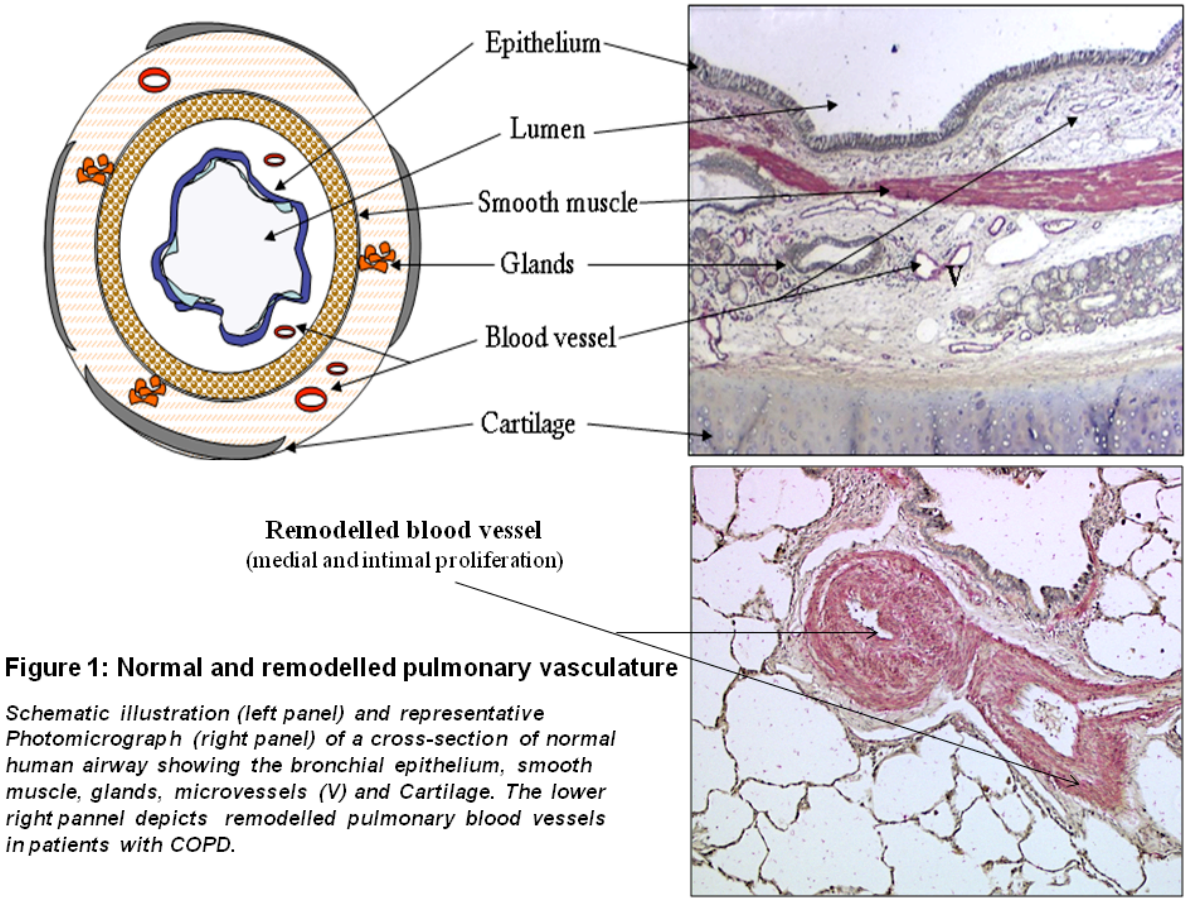

Figure 1: Normal and remodelled pulmonary vasculature

Schematic illustration (left panel) and representative Photomicrograph (right panel) of a cross-section of normal human airway showing the bronchial epithelium, smooth muscle, glands, microvessels (V) and Cartilage. The lower right pannel depicts remodelled pulmonary blood vessels in patients with COPD.

Figure 1. Cross-sections of airways with bronchial angiogenesis (microvessels) and remodelled pulmonary vasculature where vascular remodelling due to intimal and medial thickening in patients with COPD is documented.

2. The pulmonary vasculature system, cellular composition and angiogenesis

The respiratory system is permeated by a dual blood supply; the pulmonary circulation and the bronchial circulation [15]. The two circulatory systems are quite different physiologically and pharmacologically, including muscularisation at different levels and different arterial pressures: bronchial arterial (mean arterial) pressure and pulmonary artery pressure (~20 mmHg) [14,16].

2.1 The pulmonary circulation

Pulmonary arteries supply the bulk of blood flowing through the lungs. Pulmonary arteries bring relatively deoxygenated, carbon dioxide-rich blood from peripheral tissues to the lungs via the right ventricle of the heart. Pulmonary artery branches run parallel to airways to the level of the terminal bronchioles where they break off to form the capillaries in the alveolar walls. The pulmonary arteries are relatively thin-walled vessels with elastic fibres and relatively less smooth muscle than systemic arteries [16]. During hypoxic conditions in the alveolar compartments, the pulmonary arteries respond by constricting [14] causing the terminal bronchioles to dilate.

2.2 The bronchial circulation

The bronchial microvasculature plays a pivotal role in supporting airway function. The bronchial artery arises as an outgrowth from the aorta, providing oxygenated blood [16]. The vessels extend longitudinally along the airways to the lung periphery as far as the terminal bronchioles. These vessels have relatively smaller lumen and thicker walls than the pulmonary arteries associated with the same bronchus. It is a primary source of oxygenation, provides nutrients to surrounding cells and tissue and plays a role in the formation of the epithelial lining fluid. The bronchial circulation also regulates the temperature and humidifies inspired air and is the primary portal for the immune system to provide a rapid response to inhaled antigens and microbes [17,18].

2.3 Cellular composition of the pulmonary vasculature system

Mature blood vessels consist of an endothelium composed of quiescent endothelial cells sitting on a vascular basement membrane (BM). The vessel is supported by pericytes which

use web-like extremities to envelope the vessel [19] and an outer layer of vascular smooth muscle (VSM) and adventitial fibroblasts [20]. The maintenance of vessel growth and expansion within the bronchial vasculature are regulated by an array of cell types ranging from endothelial progenitor cells (EPCs), to the mural cells which support the vessels, and airway structural cells such as ASM cells and fibroblasts, as summarised in figure 2.

2.3.1. Endothelial cells, airway smooth muscle cells and fibroblasts

Endothelial cells are highly susceptible to activation and regulation by their microenvironment. In the airways, the bronchial circulation is arranged as two plexus, a submucosal plexus running through the subepithelial layer between the ASM bundles and epithelium, and a peribronchial vascular plexus which supplies the adventitia behind the ASM layer [21]. It is thought that the ASM could play an important regulatory role in the vascular remodelling within the bronchial circulatory system [22-25] via the release of different cytokines, growth factors, and the expression of adhesion molecules and deposition of extracellular matrix (ECM) components [26-29] to influence endothelial cell behaviour. The adventitia is mostly composed of alveolar sacs, which have a small layer of endothelial cells and interstitial fibroblasts. In response to tissue damage or inflammation, fibroblasts and ASM cells are also able to release angioregulatory molecules, such as VEGF, to stimulate remodelling of the vascular network [30-32]. During wound healing fibroblasts may change phenotype to a non-proliferative and extracellular matrix (ECM)-producing cell [33].

2.3.2. Pericytes and vascular smooth muscle cells

Pericytes and VSM cells act as mural cells, tightly regulating the activity of endothelial cells [34]. Pericytes not only provide a vital structural support, these cells are central to the process of angiogenesis [19]. Upon activation by inflammation, hypoxia or shear stress [32], pericytes

then activate endothelial cells to proliferate with the release of mediators [30,31], and commence the angiogenic process. VSM cells also provide support for the blood vessels, but can also activate quiescent endothelial cells by the release of pro-angiogenic factors such as VEGF, TGF-β and platelet derived growth factor (PDGF) in response to tissue damage or hypoxia [30-32,35]. Along with pericytes, VSM cells possess contractile properties for the regulation of blood flow through the pulmonary system. In response to extracellular signals the VSM cells can switch between a differentiated (contractile) state [36] and a dedifferentiated (synthetic) state, known as phenotypic switching or plasticity [37]. During the differentiated state, VSM express high levels of contractile genes and low levels of genes related to proliferation, migration and ECM synthesis, and vice versa during the dedifferentiated state. This switch is important during resolution of vascular injury and homeostasis and appears to be regulated by different miRNAs [38,39]. Chronic inflammation and tissue hypoxia are thought to induce increased vascular growth by directly effecting the VSM cells [40] the result being muscularisation and thickening of the blood vessels [13,40,41]. Factors such as tissue damage and sheer force also prompt both EPCs and endothelial cells to transdifferentiate into VSM cells [42,43]. Blood vessels of the pulmonary system are generally quite lacking in VSM at all levels of the tracheobronchial tree, however thickened bronchial vessels are reported in patients with COPD, thought to result from alveolar hypoxia [13,40]. Increased vessel muscularisation is thought to lead to increased inflammatory infiltration into the localised tissue [13,40].

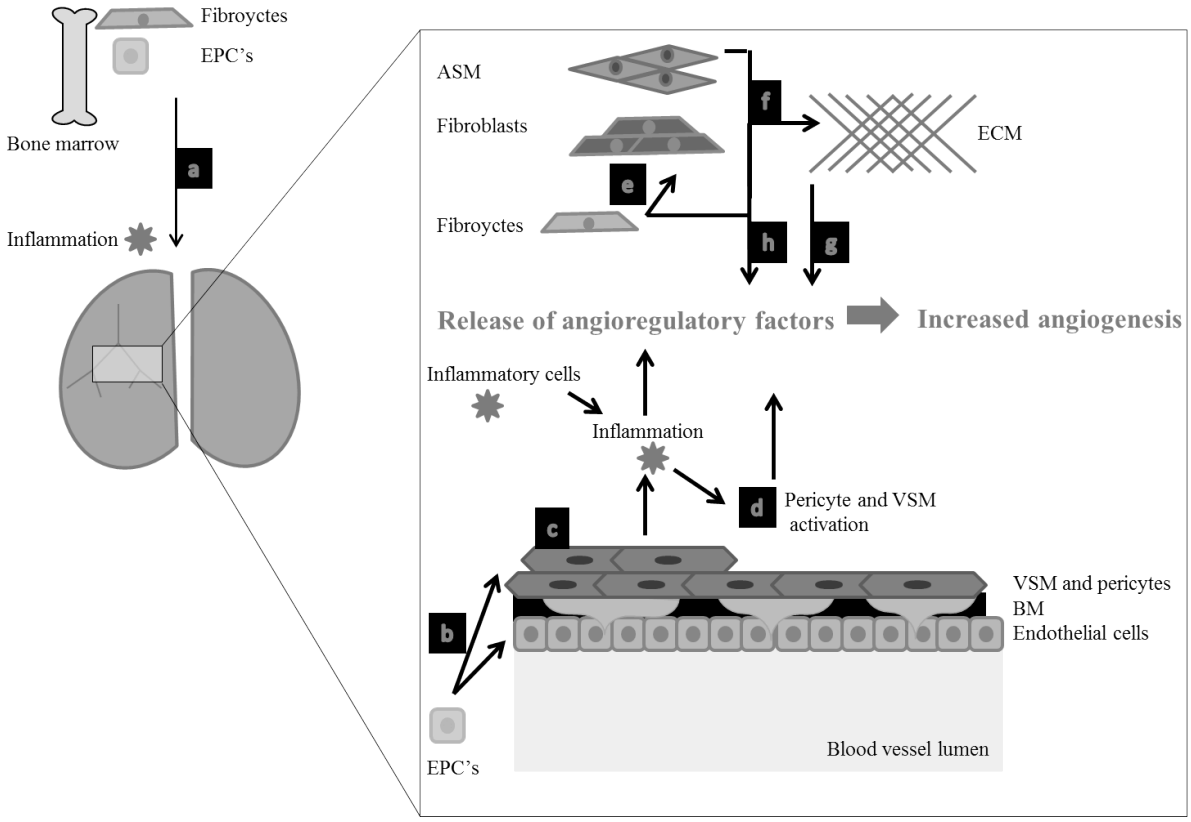

Figure 2. Cellular involvement of the vascular system. A cocktail of cellular interactions are involved in the regulation of endothelial cell behaviour and vascular growth. Under conditions of chronic inflammation multiple cell types can be activated and contribute to the stimulation and expansion of an otherwise quiescent vascular network. Chronic inflammation and pooling of potent pro-inflammatory mediators within the airway tissue stimulate increased chemotaxis of progenitor cells into the lung (a). EPCs are able to transdifferentiate into VSM cells (b), contributing to the increased muscularisation of the blood vessel wall (c), which in turns leads to increased inflammation. The source of VSM may also be VSM cells that are subject to phenotypic switching. Inflammatory mediators are also able to activate the pericytes and VSM, which surround the vessel (d) and cause the release of angioregulatory mediators inducing endothelial cell proliferation and migration and formation of an immature vessel. Fibroyctes migrate from the bone marrow, via the circulation, and transdifferentiate into fibroblasts (e) and, along with the ASM of the airway wall, increase deposition of pro-angiogenic extracellular matrix (ECM) proteins such as collagen I, II and fibronectin (f, g) at

the site of inflammation. ECM components such as heparin sulphate proteoglycans can also bind to growth factors as VEGF until required (g). The fibrocytes, fibroblasts and ASM cells themselves are also known to secrete potent proinflammatory mediators such as VEGF (h). Cellular involvement of the vascular system is very complex, with multiple cell types being a significant source of angioregulatory mediators and the system is tightly regulated by the balance of pro- and anti-angiogenic stimuli in the environment.

2.4. Angiogenic processes

Angiogenesis describes the formation of blood vessels from pre-existing ones and occurs throughout life, during wound healing and is a requirement for normal organ development [34,44]. When new capillaries are required, pro-angiogenic mediators are increased which favours angiogenesis alongside decreased levels of angiogenic inhibitors. Vascular homeostasis in healthy airways is regulated by the interaction of pro- and anti-angiogenic mediators. Several growth factors, cytokines, chemokines and other mediators have been implicated in angiogenesis and these are listed in Table 1. Dominance of angiogenic mediators is required during wound healing which are balanced by angiostatic proteins. Angiopoietin-1 (Ang-1) and -2 (Ang-2) are released from the pericytes during the angiogenic process. Ang-2 allows the migration of the immature vessel as a sprouting tip, through the localised tissue [22,23], while Ang-1 allows for the freshly formed vessel behind the sprouting tip to be stabilised with ECM deposition, formation of a new vascular BM and mural cell recruitment [24]. In fact, pericytes have the ability to sprout and form new tubes on their own, for endothelial cells to then penetrate and complete the angiogenic process [25]. However, in chronic inflammatory conditions such as asthma, the balance between angiogenic and angiostatic mediators is altered leading to unregulated vessel formation

[45,46] and it is now widely accepted that chronic inflammation is associated with angiogenesis.

2.4.1. Angiogenesis and vascular endothelial growth factor

Based on a number of clinical research studies and animal models, VEGF is the principal mediator implicated in lung neovascularisation and a potent pro-angiogenic growth factor that plays a key role in vascular remodelling both in normal and pathological conditions [13,47,48]. VEGF is a multifunctional cytokine and the VEGF family includes VEGF-A, VEGF-B, VEGF-C and VEGF-D. VEGF can induce vascular leakage, vessel permeability and angiogenesis [49]. VEGF-A isoform 165 ($VEGF_{165}$) is thought to be the most potent pro-angiogenic member of the VEGF family. VEGF induces endothelial cell proliferation and migration and is expressed in several vascularised organs including the lung [49]. Several cell populations produce VEGF including $CD34^+$ cells, macrophages, eosinophils [50], mast cells [47,50] fibroblasts [51] and ASM cells [26]. Interestingly, VEGF production by airway structural cells are induced by T_H17 cells [52] and suppressed by T_H1 cells [53]. Increased protein levels of VEGF are observed in bronchoalveolar lavage fluid (BALf), sputum and bronchial biopsies of asthma and COPD patients [11,13,48,54]. VEGF is constitutively synthesised by ASM cells *in vitro*, whereas this production can be altered in response to a number of stimuli, such as TNF-α, TGF-β1, IL-1β, angiotensin II, endothelin-1 and nitric oxide [28,55,56].

3. Vascular progenitors in pulmonary vascular changes

Vascular progenitor cells, such as EPCs and mesenchymal progenitor cells, including fibrocytes, are released from the adult bone marrow under physiological conditions, playing roles in angiogenesis and wound healing, respectively and are tightly regulated. However, in

conditions of chronic inflammation, there is increased migration and activity of these progenitor cells localised to the areas of tissue damage and the altered balance of these progenitor cells can have detrimental effects contributing to disease pathology.

3.1. Endothelial progenitor cells

EPCs are bone marrow derived-cells trafficked to the lung upon tissue injury, to play a crucial role in tissue repair [57]. These cells are able to differentiate into endothelial cells or VSM cells [42,43,58]. Increased levels of circulating EPCs have been reported in inflammatory respiratory conditions such as pneumonia or sepsis [59]. Additionally, higher levels of circulating EPCs have been found in asthmatic individuals [60], and this is also seen in the lungs of ovalbumin-sensitised mice, with EPC number increasing in response to the potent pro-inflammatory mediator TGF-β and CXCR2 [60,61]. The potent pro-angiogenic factor VEGF is a known chemo-attractant of EPCs. Levels of VEGF-A have been correlated to the number of CD34+ cells (progenitor cell marker) in the plasma of COPD patients [62]. The increased presence and persistence of circulating EPCs in patients with asthma and COPD could be significant to the increased vasculature seen in these remodelled airways.

3.2. Fibrocytes

Fibrocytes have been implicated in fibrotic airways diseases, such as interstitial lung diseases and particularly in pulmonary fibrosis (PF) [63], and have been suggested as a biomarker for idiopathic pulmonary fibrosis (IPF) severity [64]. ECM deposition by fibrocytes and fibroblasts are regulated during normal wound repair [65]. However, during chronic inflammatory processes excessive amounts of the ECM proteins collagen type-I and –II and fibronectin are deposited by fibrocytes in damaged tissue [58] which may further contribute to airway fibrosis and thickening of the airway wall. Fibrocytes also release proteases, growth

factors, and pro-inflammatory chemokines [66] including interleukin-8 (IL-8) [67,68] and VEGF [69]. Asthmatic individuals have been found to have increased levels of circulating fibrocytes [70,71], as well as more fibrocytes in the airway submucosa following an allergen challenge [72]. Fibrocytes could be playing a significant role in multiple features of the remodelled asthmatic airway. The involvement of fibrocytes in COPD pathology needs to be further evaluated.

3.3. Vascular progenitor cells

Vascular progenitor cells have been identified in pulmonary arteries of patients with COPD [73]. The endothelial layer is randomly denuded in pulmonary vessels and there is an increased amount of vascular progenitor cells in the injured endothelium in pulmonary arteries of patients with COPD [74]. These cells seem to be important for reparation of the endothelium. Changes in the number of vascular progenitor cells may lead to altered reparation capacity in the vascular wall, vascular remodelling and cardiovascular complications [75]. More studies are warranted to better understand the role of vascular progenitor cells, the mechanisms associated with their recruitment and involvement in vascular inflammation and remodelling processes.

4. Vascular inflammation and remodelling processes in asthma and COPD

4.1. Vascular inflammation and remodelling processes in asthma

Early histological studies of lung tissue taken at post-mortem from subjects who died of status asthmaticus demonstrated the presence of mucus plugs in airway passages, loss of ciliated respiratory epithelium, and thickening of BM, infiltration of eosinophils, oedema and ASM hypertrophy, now collectively termed '*airway remodelling*' [4]. Further studies in subjects with asthma were the first to confirm that changes in the airway vasculature are associated with remodelling of the airway wall [7,8]. Vessels within the submucosa plexus have since

then been reported extensively to be increased in the asthmatic airway. The subepithelial space is shortened in asthmatics and this correlates to disease severity [76], bringing the increased ASM bundles into close proximity to the submucosa plexus. Thickening of the airway wall in asthma also includes increased vessel calibre as a result of dilatation of capillary vessels, angiogenesis, oedema from microvascular leakage, increased capillary permeability and engorgement of lymphatic and blood capillaries. More recently, several reports with asthmatic subjects and in vivo animal models have confirmed these changes in the vascularisation of asthmatic airways with an increased number of vessels, increased size of vessels in the airway walls, increased vascular permeability, angiogenesis, increased vascular leakage and oedema partly due to the immature newly formed blood vessels which are leaky [5-8]. Some of these changes to the microvasculature are related to increased blood flow in asthmatic subjects [77] and associated with asthma severity [78]. The regulation of airway blood flow in mild and moderate-to-severe asthmatic subjects is different to that found in healthy airways. Basal mucosal blood flow was 1.8-fold greater in mild steroid naïve asthmatics compared to healthy non-smoking controls but in corticosteroid treated stable asthmatics, mucosal blood flow was lower and 1.4-fold greater than control subjects [77] suggesting that treatment with glucocorticosteroids may influence mucosal blood flow. Angiogenesis, vasodilation and vascular permeability are positively targeted by glucocorticosteroids [79] and the effect of glucocorticosteroids and other current therapies on features of vascular remodelling are further described later in this review.

Increased bronchial vascularisation may contribute to the increased trafficking of inflammatory cells (eosinophils, neutrophils, mast cells) observed in asthma. These changes in the microvasculature of asthmatic airways have been linked to inflammatory indices [2,80] with the number and size of airway blood vessels and levels of the pro-angiogenic mediator;

VEGF being elevated in mild-to-moderate persistent symptomatic asthmatics on inhaled glucocorticosteroid therapy compared to healthy controls. Markers of vascular permeability; perivascular albumin staining and BALf microalbumin levels were also elevated in these asthmatic subjects on high-dose inhaled corticosteroid therapy compared to healthy controls and airway albumin levels correlated with infiltrating T lymphocytes. The proportion of airway vascularity (number and size of blood vessels) and VEGF levels in this cohort of asthmatic subjects was not altered when the dose of fluticasone propionate was halved and associated with clinical asthma deterioration yet without the return of cellular inflammation [81] suggesting that vascular leakage is a feature of asthma deterioration that may precede the return of cellular inflammation.

The temporal sequence of these vascular changes and their direct impact on lung function remains to be determined. The density of vessels in the medium (2-5mm inner diameter) and small (<2mm) airways is significantly elevated in mild and moderate-to-severe asthma compared to healthy controls or subjects with COPD [7] and has been linked to asthma severity [78,82]. Furthermore, the increased density of vessels was reported to be associated with asthmatic subjects with the most hyperresponsive airways [83]. In relation to airway obstruction, as measured as the percentage predicted forced expiratory volume in the first second (%FEV_1), an inverse correlation was found with the number of vessels ($r = -0.85$) in the medium airways, but not in small airways of asthmatic subjects [7]. The temporal aspects of vascular remodelling and subsequent changes in the absence of allergen were investigated recently in an allergic sheep model [84]. This *in vivo* model demonstrates an extensive microcirculation in the small airways, which still are poorly studied unlike the large proximal airways. Using the allergic sheep model, the authors demonstrated a significant increase in blood vessel density throughout the subepithelial space and outer airway wall after 24 weeks

of weekly house dust mite challenge in a segment of lung compared to vessel density in the unchallenged control segment of the same animal and vessel density in the naïve control sheep group. Following 8 or 16 weeks of house dust mite challenge, no significant differences in vessel density were observed between the challenged or unchallenged lung segments or in the control sheep suggesting that vascular remodelling requires prolonged allergen exposure greater than 16 weeks rather than acute episodes. After a further 12 weeks of house dust mite challenge, there was a 1.27-fold increase in blood vessel density compared to the levels present in the airways at the 24-week challenge time point. However, in the pre-challenged group with a further 12 weeks of no allergen exposure, the blood vessel density continued to rise significantly by 1.17-fold compared to the 24hr challenged time-point suggesting that the increase in vessel density persists despite the lack of allergic inflammation although the total percentage of airways occupied by blood vessels was reduced in the absence of allergen exposure. Similar findings were observed in another *in vivo* model with sensitised mice after a 4-week antigen withdrawal period [85]. The mean vessel size was not found to be different between experimental or control groups in the sheep model and no significant correlations were found between vascular indices and airway function as measured by allergen-induced bronchoconstriction or airway responsiveness to methacholine [84]. The mechanisms associated with the link between repeated immunologic challenge and bronchial vascularisation are unclear. It is evident that eosinophils and chymase-positive mast cells are elevated in the airways of the house dust mite challenged sheep model [86,87] and these cells are a potential source of pro-angiogenic mediators such as VEGF, angiogenin and basic fibroblast growth factor. However, in these studies, prolonged exposure to allergen appears to be a requirement for increased airway vascularity rather than by acute challenges suggesting that inflammatory mediators required for the initiation of vessel formation and vessel maintenance must be present for a longer duration for them to significantly impact on

vascular remodelling. Interestingly, the maintenance of vascular indices in the airways despite the lack of continued allergen exposure reported in the above studies suggests that newly formed blood vessels will remain in the absence of allergic inflammation and suggests that the mediators responsible for the maintenance of blood vessels may be different to those required for initial vessel formation. Furthermore, the cells involved in initiation processes and maintenance of immature vessels may also be different with both recruited inflammatory cells and structural cells within the lung such as ASM [26] are being involved. Another possibility for the lack of reduction of vascular indices upon allergen withdrawal may be due to ECM deposition in the airways [85,88], which may be different in amount and composition in the acutely challenged airways compared to the chronically challenged airways. It would therefore be beneficial to examine the composition of ECM and levels of ECM fragments present in the airways of acutely challenged and chronically challenged animal models in future studies to identify if differences in vascular indices or the lack of reversibility upon allergen withdrawal are influenced by local ECM components or degraded ECM fragments which vary in pro and anti-angiogenic activity [88] and to evaluate these results with findings in patients with different stages of asthma. Collectively, these studies highlight that differences in vascular remodelling are apparent in acute and chronic allergen models and suggests that airway structural cells and ECM deposition may play a significant role in the maintenance of vascular remodelling after the initial inflammatory insults which may provide signals to initiate vascular remodelling but may not be sufficient to maintain it.

4.2. The role of VEGF in vascular remodelling and airway inflammation in asthma

Elevated VEGF levels have been found in sputum and BALf from asthmatic subjects compared to healthy controls as well as increased VEGF positive cells in bronchial biopsies [46,89]. Additionally, cell conditioned media from ASM cells cultured and derived from

asthmatic subjects was found to induce angiogenesis compared to ASM cell conditioned media derived from healthy controls *in vitro*, and this effect was blocked by anti-VEGF neutralising antibodies, demonstrating that VEGF was responsible for angiogenesis and implicating the ASM as a potential source of VEGF in asthmatic airways [26,46]. In addition to VEGF, angiogenin levels are also increased in BALf and in bronchial biopsies from asthmatic subjects [46,50] and are found to induce vascular endothelial cell proliferation, migration and tubule formation *in vitro* [26]. TGFβ1 and bradykinin also induce VEGF secretion in cultured human ASM cells, fibroblasts and bronchial epithelial cells [26,45,52]. In a recent study, expression of bradykinin receptors (B2R and B1R) were found to be significantly upregulated in the subepithelial space of asthmatic subjects (of mean age 62±8yr) alongside increased vessel number determined by CD31 expression. These older asthmatic patients also showed increased angiogenin levels compared to younger asthmatics (26±5yr), and age matched healthy controls [90], demonstrating that airway vascularity may further alter with age. In the same study, angiogenin expression was significantly elevated in severe asthmatic subjects compared to mild asthmatic subjects and negatively related to FEV_1. Localisation of the B2R bradykinin receptor, VEGF-A and angiogenin in asthmatic airways was found in bronchial fibroblasts. IL-32 is expressed by many cell types in the lung, including human bronchial epithelial cells. Levels of VEGF secreted by bronchial epithelial cells are significantly elevated in IL-32 knockdown cells suggesting that IL-32 may be an important regulator of VEGF [91]. In transgenic mice models, overexpression of $VEGF_{165}$ isoform in the airways results in increased angiogenesis with newly formed vessels being larger than control airways, increased myocyte hyperplasia with enlarged ASM bundles, increased oedema and inflammation with increased mononuclear cells, B lymphocytes, eosinophils and CD4+ and CD8+ T cells within 7 days of $VEGF_{165}$ overexpression via an IL-13-dependent and -independent pathway. Within this timeframe, $VEGF_{165}$ was also found to

induce TGFβ1 production and activation. After 4 months of $VEGF_{165}$ overexpression, prominent collagen deposition and features of vascular remodelling, parenchymal remodelling and physiologic dysregulation were found [92]. Physiologic responses to methacholine were also enhanced demonstrating hyperresponsive airways in the $VEGF_{165}$overexpressed mice. Interestingly, after cessation of $VEGF_{165}$, many of the VEGF-induced alterations were reversible except for the ASM and physiologic abnormalities suggesting that VEGF independent mechanism may be responsible for the observed increase in ASM mass or that the VEGF mediated effect is irreversible. In another allergic animal model, BALf from sensitised mice treated with ovalbumin had increased levels of VEGF compared to BALf from control mice. When these animals were prior treated with 2-chloroadenosine, 75% of alveolar macrophages were depleted in the BALf without affecting other cell types and with a concomitant decrease in VEGF levels by 95%. In sensitised mice, the expression of the non-coding small RNA miR-20b was significantly lower in alveolar macrophages compared to control mice and VEGF expression was negatively regulated by endogenous miR-20b. Upon ovalbumin challenge, either macrophage depletion or treatment with an aerosolized VEGF-neutralising antibody suppressed cellular inflammation, and levels of the Th2-cytokines IL-4, IL-5 and IL-13 in BALf as well as significant inhibition of airway hyperresponsiveness to inhaled methacholine implicating alveolar macrophage-derived VEGF [93].

The human VEGF gene is located on chromosome 6p21 with 140 reported single nucleotide polymorphisms (SNP). A study by Sharma *et al*., explored whether VEGF SNP were associated with childhood asthma, lung function, airway responsiveness and whether treatment with inhaled corticosteroids influenced the outcome 4 years later. They reported that of the seventeen SNPs detected in the VEGF gene, the VEGF variant rs833058 was found to be associated with asthma in both children and their families from two geographically

distinct cohorts [94]. A study by Kreiner-Møller *et al*., demonstrated that specific VEGF gene variants are associated with FEV_1 in children of school age but not at birth and independent of asthma suggesting that VEGF may be implicated in lung function development [95] and not associated with the early onset of asthma. A number of factors are thought to contribute to the development of asthma in children and progression of this condition into adulthood. Research has indicated the significance of SNP's, genetic disposition, environmental exposures, respiratory infection, and epigenetic factors could be acting together to orchestrate the development of asthma and other airway conditions.

Collectively, these studies implicate several sources of VEGF in the airways and therapeutically targeting VEGF and its receptors in combination with mediators inducing VEGF and miR-20b may be beneficial to prevent vascular remodelling and inflammation in asthma. Microvascular changes in the airways of COPD subjects have also been reported and these are discussed next.

5. Vascular inflammation and remodelling in COPD

A common co-morbidity in patients with COPD is cardiovascular disease [96,97]. Pathological changes in the pulmonary vasculature have an important role in symptoms associated with declined lung function and fibrosis in the distal parts of the lungs. This includes injuries and loss of alveolar cells (emphysema), accumulation and activation of inflammatory cells and increased ECM deposition [1].

5.1. Inflammatory cells in the pulmonary circulation

Characteristic for COPD is an increased amount of inflammatory cells in the lung, such as $CD8^+$ T-lymphocytes, neutrophils and macrophages, which can contribute to the changes in pulmonary vessels. Patients with COPD have an increased number of infiltrating $CD8^+$ T-lymphocytes in adventitia in pulmonary arteries [41]. It has been shown that cigarette smoke may increase the number of circulating neutrophils in the blood vessels and in the airways, with neutrophils assembling in pulmonary capillaries, sputum and BALf [98]. Circulating neutrophils in stable COPD patients appear to have abnormal neutrophil activity with increased production of reactive oxygen radicals. These neutrophils respond stronger to chemotactic factors and attach easier to the endothelial cell layer, which may promote on-going inflammation and emphysema formation in the lung [99]. In patients with COPD there are an increased number of macrophages in the airways, lung parenchyma and BALf and the numbers of macrophages correlates well with the different GOLD stages of COPD [100]. Cigarette smoke activates macrophages to release inflammatory mediators, reactive oxygen radicals and proteases. Macrophages from COPD patients are more active and secrete more inflammatory proteins, such as chemokines, metalloproteinases and cytokines. The increased amount of macrophages may depend on enhanced recruitment of monocytes from the circulation which been found in BALf from patients with chronic bronchitis [101]. Chemotactic factors, such as leukotriene B_4 (LTB_4) are increased in sputum from COPD patients prior to exacerbations and LTB_4 could be used as a biomarker to predict forthcoming exacerbation and worsening of COPD [102]. LTB_4 is also implicated in pulmonary vascular remodelling in patients with pulmonary arterial hypertension [103].

5.2. Inflammatory lipid mediators and remodelling

Altered function and structure of the pulmonary circulation may impair gas exchange in the alveolar compartments and result in pulmonary hypertension in some COPD patients [14].

Prostacyclin is a potent vasodilator and has anti-inflammatory properties, with prostacyclin analogues used as treatment for pulmonary hypertension [104]. Prostacyclin is generated via the cyclooxygenase (COX) pathway and is an important regulator of vascular tone and thrombocyte function [105]. The prostacyclin receptor, IP, is expressed in high amounts by endothelial cells but also by fibroblasts in the human lung [106]. Treatments with prostacyclin analogues have shown positive effects on gas exchange and improved lung function capacity in a small study with COPD patients [107,108]. Prostacyclin and other cAMP generating substances appear to have a central role in remodelling processes since prostacyclin reduces fibroblast activity and migration and reduced collagen type-I synthesis [106,109,110]. Prostacyclin may also induce synthesis of VEGF from human lung fibroblasts *in vitro* [51] and promote angiogenesis. The COX-2 pathway has been shown to play a role in VEGF-induced angiogenesis via p38 and JNK kinase activation pathways in endothelial cells [111]. *In vitro* studies with pulmonary fibroblasts from patients with IPF showed an altered balance in the synthesis of prostacyclin and thromboxane, with reduced levels of prostacyclin [112], while lung fibroblasts from patients with severe COPD (GOLD IV) synthesised an increased amount of prostacyclin [106]. Nevertheless, the distal lung fibroblasts from severe COPD patients appear to have altered fibroblast function and defect repair mechanisms in the ECM structure of the collagen network assembly in response to prostacyclin, which may thereby affect emphysema progression [106]. Taking the data together, depending on state of disease progression, prostacyclin may therefore have different outcomes. COPD patients in earlier stages of the disease and with peribronchial fibrosis may respond more favourable to prostacyclin treatment compared to severe COPD patients with emphysema. However, additional preclinical and clinical studies with patients with different disease stages of COPD are warranted to address this hypothesis. Prostacyclin is in homeostatic balance with thromboxane in the circulatory system [113,114], where thromboxane is mainly produced by

thrombocytes via the COX pathway and known as a potent vaso- and bronchoconstrictor in both human and animals [115,116]. Patients with COPD have an increased amount of thromboxane metabolites in their urine [117], probably due to hypoxia and increased platelet aggregation, which correlates with the augmented amount of plaque in the vessels of these patients [118]. These data suggest that prostacyclin and thromboxane may be important markers in vascular inflammation and regulation of ongoing ECM changes in COPD.

5.3. Vascular changes and growth factors in COPD

In COPD, vascular remodelling involves angiogenesis, heterogenic bronchial vascularisation and structural changes of the vascular wall [119]. Patients with COPD present vessels with increased arterial stiffness [96]; the vessels are less elastic and correlate with disease severity [120], probably due to altered ECM structure [121]. The systemic inflammation may affect elasticity in the pulmonary vessels and C - reactive protein (CRP; high levels indicate infection or inflammation) in serum correlate positively with arterial stiffness [122]. Also the bronchial wall is more vascularised in COPD patients compared to healthy individuals [11]. Changes in VEGF expression together with hypoxia has been shown to be important in the onset of vascular remodelling [123], and may have an important role in the pathophysiology of COPD. Basic FGF and VEGF are key mediators in angiogenesis processes in the airways and promote proliferation of endothelial cells *in vitro* [124] and may therefore have a central role in angiogenesis in COPD. FGF receptor-1 is expressed in the endothelium of the bronchial vasculature and correlates with exposure to cigarette smoke in patients with COPD [11]. Kranenburg et al. reported that the wall thickness of large vessels (>200 μm in internal diameter) is increased in airways of COPD patients. In these vessels, an increased expression of FGF-2 and its receptor was observed [10]. The same group has shown that bronchial angiogenesis is associated with enhanced VEGF expression and inversely correlated with the

lung function in patients with COPD [10,11]. Furthermore, FGF-1 and FGF-2 induce ASM cells *in vitro* to express VEGF-A and its major variants $VEGF_{121}$ and $VEGF_{165}$, followed by an enhanced release of VEGF protein that is mediated by ERK1/2 and $p38^{MAPK}$ pathways [125]. Studies show different results regarding VEGF, in some cases VEGF may be protective and in others detrimental. COPD patients with chronic bronchitis had increased levels of VEGF in sputum, whereas COPD patients with more emphysema had low levels of VEGF [126] and also show decreased expression of VEGF receptors in pulmonary arteries [127], which indicate that also VEGF may have different roles depending on disease progression and disease severity. There exists an interesting link between VEGF and prostacyclin. VEGF synthesis is increased by prostacyclin in human lung fibroblasts *in vitro* [51] and this cross talk may be important during inflammation and hypoxic conditions in the distal lung promoting angiogenesis in areas with poor circulation and ventilation. It has been shown that patients with acute exacerbations present high levels of VEGF in the circulation compared to stable COPD patients and healthy individuals [128]. In a small study with bronchial biopsies, patients without glucocorticosteroid treatment showed more bronchial vascularisation, larger vessels and more VEGF receptors expressed in the tissue compared to COPD patients that were treated with inhaled glucocorticosteroids [119]. Interestingly, VEGF has been shown to act both as a promoter of endothelial cell function and a negative regulator of VSMCs and vessel maturation in combination with PDGF [34], highlighting the complex role of VEGF in vascular remodelling. The vascular changes in COPD patients seem to depend on VEGF as an important regulator in local and systemic inflammation and in interactions with TGF-β in regulation of ECM production [1]. Based on the accumulating evidence, both VEGF, FGF-1 and FGF-2 signalling pathways are involved in the airway remodelling, at the cellular structural level, as well as vasculature level. The next question will be whether these

pathways can be used as a potential target to control the initiation, duration and outcome of vascular changes in asthma and COPD.

6. Impact of therapeutic strategies on pulmonary vascular changes

Many therapeutic approaches are currently developed for tackling airway inflammation in asthma and COPD. Therapeutic strategies that also affect the vascular remodelling are warranted and under progression [129]. Airway inflammation and perpetuation being the hallmark in vascular remodelling, most of the therapies available so far are anti-inflammatory therapies. Despite a great diversity of possible therapeutic agents, a combination of inhaled corticosteroids and long-acting β_2-adrenergic agonists remains the treatment of choice for obstructive airway diseases [130-132]. In asthmatic airways, angiogenesis, vasodilation and vascular permeability are positively targeted by glucocorticosteroids [79] and down regulate vascular remodelling by acting on proangiogenic factors, whereas long-acting β2-agonists seem to be mostly effective in decreasing vascular permeability [129,133]. However, this combination therapy is less effective in corticosteroid-resistant or severe asthmatics and COPD patients [134].

6.1 Macrolides

Interestingly, treatment with macrolides is moving into the clinical practice for chronic lung disorders. The potential of antimicrobial macrolides in treating chronic airways diseases was first discovered in a case report of a corticosteroid-dependent asthma patient [135]. This class of drugs was recently shown to be effective in (i) preventing COPD exacerbations and, (ii) in the treatment of *neutrophilic* asthma [136,137], and especially (iii) in preventing *neutrophilic* reversible allograft dysfunction. The latter disease is a subtype of bronchiolitis obliterans syndrome, the most devastating complication after lung transplantation [138]. Azithromycin

seems to inhibit the IL-17 induced IL-8 production in the airways of these patients [138,139], making it a potentially interesting anti-inflammatory drug. However, until recently the anti-angiogenic effect of macrolides in reducing VEGF production during chronic airway inflammation was not yet thoroughly explored. Azithromycin has recently been shown to reduce the activation of $p38^{MAPK}$ pathway in ASM cells treated with FGF-1 or FGF-2, leading to a significant reduction in VEGF protein release from ASM cells [140]. The MAPK pathways, in particular $p38^{MAPK}$, are also involved in the regulation of the synthesis of inflammatory mediators at the level of transcription and translation, making them potential targets for anti-inflammatory agents [140,141]. These findings suggest a potential effect of azithromycin not only as an immunomodulatory, but also as an anti-angiogenic agent, at least driven by ASM cells [125,142]. Due to their known side effects, long-term treatment with systemic corticosteroids is not advised in patients. Since azithromycin shares the same signalling pathway as dexamethasone in exerting its inhibitory effect on VEGF release by ASM cells *in vitro* [139,140], it is acceptable to hypothesize that the combined treatment of corticosteroid and azithromycin could allow a lower dosage of corticosteroids, hence decreasing their side-effects. On the other hand, however, the long-term use of azithromycin in general could enhance anti-microbial resistance to this drug. Therefore, more studies are needed to investigate the safety and effectiveness of combining both drugs.

6.2. Phosphodiesterase-inhibitors

A number of recent studies explored different therapeutic approaches using pulmonary endothelial cells *in vitro*. Intracellular cAMP levels are regulated by synthesis by adenylyl cyclases and hydrolysis by cyclic nucleotide phosphodiesterases (PDEs). Vascular endothelial cells express variable levels of PDE-2, PDE-3, PDE-4, and PDE-5 in cells derived from

different sources aorta, umbilical vein, and microvascular structures [143]. Selective PDE inhibitors are used in the treatment of pulmonary hypertension [144], which make them interesting for treatment of also other pulmonary diseases with vascular involvements. Currently, selective PDE inhibitors are in clinical trials for treatments of respiratory diseases, such as asthma and COPD [145] and a specific PDE4 inhibitor (roflumilast) is on the market for treatment of severe COPD as add-on therapy [145]. Selective inhibition of PDE-4 showed an increase in cAMP levels and inhibition of endothelial cell migration *in vitro***,** which demonstrated that PDE-4 inhibitors may serve as novel therapeutic agent to limit angiogenesis in complex human diseases like COPD and asthma. A recent study shows that PDE-4 inhibition augments prostaglandin E2 stimulated VEGF production in human lung fibroblasts *in vitro* indicating for yet another therapeutic strategy for patients with chronic airway diseases, like COPD [146]. PDE-5 inhibitors are selective blockers of PDE-5, which catalyzes the hydrolysis of cyclic guanosine monophosphate (cGMP) to its corresponding monophosphates. cGMP is a potent vasodilator and nitric oxide donor and in turn induces angiogenesis [55]. PDE-5 inhibitors increase endothelial cell cGMP and promote angiogenesis. However, not all endothelial cell phenotypes express PDE5. Zhu and colleagues studied in pulmonary microvascular endothelial cells by stable transfection with human full-length PDE5 cDNA and found that overexpression reduced the basal and atrial natriuretic peptide stimulated cGMP concentrations and displayed attenuated blood vessel network formation on matrigel *in vitro* [147]. Therapeutic treatment directed towards the control of bronchial vascular remodelling could prove to be an important step in the development of a new generation of drugs for asthma and COPD. In COPD, there is less available experimental evidence on the effect of the currently used drugs on airway microvascularity changes [129,133]. Research emerged in recent years provides evidence that by tackling an important regulator of angiogenesis and vascular remodelling, VEGF both at ligand and receptor level

could be a potential way forward in controlling the airway vascular remodelling in patients with chronic airway diseases like asthma and COPD.

7. Conclusions and a forward-looking perspective

Ongoing inflammatory processes in asthma and COPD may result in structural changes in the pulmonary vasculature and contribute to an influx of progenitors and increased synthesis of vascular mediators. However, more studies are warranted to increase the knowledge of the cells and mediators associated with the vascular changes involved in the development of airway obstruction and remodelling processes in asthma and COPD. Research areas that need further attention and where we have black holes are: I. Cellular plasticity, including phenotypic switching and recruitment of progenitor cells, and regulation of pulmonary vascular events. miRNA has an important role in molecular regulation, however which specific miRNAs that are relevant for pulmonary vascular dysfunction and resolution has to be elucidated. II. The involvement and impact of ECM proteins in cellular plasticity and interactions with inflammatory mediators needs to be highlighted in pulmonary vascular remodelling processes. III. The onset or origin of pulmonary vascular remodelling is still unclear. Current ideas suggest that obstructive lung diseases, such as asthma, may have a developmental/perinatal basis and these ideas needs to be further evaluated in asthmatic subjects and in animal models. IV. Much of the work regarding angiogenesis and vascular remodelling in asthma refers to the bronchial vasculature. So far little is known if or how the pulmonary circulation is affected in patients with asthma. Pulmonary vascular dysfunction in the peripheral airways and pulmonary circulation is complicated to investigate and relevant *ex vivo* and *in vivo* models are needed. V. Current animal models do not perfectly mimic ongoing vascular changes in patients with obstructive lung diseases. Finding better animal models that better mimic the airway pathology of those with respiratory disease under allergic

and non-allergic conditions to address the timeframe for bronchial vascularisation and the factors that regulate these processes are warranted. The allergic sheep model may be a useful tool to study the immunological and physiological mechanisms of allergic asthma and timeframes involved for structural remodelling in humans. Quite some research is going on in the field of pulmonary vascular disorders, and of importance is that some of the *in vitro* works are confirmed *in vivo*. Also more preclinical and clinical studies of patients with different stages of asthma and COPD, and in collaboration with the cardiologists, are warranted to better understand the underlying mechanisms and consequences of vascular changes in the pulmonary system with the emphasis to identify current or novel therapies that have an impact on these changes.

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