

# VEGF, Angiopoietin-1 and -2 in Bronchial Asthma: New Molecular Targets in Airway Angiogenesis and Microvascular Remodeling

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Received: July 31, 2006; Accepted: October 31, 2006; Revised: November 2, 2006

**Abstract:** Airway angiogenesis and microvascular remodeling are known features of bronchial asthma, but the mechanisms of these structural alterations are just beginning to be elucidated. Vascular endothelial growth factor (VEGF), one of the most potent angiogenic factors, stimulates endothelial cell proliferation and induces the angiogenesis. Recently, considerable attentions have been devoted to the physiological roles of angiopoietin (Ang)-1 and -2 as regulatory factors of VEGF. Ang-1 has been shown to induce the migration and sprouting of endothelial cells, and coexpression of Ang-1 and VEGF enhanced angiogenesis. In the presence of high levels of VEGF, Ang-2 also promotes rapid increase in capillary diameter, remodeling of the basal lamina, proliferation and migration of endothelial cells, and stimulates sprouting of new blood vessels. Thus, VEGF, Ang-1 and -2 may play complementary and coordinated roles in airway angiogenesis and microvascular remodeling, and these structural changes are potentially reversible by therapeutic intervention. The scope of the present review is to discuss from a clinical point of view the potential interactions between VEGF and angiopoietins in the asthmatic airways, and focus on the therapeutic implications targeting for these angiogenic factors. Recently, there is an increasing number of patents which have been focused on the inhibitors of VEGF action. These inhibitors are directed towards the receptors of VEGF or intracellular substrates for the receptors. We will also discuss several patents regarding inhibitors of VEGF action in the present review.

**Keywords:** Bronchial asthma, angiogenesis, microvascular remodeling, vascular endothelial growth factor, angiopoietin-1, angiopoietin-2, Tie-2.

## INTRODUCTION

Angiogenesis and microvascular remodeling are elements of the tissue remodeling in chronic inflammatory diseases [1]. Both types of change in the microvasculature result from endothelial cell proliferation and often occur together, but they represent different phenomena and responses to different stimuli. Angiogenesis denotes the formation of new blood vessels from pre-existing vessels. Physiological angiogenesis, which is required for embryonic development and wound healing, is characterized by tight regulation both spatially and temporally. In contrast, several pathological conditions, such as rheumatoid arthritis and bronchial asthma, are characterized by excessive angiogenesis where vessels develop in an uncontrolled or disorganized manner [2]. Angiogenic factors, such as fibroblast growth factor [3] and vascular endothelial growth factor (VEGF) [4] (Fig. 1), stimulate endothelial cells to secrete several proteases and plasminogen activators, resulting in the degradation of the vessel basement membrane, which in turn allows cells to invade the surrounding matrix. The endothelial cells migrate, proliferate and eventually differentiate to form a new, lumen-containing vessel. Finally, the endothelial cells deposit a new basement membrane and secrete growth factors, which attract supporting cells such as pericytes, ensuring the stability of the new vessel. This is a complex process that involves the concerted action of several other factors that act

on specific receptors to regulate vessel stability. Moreover, chronic inflammatory diseases evolve once it has acquired the ability to disturb the balance between the production of angiogenic and angiostatic factors, thereby promoting the angiogenic switch (Fig. 2) [5]. On the other hand, microvascular remodeling involves structural alterations of arterioles and capillaries without the formations of new vessels. As chronic inflammatory diseases evolve, the microvasculature also undergoes progressive changes in structure and function. Therefore, angiogenesis and microvascular remodeling in the airway wall may be one of the main aspects characterizing the chronic inflammatory diseases. However, the literature on angiogenesis and microvascular remodeling in human airway diseases is relatively sparse.

## ANGIOGENESIS AND MICROVASCULAR REMODELING IN ASTHMA

Asthma is a chronic airway inflammatory disease associated with airway wall remodeling. Structural alteration of airway walls is an essential feature of asthma, and includes hypertrophy and hyperplasia of airway smooth muscle, mucous gland hyperplasia, thickening of the reticular basement membrane, and qualitative and quantitative changes of airway blood vessels [6]. It was recognized many years ago that the airway mucosa in fatal asthma is edematous and contains dilated, congested blood vessels [7]. Early studies also showed that the airway wall of subjects with asthma is abnormally thick [8], a feature that has been confirmed by morphometric studies [9] and computed tomography [10]. Subsequently, it was reported that both the total number of vessels and vascular area in biopsy specimens taken from

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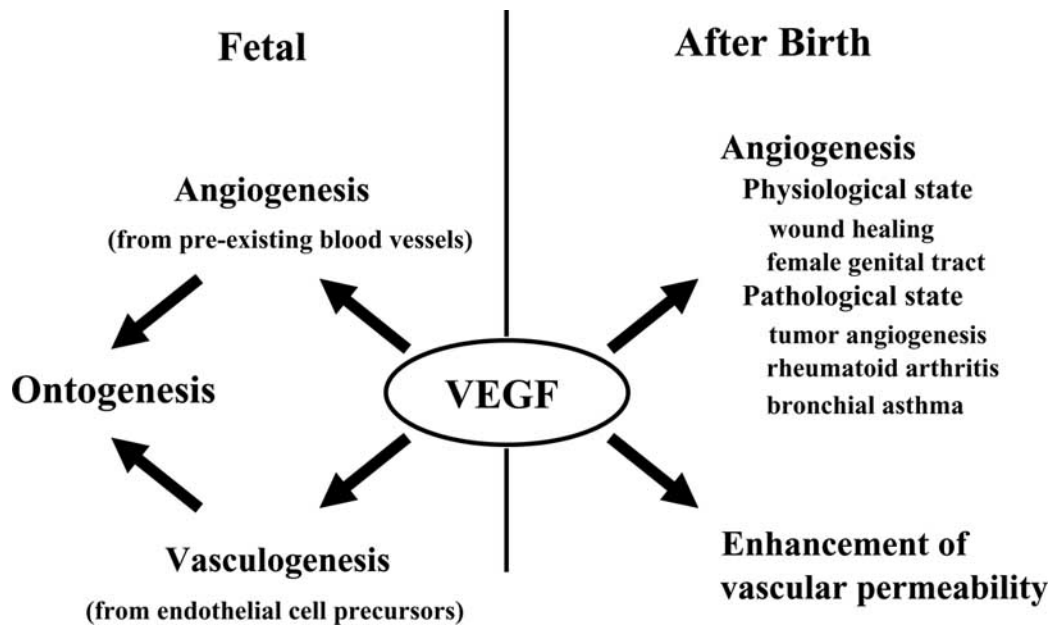


Fig. 1. Physiological roles of VEGF (vascular endothelial growth factor).

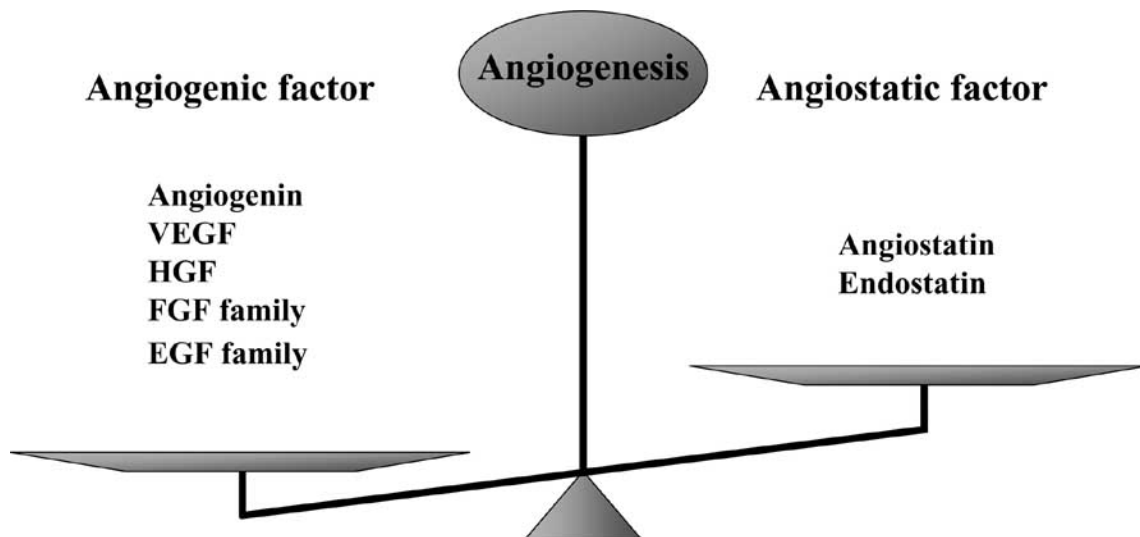


Fig. 2. Angiogenesis regulated by balance between angiogenic factors and angiostatic factors.

VEGF = vascular endothelial growth factor, HGF = hepatocyte growth factor, FGF = fibroblast growth factor, EGF = epidermal growth factor.

asthmatic patients were increased compared with those in normal control subjects [11]. These observations have shown that microvascular leakage and vasodilation lead to the wall thickness, and therefore this vascular contribution is functionally important, because even modest increase in wall thickness can amplify decreases in airway conductance produced by bronchoconstriction [12-14]. Thus, it is likely that angiogenesis and microvascular remodeling, and consequent thickening of the airway wall mucosa, contribute to the pathogenesis of asthma [15].

Several early studies of the pathology of asthma probably missed changes in the airway vasculature because the tiny mucosal blood vessels are inconspicuous in conventional histological sections. However, immunohistochemical methods using antibodies to vascular markers have made it

much easier to visualize airway vessels in bronchial biopsies and autopsy specimens, and changes in the airway microvasculature in human inflammatory airway diseases are now better documented. The presence of angiogenesis in asthma and other airway diseases is being documented by an increasing number of studies [16,17]. Furthermore, blood vessels previously described as enlarged, congested capillaries are known to be a manifestation of angiogenesis and microvascular remodeling instead of simple vasodilation [18]. The mechanism responsible for the formation of new vessels and the remodeling of the existing ones is unknown. Mediators and inflammatory cells can be involved in different ways. Among endothelial cell-specific growth factors, VEGF has shown potent and clinically relevant effects on the microvasculature. Mast cells can also play a

role in inducing the neovascularization process through the release of proangiogenic factors. Histamine, the major preformed mast cell mediator, stimulates new vessel growth by acting through H1 and H2 receptors [19], and heparin, the main glycosaminoglycan constituent of mast cells granules, possesses a proangiogenic activity [20]. The evidence that mast cells are positively related to the number of vessels in patients with asthma supports the significant roles of mast cells in angiogenesis. Moreover, mast cells produce and secrete VEGF, which has been shown to stimulate mast cell migration at sites of angiogenesis [21]. Nonetheless, the mechanism and therapeutic implications of alterations in airway blood vessels are just beginning to be elucidated, and changes in the microvasculature still represent an important gap in the understanding of the pathophysiology of asthma.

### VEGF IN ASTHMA

VEGF has clinically relevant actions on the microvasculature in the airway of asthmatic subjects. VEGF is expressed by epithelial cells of the distal airways in the fetal and postnatal lung [22,23]. Hypoxia, numerous growth factors, and inflammatory cytokines regulate VEGF expression [24]. VEGF has several isoforms that are produced by alternative splicing of the primary mRNA. The splice variants are differentially expressed in lung development and injury [25]. VEGFR1 and VEGFR2 are the two main receptors for VEGF signaling in human airways [26]. These receptors are regulated by autocrine and paracrine mechanisms. Both receptors are located on the vascular endothelium, but have different functions *in vivo*. VEGFR2 has been demonstrated to be the active receptor involved in the mediation of major growth and permeability actions of VEGF, whereas VEGFR1 has been postulated to act as a modulating decoy to VEGFR2, thereby inhibiting VEGFR2-VEGF bindings. With the use of receptor knockout models, the VEGF receptors have been found to play important roles in regulation of endothelial cell proliferation and vessel formation [27]. Indeed, VEGFR1 inhibits the excessive proliferation and migration of vascular endothelial cells, whereas VEGFR2 allows the proliferation and differentiation of vascular endothelial cells.

Angiogenesis is a complicated multi-step process, which includes the dynamic changes of cell-cell and cell-matrix interactions, endothelial cell proliferation and migration, recruitment of the perivascular supporting cells, and the maturation process. These steps are strictly regulated by several angiogenic factors (Fig. 3). VEGF is one of the most potent angiogenic factors, and stimulates endothelial cell proliferation and induces angiogenesis. In fact, VEGF administration can initiate vessel formation, but by itself promotes formation of only leaky, immature and unstable vessels [28]. VEGF is widely expressed within many different highly vascularized organs, including the lung [29]. The possibility that VEGF plays an active role in the angiogenesis and microvascular remodeling process was recognized by Hoshino and coworkers [30], who found that VEGF expression increases in the airways of subjects with asthma and correlates with mucosal vascularity. Moreover, we recently found that VEGF level in induced sputum from asthmatic patients is increased compared with that in normal control subjects, and that it is inversely correlated with degree of airway obstruction [31]. Angiogenic sprouting is perhaps the predominant mechanism by which a vascular bed such as the airway vasculature is thought to grow [32]. Immunohistochemical evidence from previous studies suggests that this may be true in the airways [33]. The classic sprouting process involves endothelial cell migration, proliferation, and tube formation [34]. VEGF contributes to this process by stimulating vascular splitting and sprouting.

### ANGIOPOIETIN-1 AND-2 IN ASTHMA

The Tie (tyrosine kinase with immunoglobulin-like loop and epidermal growth factor homology domain) represents a novel class of receptor tyrosine kinases that are mostly expressed by vascular endothelial cells. There are currently two known members in this class: Tie-1 and Tie-2 [35]. During embryonic development, endothelial cells express both Tie-1 and Tie-2 receptors. Tie-2 receptor is also expressed in quiescent endothelial cells in adult tissues, and is essential for angiogenesis. Given that Tie-2 receptor mRNA and protein are most abundant in the lung, we believe that the lung is uniquely dependent on Tie-2 signaling [36].

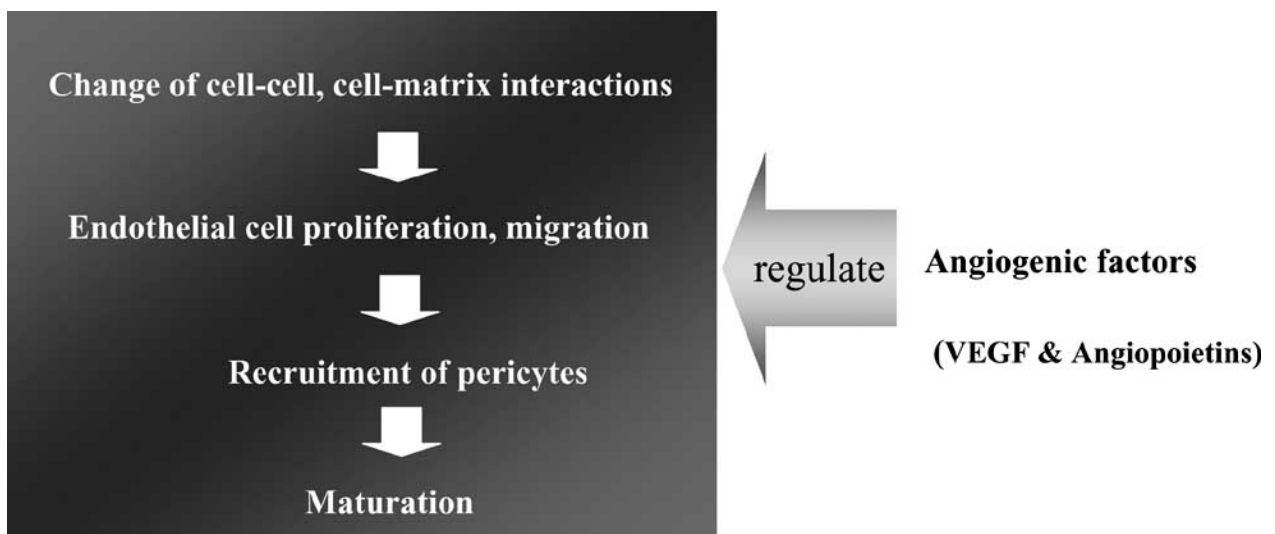


Fig. 3. Multi-step mechanism of angiogenesis regulated by angiogenic factors.

The two of the ligands for Tie-2 receptor are angiopoietin-1 and -2 (Ang-1 and Ang-2), both of which bind to Tie-2 receptor with equal affinity but result in distinct effects. Ang-1 and -2 are nearly 70 kDa with a considerable sequence homology, which consists of a single peptide, an N-terminal coiled-coil domain, a short linker peptide region, and a C-terminal fibrinogen homology domain [37]. The coiled-coil region is responsible for dimerization or multimerization of angiopoietins, and the fibrinogen homology domain binds to Tie-2 receptor [38]. Both Ang-1 and -2 form dimers and oligomers [39]. Furthermore, expression profiling studies have shown that the main sources of Ang-1 and -2 are pericytes and endothelial cells, respectively [40]. Ang-2 levels can be transcriptionally and post-transcriptionally regulated by hypoxia or exposure to growth factors, such as VEGF [41]. In addition, Ang-2 protein is stored inside endothelial cells and can be secreted within minutes after stimulation by thrombin or histamine [42].

Ang-1 is essential for development of the vasculature but in a different way than VEGF. Ang-1 is a ligand of the endothelial cell-specific tyrosine-kinase receptor Tie-2 and is an essential mediator of angiogenesis [43]. Through activation of the Tie-2 receptor and stimulation of the Akt/survivin pathway, Ang-1 protects against endothelial cell apoptosis via phosphatidylinositol 3-kinase/Akt pathway and also functions to recruit and sustain peri-endothelial supporting cells, allowing endothelial cells to stabilize the structure and modulate the function of blood vessels [44]. Several studies have offered possible mechanisms for the proangiogenic effect of Ang-1. Although Ang-1 does not stimulate the proliferation of endothelial cells, it stimulates endothelial cell migration, induces the capillary-like tubule formation, and promotes survival of endothelial cells. Ang-1 induces tyrosine phosphorylation of Tie-2 receptor and promotes recruitment of the pericytes and smooth muscle cells, thereby playing a role in establishing and maintaining the vascular integrity and quiescence. The transgenic mice overexpressing Ang-1 displayed increased vascularization and

decreased adult vasculature leakage. Together, those results indicated that Ang-1 plays indispensable role in the formation of blood vessels by recruiting and maintaining peri-endothelial supporting cells. Mice lacking Ang-1, or its tyrosine kinase receptor Tie-2, die because primitive endothelial cell tubes do not evolve into mature vessels [45]. Thus, Ang-1 appears to be essential for maturation of the vasculature from primitive tubes into a hierarchical network of vessels composed of endothelial cells and pericytes or smooth muscle cells. Therefore, Ang-1 is known to stabilize nascent vessels and make them leak-resistant, presumably by facilitating communication between pericytes and endothelial cells [46]. On the basis of these findings, the mechanism of vessel maturation by Ang-1 is clear.

A second angiopoietin, Ang-2 antagonizes the effects of Ang-1 on Tie-2 receptor and in some contexts acts as a natural inhibitor of Ang-1 (Fig. 4) [47]. Whereas Ang-1 is widely expressed in normal adult tissues, Ang-2 is expressed mainly at sites of vascular remodeling such as chronic inflammation or tumors [48]. Ang-1 induces the autophosphorylation of Tie-2 receptor while Ang-2 is capable of competitively inhibiting this kinase activation. As an antagonist of Ang-1, Ang-2 competes with Ang-1 for binding of Tie-2 receptor, blocks the phosphorylation of Tie-2 receptors induced by Ang-1, and loosens the interactions between endothelial and perivascular supporting cells and extracellular matrix. Targeted disruption of Ang-1 and Tie-2 receptor and over-expression of Ang-2 resulted in embryonic death with the similar vascular defects. Those mice have normal primary vascular development, but the remodeling and maturation of the vasculature are defective.

#### POTENTIAL INTERACTIONS BETWEEN VEGF AND ANGIOPOIETINS IN ASTHMA

Angiopoietins play important roles in the angiogenesis and microvascular remodeling process [49]. Ang-1 is important in maintaining the quiescence and stability of the mature vasculature. In the adult, disruption of Ang-1

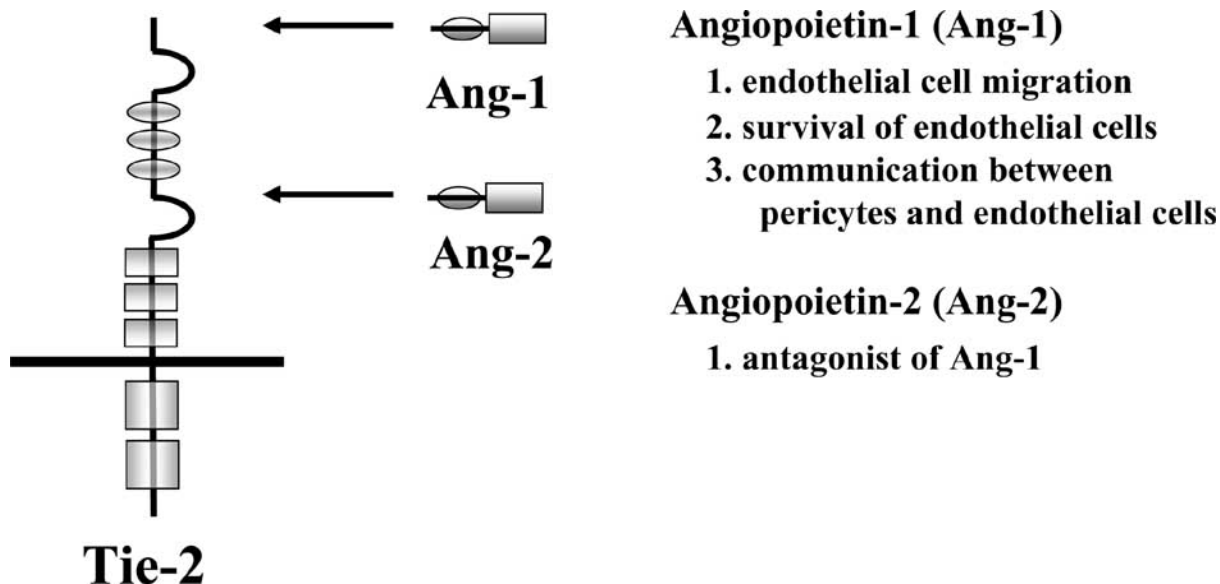


Fig. 4. Physiological roles of Ang-1 and -2 via Tie-2 receptor. Ang-1 = angiopoietin-1, Ang-2 = angiopoietin-2.

stabilization corresponds to the re-initiation of vascular remodeling under the influence of coincident VEGF (i.e., increased sprouting), as occurs in the estrous adult female reproductive system or in tumors. However, the role of Ang-2 appears to be more complex (Fig. 5). In the presence of low levels of VEGF, Ang-2 acts as an antagonist of Ang-1 and destabilizes vessels, ultimately leading to vessel regression. In contrast, in the presence of high levels of VEGF, Ang-2 facilitates vascular sprouting [50]. Therefore, we hypothesized that interaction between VEGF and angiopoietins concomitantly play an important role in airway remodeling, particularly in angiogenesis and microvascular remodeling. Although the importance of both the angiopoietins and VEGF synergistically affecting angiogenesis has been established, angiopoietins have remained largely unexplored in asthma.

Recently, Feltis *et al.* have examined VEGF and Ang-1 in the airways of subjects with asthma and contrasted these results with findings in normal controls [51]. They obtained bronchial biopsy and bronchoalveolar lavage (BAL) samples from 35 subjects with mild to moderate asthma and from 22 normal control subjects. And then they performed immunohistochemistry and image analysis to obtain quantitative measures of VEGF and Ang-1 staining in airway biopsies, and enzyme-linked immunosorbent assay (ELISA) to assess VEGF concentration in the BAL fluid. As a results, VEGF staining and VEGF levels in BAL fluid were elevated in the airway of subjects with asthma and were related to the number of vessels; Ang-1 staining was similarly increased. Moreover, angiogenic sprouts (early-forming vascular structures) were increased in number in subjects with asthma. These findings suggest that VEGF and Ang-1 are likely to be important in vascular changes in the airways of patients with asthma. Furthermore, there are observable structures in the vessel walls of asthmatic airways that could present ongoing evidence of increased angiogenic activity. We also examined VEGF and Ang-2 levels in induced

sputum obtained 17 asthmatic patients and 10 normal control subjects [52]. Both levels were significantly higher in asthmatics than in normal controls (Table 1). Moreover, Ang-2 levels were significantly correlated with VEGF levels. Our findings suggest that interaction between VEGF and Ang-2 in asthmatic airways may exist.

**Table 1. Concentration of VEGF and Ang-2 in induced sputum**

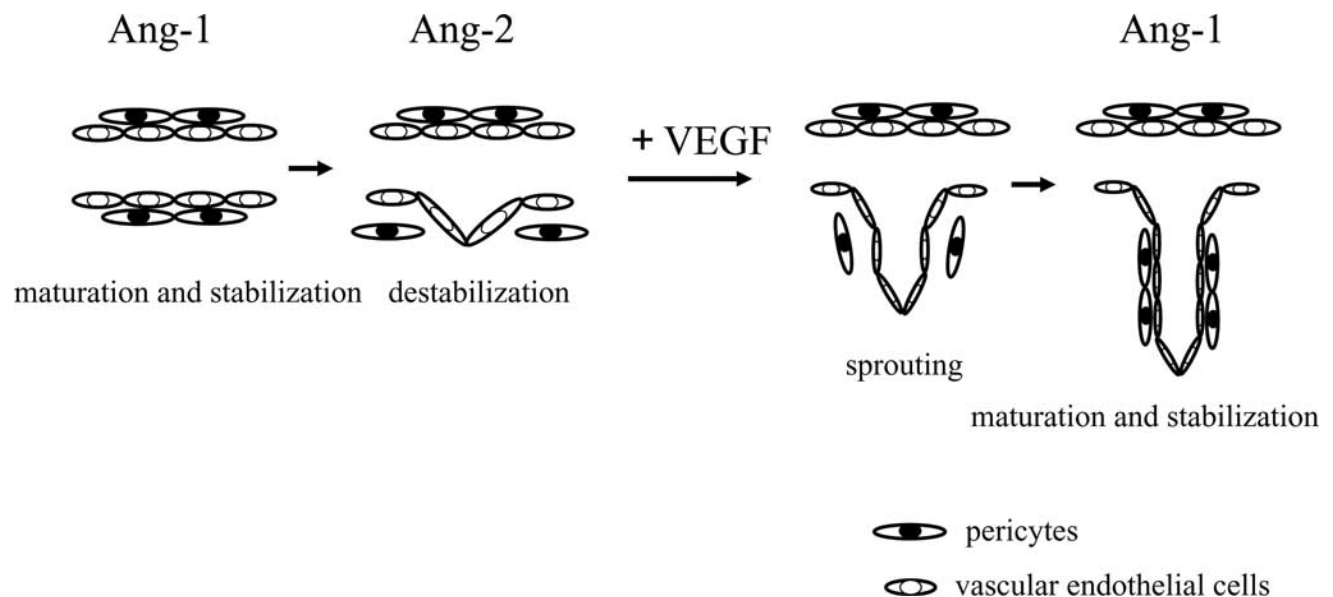
	Normal controls (n=10)	Asthmatic patients (n=17)
Before BDP therapy		
VEGF in sputum (pg/mL)	1480 (1290)	6680 (1850)
Ang-2 in sputum (pg/mL)	230 (220)	750 (460)
VEGF/Ang-2	5.4 (3.5)	11.6 (6.3)
After BDP therapy		
VEGF in sputum (pg/mL)	N.D.	4100 (1750)*
Ang-2 in sputum (pg/mL)	N.D.	750 (440)
VEGF/Ang-2	N.D.	6.5 (3.3)*

Abbreviations: BDP = beclomethasone dipropionate, VEGF = vascular endothelial growth factor, Ang-2 = angiopoietin-2, N.D. = not determined.

All data represent mean (SD).

\* p < 0.01 compared with pre-BDP therapy.

Studies of the mechanism of the angiogenic effects of Ang-1 and -2 are just beginning. Angiopoietins and VEGF apparently act in a complementary and coordinated manner



**Fig. 5.** Interaction between VEGF, Ang-1 and -2 in the asthmatic airways. VEGF = vascular endothelial growth factor, Ang-1 = angiopoietin-1, Ang-2 = angiopoietin-2.

in the process of angiogenesis and microvascular remodeling. In the presence of high levels of VEGF, Ang-2 promotes rapid increase in capillary diameter, remodeling of the basal lamina, proliferation and migration of endothelial cells, and stimulates sprouting of new blood vessels. On the other hand, Ang-2 has been reported to promote endothelial cell apoptosis and vessel regression in the presence of low levels of VEGF [53]. Holash *et al.* reported that vessel regression by an Ang-2-dependent apoptotic mechanism seems to be involved in disrupted interactions between endothelial cells and the surrounding extracellular matrix and supporting cells [54]. Thus, it is of importance to evaluate the balance between VEGF and Ang-2 levels in asthmatic airways. Therefore, we used VEGF/Ang-2 ratio as one tool to indicate the balance between VEGF and Ang-2 levels. We have already determined that the VEGF/Ang-2 ratio was higher in asthmatic patients than in normal controls. These findings suggest that higher levels of VEGF/Ang-2 may play important roles in stimulating the sprouting of new blood vessels in asthmatic airways. On the basis of these findings, we speculate that high levels of VEGF and Ang-2 in asthmatic patients indicate that blood vessels in the asthmatic airways are in a hypervascularized, destabilized state, and that this contributes to upregulation of airway vascular hyperpermeability.

#### THERAPEUTIC INTERVENTION FOR ANGIOGENESIS AND MICROVASCULAR REMODELING IN ASTHMA

In lung-targeted VEGF transgenic mice, VEGF induced an asthma-like phenotype with microvascular remodeling [55]. They established the reversibility of VEGF-induced alteration, since angiogenesis had returned to basal levels by cessation of transgenic VEGF elaboration. These findings indicate that even severe structural remodeling of the airway microvasculature is reversible in animal models. Related findings in subjects with asthma suggest that some reversal is also possible in human airway diseases [56].

Current guidelines for asthma recommend inhaled corticosteroids as first-line control therapy for asthma. Inhaled corticosteroids are able to downregulate several airway inflammatory cytokines, and to reduce cell infiltration of bronchial walls. Thus, inhaled corticosteroids are the most effective anti-asthma drugs because they act on airway cellular inflammation [57] and may reduce the thickness of the basement membrane [58]. However, to date, studies on the effect of inhaled corticosteroids on angiogenesis and microvascular remodeling in patients with asthma are scant. In a cross-sectional study, Orsida and coworkers found that patients with asthma who did not receive inhaled corticosteroids did not differ from those who received inhaled beclomethasone dipropionate (BDP) in terms of number of vessels in the airway wall [59]. However, patients with high dose of inhaled steroids tended to have a reduced number of vessels, and in the overall group of treated patients the percentage of vascular area are inversely related to the dosage of the drug. In a controlled longitudinal study, Hoshino and coworkers showed that a 6-month treatment with a daily dose of 800 µg BDP significantly reduced the number of vessels and the vascular area in patients with asthma [60]. Moreover, Chetta *et al.* showed that in patients

with mild to moderate asthma, high dose of inhaled fluticasone propionate given over 6 weeks can significantly affect airway remodeling by reducing both submucosal vascularity and basement membrane thickness [61]. 2-adrenergic receptor agonists are commonly used in the treatment of chronic airway diseases and are known to inhibit plasma leakage evoked by a variety of stimuli [62]. Moreover, the long acting 2-agonist salmeterol may reduce angiogenesis and microvascular remodeling in the airways of individuals with asthma [63].

Leukotriene receptor antagonists have been suggested both as suitable monotherapy and add-on therapy to inhaled corticosteroids for the treatment of asthma. The cysteinyl leukotrienes induce bronchoconstriction, mucus hypersecretion, mucosal edema, and enhance airway hyperactivity. Therefore, it is not surprising that leukotriene receptor antagonists improve lung function, attenuate bronchial hyperresponsiveness, and reduce the number of exacerbations in patients with mild to moderate asthma. Moreover, addition of leukotriene receptor antagonists to inhaled corticosteroids results in better control of asthma and can decrease the requirement for inhaled corticosteroids [64,65]. We have already determined that pranlukast, a selective leukotriene receptor antagonist, decreases airway VEGF level in asthmatic patients [66]. It is likely that leukotriene antagonists reduce angiogenesis and microvascular remodeling *via* reduction in airway VEGF level. Moreover, we have reported that VEGF levels in induced sputum were significantly decreased after 8 weeks of inhaled BDP therapy. Indeed, several previous studies also showed that transcription of VEGF mRNA and VEGF secretion were down-regulated in the presence of corticosteroids [67]. However, there are no reports on the effect of any drug on Ang-1 and -2 levels in asthmatic airways. We firstly found that VEGF/Ang-2 ratio after BDP therapy in asthmatic patients was significantly decreased to the same level as in controls, and that VEGF/Ang-2 ratio may be responsible for the increase in airway vascularity.

Recently, an intense research has been focused on the inhibitors of VEGF action [68]. These inhibitors are directed towards the receptors of VEGF or intracellular substrates for the receptors [69]. VEGFR2 has been demonstrated to be the active receptor [70], whereas VEGFR1 has acted as a decoy to VEGFR2 [71]. Interestingly, since decoy receptor VEGFR1 has the high affinity for the VEGF ligand, VEGFR1 may have the potential strategic importance for the inhibition of VEGF action. In future, the VEGF receptor inhibitors such as SU 5614 or SU 1498, will be required to examine their potential therapeutic effects [72]. Furthermore, endogenous and exogenous angiogenic inhibitors, which can act by preventing growth factor function, have also been described [73,74].

#### CURRENT & FUTURE DEVELOPMENTS

In this review we have shown that expression of VEGF, Ang-1 and -2 are up-regulated in bronchial asthma and is associated with angiogenesis and microvascular remodeling. Recently, Bhandari *et al.* determined that exaggerated VEGF production play an important role in the pathogenesis of asthma *via* nitric oxide-dependent and -independent pathway [75]. Thus, we have provided important new insights regar-

ding the angiogenesis and microvascular remodeling in the asthmatic airways. One of the major challenges to researchers in the angiogenesis field has been to identify the crucial signal transduction pathway by which VEGF and angiopoietins modulate angiogenesis [76]. Cell culture models have provided a plethora of data regarding VEGF and angiopoietins signal transduction pathways and physiological roles [77]. The future lies in identifying the crucial genes activated by the VEGF and angiopoietins signaling pathways that are responsible for angiogenesis [78]. As Wohlfahrt *et al.* have already showed that an approach to applying a DNA microarray technique give new insights into the pathophysiology of asthma [79], the advent of microarray technology and serial analysis of gene expression, where gene profiles for specific growth factors can be studied, will enable the identification of the crucial angiogenic genes whose expression are regulated by VEGF and angiopoietins, and the signaling pathways involved. Such knowledge will herald a new era in angiogenic signaling and facilitate the generation of angiogenic inhibitors that can specifically target the angiogenesis and microvascular remodeling in asthma. As with interesting discoveries, our study raises many questions, and makes it clear that more work is needed to understand the complexity of the control of angiogenesis and microvascular remodeling under pathological conditions, such as asthma.

#### ACKNOWLEDGEMENTS

We thank Miss Yukari Matsuyama for her help in the preparation and editing of the manuscript.

#### CONFLICT OF INTEREST

There is no conflict of interest in this study.

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