Expression of vascular endothelial growth factor, basic fibroblast growth factor, and angiogenin immunoreactivity in asthmatic airways and its relationship to angiogenesis

Makoto Hoshino, MD, Miki Takahashi, MD, and Nozomi Aoike, MD *Tokyo, Japan*

Background: Angiogenesis is a prerequisite for airway remodeling in bronchial asthma. Several growth factors may play important roles in inflammation and angiogenesis through effects on inflammatory cell infiltration or neovascularization.
Objective: We sought to compare bronchial vascularity and expression of vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), and angiogenin in bronchial biopsy specimens from asthmatic and healthy control subjects.
Methods: Bronchial biopsy specimens were obtained from 16 asthmatic subjects and 9 normal control subjects. The number of vessel profiles and the vascular area per unit area on a histologic section were estimated by using computerized image analysis after staining for type IV collagen in vessel walls. Numbers of VEGF$^+$, bFGF$^+$, and angiogenin$^+$ cells were determined by means of immunoreactivity.
Results: The airways of asthmatic subjects had significantly more vessels ($P < .05$) and greater vascular area ($P < .001$) than that observed in control subjects. Asthmatic subjects exhibited higher VEGF and bFGF and angiogenin immunoreactivity in the submucosa than did control subjects ($P < .001$, respectively). Significant correlations were detected between the vascular area and the numbers of angiogenic factor–positive cells (VEGF: $r_s = 0.93$, $P < .001$; bFGF: $r_s = 0.83$, $P < .001$; angiogenin: $r_s = 0.88$, $P < .001$) within the asthmatic airways. Furthermore, the degree of vascularity was inversely correlated with airway caliber and airway responsiveness. Colocalization analysis revealed that the angiogenic factor–positive cells were CD34$^+$ cells, eosinophils, and macrophages.
Conclusion: Our results suggest that increased vascularity of the bronchial mucosa in asthmatic subjects is closely related to the expression of angiogenic factors, which may then contribute to the pathogenesis of asthma. (J Allergy Clin Immunol 2001;107:295-301.)

Key words: *Angiogenesis, vascular endothelial growth factor, basic fibroblast growth factor, angiogenin, airway vascularity, airway remodeling*

Abbreviations used
APAAP: Alkaline phosphate–antialkaline phosphatase
bFGF: Basic fibroblast growth factor
Dmin: Minimum cumulative dose
MBP: Major basic protein
VEGF: Vascular endothelial growth factor

Chronic inflammation of the airways and airway-tissue remodeling are the most common histopathologic features of bronchial asthma. Airway remodeling is thought to lead to irreversible airway obstruction, which is one of the factors that makes treatment of asthmatic patients difficult. The formation of new blood vessels (angiogenesis) may play an important role both in the development of bronchial asthma and in the physiologic repair process. In addition, some of the cytokines and growth factors associated with airway inflammation may also play roles in airway remodeling[1-4] and may be responsible for the increased vascularity found in asthmatic airways. Recently, several angiogenic factors, such as fibroblast growth factor,[5] hepatocyte growth factor,[6] platelet-derived growth factor,[7] and angiogenin, have been identified.[8] Vascular endothelial growth factor (VEGF), which is involved in vascular permeability, is a potent multifunctional cytokine that has several important effects on angiogenesis.[9-11] Vasculogenesis, the differentiation of mesenchymal cells into hemangioblasts and primitive vessels, and angiogenesis, the formation of new blood vessels from preexisting endothelium, are essential components of tissue growth and remodeling. However, no reports have been published describing possible associations between cytokines and angiogenesis in bronchial asthma.

We hypothesized that cytokines may play an important role in airway remodeling, particularly in angiogenesis associated with bronchial asthma. To investigate the development of angiogenesis in asthmatic airways, we examined the levels of VEGF, basic fibroblast growth factor (bFGF), and angiogenin protein immunoreactivity in endobronchial biopsy specimens obtained from patients with asthma and age-matched control subjects. The relationships between vascularity, angiogenic factor expression, and clinicopathologic features were then analyzed.

METHODS

Study patients

The 16 atopic asthmatic patients were defined according to the criteria of the American Thoracic Society. All patients had typical clinical symptoms, a documented reversible airway obstruction (>20%

From the Second Department of Internal Medicine, Toho University School of Medicine, Tokyo.
Received for publication May 30, 2000; revised September 26, 2000; accepted for publication September 26, 2000.
Reprint requests: Makoto Hoshino, MD, Second Department of Internal Medicine, Toho University School of Medicine, 6-11-1, Omori-nishi, Ota-ku, Tokyo, 143-8541, Japan.

0091-6749/2001 $35.00 + 0 **1/83/111928**
doi:10.1067/mai.2001.111928

TABLE I. Clinical characteristics of asthmatic and normal control subjects

	Asthmatic subjects (n = 16)	Control subjects (n = 9)
Age (y)	32 (18-52)	36 (23-49)
Sex (M:F)	8:8	5:4
FEV_1 (% predicted)	72.3 (50.6-89.2)	95.6 (89.2-102.0)
Dmin (U)	1.12 (0.19-3.38)	>50
IgE (IU/mL)	498.0 (48.5-2306.7)	20 (3.0-45)

Values are expressed as mean (range). A Dmin unit is equal to 1 minute of 1.0 mg/mL aerosol inhalation of methacholine.

improvement in FEV_1), and increased airway responsiveness to methacholine. Nine nonatopic normal subjects were recruited from a hospital outpatient department. None of the asthmatic subjects had received inhaled or oral corticosteroid therapy in the 3 months before the study, and all required intermittent inhaled β_2-agonist alone. All subjects were nonsmokers, and control subjects had no evidence of any other pulmonary disease and had not experienced any upper respiratory tract infections during the 2 weeks preceding the study. The study was approved by the Ethics Committee of the Toho University School of Medicine, and all subjects gave written informed consent. Seven days after the initial visit, patients returned, having omitted inhaled β_2-agonist for at least 12 hours previously. Spirometry and airway methacholine responsiveness were measured with an Astograph device (TCK-6000CV; Chest MI Co, Tokyo, Japan), according to the method of Takishima et al.[12] Bronchial hyperresponsiveness was evaluated as the minimum cumulative dose (Dmin) of methacholine for which the reciprocal of respiratory resistance decreased linearly. Dmin was calculated so that 1 unit of Dmin equaled 1 minute of inhalation of aerosol solution at 1.0 mg/mL. Seven days later, fiberoptic bronchoscopy was performed on all patients. The clinical and demographic characteristics of the subjects are shown in Table I.

Fiberoptic bronchoscopy

Premedication was provided by intramuscular injection of 0.5 mg of atropine sulphate and 15 mg of pentazocine. After the throat had been anesthetized with 4% lidocaine spray, the subjects inhaled 200 μg of salbutamol (GlaxoWellcome, Greenford, UK) to prevent bronchoconstriction. A bronchoscope (BF type 1T-30; Olympus Co, Tokyo, Japan) was inserted through the mouth, and the pharynx, trachea, and bronchi were anesthetized with 2% lidocaine. Oxygen at 4 L/min was administered through the nasal cannulas throughout the procedure. Biopsy forceps (FB-15C, Olympus) were used to collect 2 to 3 biopsy specimens from the spur of a basal segment bronchus of the right lower lobe and from the subcarina of the right upper lobe. After the procedure, patients inhaled 200 μg of salbutamol. Biopsy specimens were placed in OCT medium, snap-frozen in liquid nitrogen–cooled isopentane, and stored at –70°C until use.

Sample processing

For immunocytochemistry, biopsy tissues were immersed in 15% PBS and cooled on ice for 20 minutes. Tissues were then blocked with OCT medium, and 4-μm cryostat sections were cut, air-dried for 1 hour, and then fixed by immersion in a mixture of acetone-methanol (60:40) for 7 minutes. Slides were air-dried and stored at –70°C until use.

Immunocytochemistry

Immunostaining was performed by using the alkaline phosphate–antialkaline phosphatase (APAAP) method, as previously described.[13] Vessels were identified by means of anti-collagen type IV antibody (1:50 dilution; Monosan, Uden, The Netherlands) staining of the basement membranes supporting the endothelium. Immunostaining for VEGF was performed by using the rabbit anti-human polyclonal antibody A-20 (1:200 dilution; Santa Cruz Biotechnology, Santa Cruz, Calif) that recognizes the 121, 165, and 189 isoforms of VEGF. Slides were also incubated with a rabbit anti-human polyclonal antibody against bFGF at a dilution of 1:200 (Yanaihara Institutes Inc, Shizuoka, Japan) and an mAb against angiogenin at dilution of 1:50 (Biomedical Technology Inc, Stoughton, Mass).

Double immunocytochemistry

To confirm the phenotype of $VEGF^+$, $bFGF^+$, and $angiogenin^+$ cells, double sequential immunocytochemistry was performed as previously described.[14] Briefly, endogenous peroxidase activity in biopsy specimen cryostat sections was blocked with 1% H_2O_2 and 0.02% sodium azide in TRIS-buffered saline for 30 minutes. A mixture of primary antibodies was then applied that consisted of either polyclonal anti-VEGF antibody, polyclonal anti-bFGF antibody, or monoclonal anti-angiogenin antibody to detect VEGF, bFGF, and angiogenin immunoreactivity, respectively. Appropriate mAbs to detect eosinophils (anti-major basic protein; Harlan Sera-lab, Ltd, Sussex, United Kingdom), mast cells (anti-AA1; Dako Ltd, High Wycomb, United Kingdom), T cells (anti-CD3; Becton Dickinson, Oxford, United Kingdom), macrophages (anti-CD68; Dako), and $CD34^+$ cells (anti-CD34; Biomeda Corp, Foster, Calif) were then added. After incubation with the appropriate secondary antibodies, a tertiary layer of streptavidin peroxidase and murine APAAP conjugate was then applied. Sections were developed sequentially in Fast Red (APAAP substrate) and diaminobenzidine (peroxide substrate). Appropriate negative controls were included in all immunochemical studies, which included TRIS-buffered saline alone, omission of the primary antibodies, and the use of an irrelevant mouse or rabbit IgG antibody. A total of 500 cells from randomly selected areas in the sample tissues were examined. Cell counts were expressed as the percentage of angiogenic factor–positive cells that colocalized with major basic protein (MBP), AA1, CD3, CD68, or CD34.

Quantitation

For immunocytochemical analysis, sections were coded and counted in a blind fashion by using an Olympus microscope with eyepiece graticule at 200× magnification. A computerized colorometric image analyzer (Win ROOF; Mitani Corp, Fukui, Japan) was used to quantify collagen type IV immunoreactivity in tissue. The entire submucosa beneath the epithelial basement membrane was outlined on the color monitor by means of an electronic mouse cursor. The number of vessel profiles per unit area was then divided by the submucosal area to determine the number of vessels per square millimeter of submucosa. All structures internal to the vessel endothelial basement membrane were evaluated as vascular area. The mean size of vessels was estimated by dividing the total vascular area by the total number of vessels for each subject. Because vessels are 3-dimensional structures, it is difficult to accurately count the number of vessels on a single histologic section. However, the percentage of vascular area determined by dividing the vascular area by the submucosal area is a more important measure of angiogenic change than the number of vessel profiles. Therefore angiogenic factor expression was compared with vascular area. Numbers of positively stained cells were counted for the entire submucosal area, excluding mucosal glands and smooth muscle. Results were expressed as the number of positive cells per square millimeter of submucosa.

Statistical analysis

Between-group comparisons were performed by using the Mann-Whitney *U* test with the Bonferroni correction. The same observer performed at least 3 replicate measurements, and intraobserver reproducibility was assessed by using the coefficient of variation for repeat

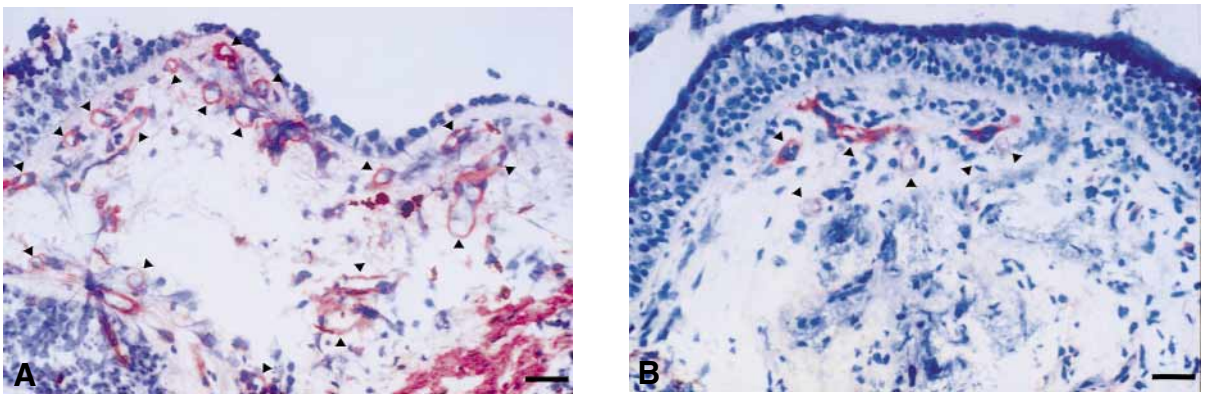

FIG 1. Bronchial biopsy specimens stained with anti-collagen IV from an asthmatic patient **(A)** and a control subject **(B)**. *Arrowheads* indicate vessels (bar = 25 μm).

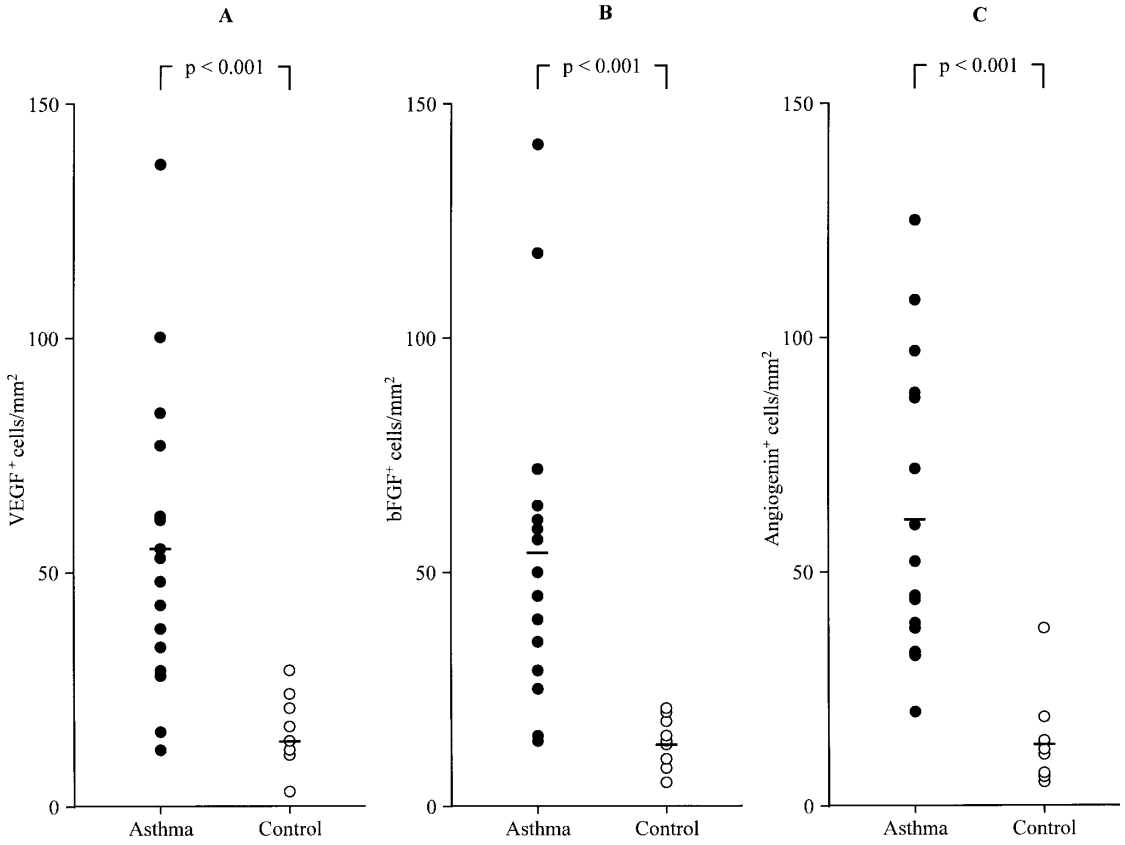

FIG 2. Number of VEGF+ **(A)**, bFGF+ **(B)**, and angiogenin+ **(C)** cells within the submucosa of bronchial biopsy specimens obtained from asthmatic and control subjects. *Horizontal bars* represent mean values.

measurements. The intraobserver coefficient of variation ranged from 4% to 12% for the number of vessel profiles per unit area and percentage vascularity and from 1% to 5% for cell positivity. Correlation coefficients were obtained by using the Spearman rank-order method. A P value of less than .05 was considered statistically significant.

RESULTS

Vascularity

The number of vessel profiles per unit area in asthmatic patients was increased compared with that of the control subjects (mean ± SD, 307 ± 125/mm^2 vs 210 ± 78/mm^2; $P < .05$). The percentage vessel area was greater in asthmatic subjects than in control subjects (12.4% ± 4.4% vs 3.4% ± 1.3%, $P < .001$). However, there was no significant difference in mean vessel size for asthmatic subjects compared with control subjects (279.8 ± 95.5 mm^2 vs 239.1 ± 78.4 mm^2). There was an inverse correlation between FEV_1 percent predicted and the percentage of vessel area ($r_s = –0.88$, $P < .001$), and we also observed an inverse correlation between airway respon-

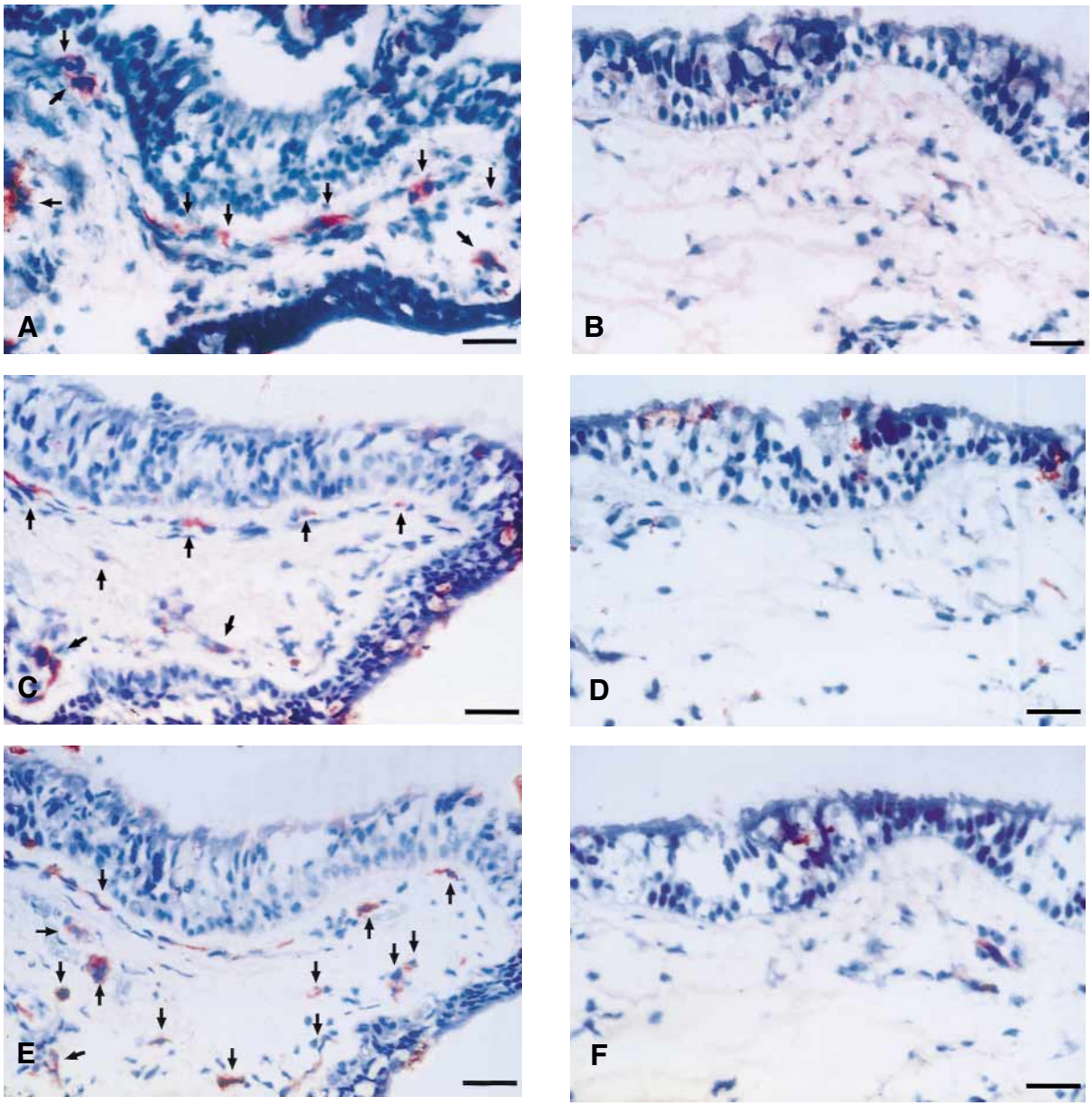

FIG 3. Photomicrographs showing staining of bronchial mucosal biopsy specimens with antibodies to angiogenic factors: VEGF (**A,** asthma; **B,** control), bFGF (**C,** asthma; **D,** control), angiogenin (**E,** asthma; **F,** control). *Arrows* indicate some of the cells that react with each angiogenic antibody. Colocalization of VEGF immunoreactivity (red) and anti-CD34$^+$ cells (brown; **G**), bFGF immunoreactivity (red) and anti-MBP$^+$ cells (eosinophils, brown; **H**), angiogenin immunoreactivity (red) and anti-CD68$^+$ (macrophages, brown; **I**) in asthmatic bronchial mucosa (bar = 25 μm).

siveness and the percentage of vessel area (r_s = –0.92, P < .001). An example of anti-collagen IV–positive staining of biopsy specimens taken from asthmatic and control subjects is shown in Fig 1, *A* and *B*.

Immunoreactivity of VEGF, bFGF, and angiogenin

Immunocytochemical staining revealed increased numbers of VEGF-immunoreactive cells in the bronchial mucosa of asthmatic subjects compared with control subjects (54.8 ± 31.4/mm^2 vs 14.8 ± 8.3/mm^2, P < .001; Fig 2, *A*). Cells expressing bFGF immunoreactivity were also significantly increased in the bronchial mucosa of asthmatic subjects compared with control subjects (53.7 ± 33.3/mm^2 vs 13.7 ± 5.1/mm^2, P < .001; Fig 2, *B*). The numbers of angiogenin-immunostaining cells were significantly elevated in asthmatic subjects compared with control subjects (60.7 ± 30.5/mm^2 vs 13.7 ± 9.4/mm^2, P < .001; Fig 2, *C*). Examples of staining for each of the angiogenic factors are shown in Fig 3, *A*-*F*.

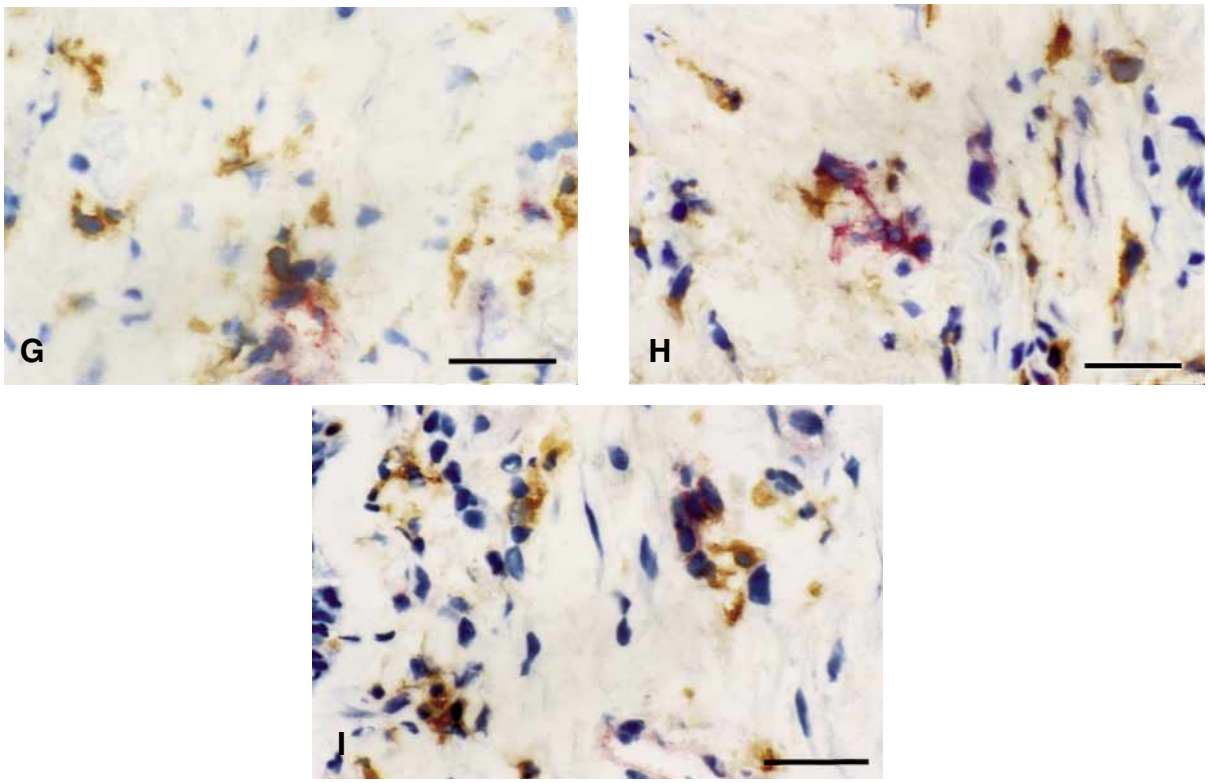

FIG 3. Continued

Relationship between vascularity and angiogenic factors

Statistically significant correlations were detected between the percentage of vessel area vascularity in each subject and positive cellular immunostaining for VEGF (r_s = 0.93, P < .001), bFGF (r_s = 0.83, P < .001), and angiogenin (r_s = 0.88, P < .001; Fig 4).

Cell sources of VEGF, bFGF, and angiogenin

Colocalization studies were performed to phenotype cells producing the angiogenic factors in the asthmatic airway (Fig 3, *G-I*). Results demonstrated that CD34$^+$ cells, MBP$^+$ eosinophils, and CD68$^+$ macrophages colocalized with angiogenic factor expression (n = 9, Table II).

TABLE II. Percentages of each cell type coexpressing angiogenic factor for VEGF, bFGF, and angiogenin within the submucosa of bronchial biopsy specimens from asthmatic subjects (n = 9)

Cell markers	VEGF$^+$ cells (%)	bFGF$^+$ cells (%)	Angiogenin$^+$ (%)
MBP$^+$ eosinophils	22.8 ± 5.4	21.2 ± 5.8	23.1 ± 5.3
AA1$^+$ mast cells	9.9 ± 5.0	10.1 ± 2.9	11.1 ± 4.3
CD3$^+$ T cells	15.9 ± 2.9	19.1 ± 4.0	14.6 ± 4.1
CD68$^+$ macrophages	22.7 ± 5.2	20.7 ± 3.0	23.0 ± 4.9
CD34$^+$ cells	26.7 ± 2.5	26.8 ± 2.8	28.2 ± 5.6

Results are expressed as means ± SD.

DISCUSSION

The present study showed that (1) angiogenic factor levels were significantly increased in the airways of asthmatic subjects compared with control subjects; (2) there were significant correlations between vessel area and amounts of angiogenic factors in the asthmatic airways; and (3) the angiogenic factor–positive cells in the submucosa were CD34$^+$ cells, eosinophils, and macrophages. Also, the submucosal vessel area was closely correlated with both airway caliber and airway responsiveness in asthmatic subjects. Taken together, these results provide the first evidence that VEGF, bFGF, and angiogenin may play important roles in asthmatic airway angiogenesis.

The contribution of the increased vessel number and the percentage of vessel area to airway remodeling has not been fully elucidated. Previously, Dunnill[15] reported that dilatation of capillary blood vessels was a striking feature of bronchial mucosa in fatal asthma. Morphometric studies[16] have confirmed these early observations and have shown that enlarged, congested, mucosal blood vessels contribute to increased airway wall thickness in asthma. The inflammatory process also involves increased vascularity, which may again be enhanced by such structural changes. This study confirms previous findings[17] of increased bronchial vascularity in subjects with mild-to-moderate asthma, both in terms of the number of vessels and the area of submucosa occupied by vessels. It has been suggested that structural changes within the airways of

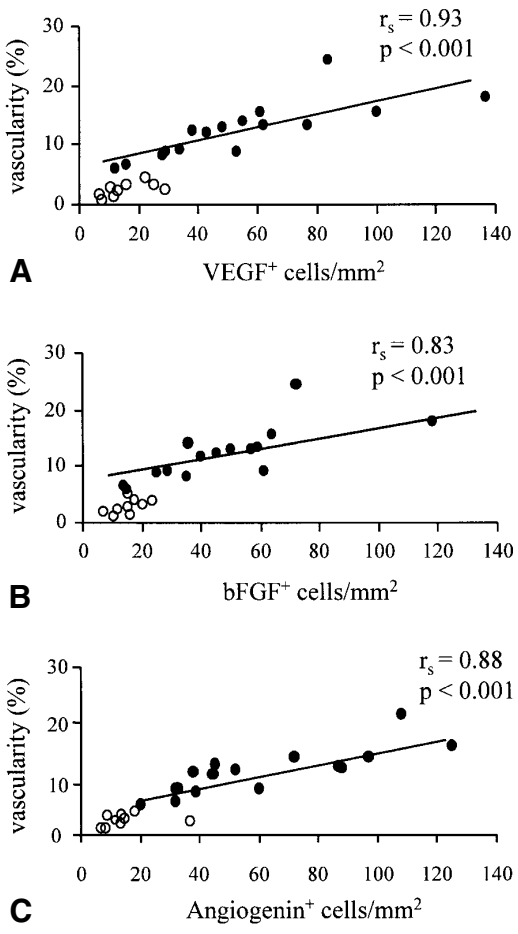

FIG 4. Correlations between the percentage of vessel area and VEGF+ cells **(A)**, bFGF+ cells **(B)**, and angiogenin+ cells **(C)** in bronchial mucosa. *Open circles* represent data from control subjects, and *closed circles* represent data from asthmatic patients. Correlations were significant only on asthmatic patients.

asthmatic subjects contribute to the development of persistent airway hyperresponsiveness[1] and chronic alterations in airflow obstruction.[18] It has been reported that collagen III, collagen V, and tenascin deposition are involved in reticular layer thickening.[19,20] It is also thought that increased vascularity, with consequent thickening of the airway wall, may lead to the narrowing of the bronchial lumen, increased airway resistance, and decreased forced expiratory flow rates.[21] In our study the percentage of vessel area was significantly correlated with airway caliber and airway responsiveness in asthmatic subjects. These results agree with previous findings[22] and confirm that increased bronchial vascularity caused by airway remodeling is a specific characteristic of asthma.

A number of growth factors with proven or potential roles in vascular development and remodeling have been identified, including those associated with various disease conditions.[23] In the present study we focused on the expression of VEGF, bFGF, and angiogenin among a variety of angiogenic factors because asthmatic airway mucosa includes abundant vessels and fibrotic components. VEGF is a highly specific mitogen for endothelial cells in vitro and has angiogenic properties in vivo.[9,10,24,25] Moreover, VEGF has been reported to have a narrow target cell range and is specific for endothelial cells because VEGF binds to the high-affinity cell surface receptors KDR/Flk and Flt, which are predominantly expressed by endothelial cells.[26,27] VEGF is also the most potent inducer of vascular hyperpermeability and angiogenesis, phenomena that are consistently found in inflammatory[28,29] and neoplastic disorders. bFGF belongs to a group of heparin-binding growth factors that stimulate endothelial cell proliferation and migration in vitro and angiogenesis in vivo.[11] bFGF plays a significant role in inflammatory conditions, including wound healing[30] and pulmonary fibrosis.[31] The tissue distribution of bFGF, combined with in vitro evidence that bFGF can be selectively released by proteases[32] or by cellular injury,[33] suggests a role for bFGF in angiogenesis. Angiogenin, a polypeptide first isolated from conditioned media of a human adenocarcinoma cell line, is a potent stimulator of angiogenesis.[34] Although both heparin-binding growth factors and angiogenin are potent stimulators of angiogenesis, the molecules are unrelated. A statistically significant correlation was found between the number of angiogenic factor–positive cells and the percentage vessel area in asthmatic airways. This suggests that angiogenic factors may activate the corresponding cognate receptors on endothelial cells, thus establishing a paracrine mechanism that could ultimately lead to blood vessel neoformation. Our findings are consistent with previous reports that vascular density correlated with VEGF immunostaining in neoplastic disease[35] and bFGF immunostaining in disc herniation.[36,37]

Recently, Haley and Drazen[38] have questioned the implied relationship between the presence of inflammatory cells in the airways and airway responsiveness. Inflammatory cells in asthmatic airways release not only short-lived mediators, such as histamine, leukotrienes, and platelet-activating factor, but also cytokines and chemokines, which may lead to more chronic effects. It is possible that these latter factors may contribute to airway hyperresponsiveness by inducing chronic airway remodeling. Thus we hypothesize that angiogenic factors may be one of the most important mediators of irreversible airway function in asthma.

Using double immunocytochemistry, we phenotyped the cells expressing VEGF, bFGF, and angiogenin and found that CD34+ cells, eosinophils, and macrophages within the bronchial mucosa were the most significant cellular sources of these angiogenic factors in asthmatic individuals. CD34 is a surface marker expressed on hemopoietic progenitor cells, normal vascular endothelium, and fibroblasts. CD34 is expressed most strongly on immature hemopoietic precursor cells and is progressively lost as cells differentiate.[39,40] Vascular endothelial cells do not express VEGF but are known to be target cells for VEGF.[41] Thus mechanisms regulating angiogenesis in the airways are complex, involving antigen-presenting cells and T cells that process antigen and

orchestrate the immune response, with eosinophils as effector cells and endothelial cells as target cells.

In conclusion, we have demonstrated that VEGF, bFGF, and angiogenin immunoreactivity is upregulated in bronchial asthma compared with healthy control subjects and is associated with the degree of airway vascularity. Our results suggest that these angiogenic factors play a role in the angiogenic alterations associated with asthma. Therefore novel therapeutic agents that prevent angiogenic factor production may be of use in restoring normal lung function in asthmatic subjects.

REFERENCES

1. James AL, Pare PD, Hogg JC. The mechanics of the airway narrowing in asthma. Am Rev Respir Dis 1989;139:242-6.
2. Minshall EM, Leung DYM, Martin RJ, Song YL, Cameron L, Ernst P, et al. Eosinophil-associated TGF-β_1 mRNA expression and airways fibrosis in bronchial asthma. Am J Respir Cell Mol Biol 1997;17:326-33.
3. Ohno I, Nitta Y, Yamauchi K, Hoshi H, Honma M, Woolley K, et al. Eosinophils as a potential source of platelet-derived growth factor B-chain (PDGF-B) in nasal polyposis and bronchial asthma. Am J Respir Cell Mol Biol 1995;13:639-47.
4. Amishima M, Munakata M, Nasuhara Y, Sato A, Takahashi T, Homma Y, et al. Expression of epidermal growth factor and epidermal growth factor receptor immunoreactivity in the asthmatic human airway. Am J Respir Crit Care Med 1998;157:1907-12.
5. Montesano R, Vassalli J-D, Baird A, Guillemin R, Orci L. Basic fibroblast growth factor induces angiogenesis in vitro. Proc Natl Acad Sci USA 1986;83:7297-301.
6. Nakamura T, Nawa K, Ichihara A. Partial purification and characterization of hepatocyte growth factor from serum of hepatectomized rats. Biochem Biophys Res Commun 1984;12:1450-9.
7. Ishikawa F, Miyazano K, Hellman U, Drexler H, Wernstedt C, Hagiwara K, et al. Identification of angiogenic activity and the cloning and expression of platelet-derived endothelial cell growth factor. Nature 1989;338:557-62.
8. Klagsbrun M, D'Amore PA. Regulators of angiogenesis. Annu Rev Physiol 1991;53:217-39.
9. Leung DW, Cachianes G, Kuang W-J, Goeddel DV, Ferrara N. Vascular endothelial growth factor is a secreted angiogenic mitogen. Science 1989;246:1306-9.
10. Keck PJ, Hauser SD, Krivi G, Sanzo K, Warren T, Feder J, et al. Vascular permeability factor, an endothelial cell mitogen related to PDGF. Science 1989;266:1309-12.
11. Folkman J, Klagsbrun M. Angiogenic factors. Science 1987;235:442-7.
12. Takishima T, Hida W, Sasaki H, Suzuki S, Sasaki K. Direct-writing recorder of the dose-response curves of the airway to methacholine. Chest 1981;80:600-6.
13. Durham SR, Ying S, Varney VA, Jacobson MR, Sudderick RM, Mackay IS, et al. Cytokine messenger RNA expression for IL-3, IL-4, IL-5, and granulocyto/macrophage-colony-stimulating factor in the nasal mucosa after local allergen provocation: relationship to tissue eosinophilia. J Immunol 1992;148:2390-4.
14. Moqbel R, Ying S, Barkans J, Newman TM, Kimmitt P, Wakelin M, et al. Identification of messenger RNA for IL-4 in human eosinophils with granule localization and release of the translated product. J Immunol 1995;155:4939-47.
15. Dunnill MS. The pathology of asthma with special reference to changes in the bronchial mucosa. J Clin Pathol 1960;13:27-33.
16. Kuwano K, Bosken CH, Pare PD, Bai TR, Wiggs BR, Hogg JC. Small airways dimensions in asthma and in chronic obstructive pulmonary disease. Am Rev Respir Dis 1993;148:1220-5.
17. Li X, Wilson JW. Increased vascularity of the bronchial mucosa in mild asthma. Am J Respir Crit Care Med 1997;156:229-33.
18. Van Schayck CP, Dompeling E, Van Herwaarden CLA, Wever AMJ, Van Weel C. Interacting effects of atopy and bronchial hyperresponsiveness on the annual decline in lung function and the exacerbation rate in asthma. Am Rev Respir Dis 1991;144:1297-301.
19. Roche WR, Beasley R, Williams JH, Holgate ST. Subepithelial fibrosis in the bronchi of asthmatics. Lancet 1989;1:520-4.
20. Hoshino M, Nakamura Y, Sim JJ, Shimojo J, Isogai S. Bronchial subepithelial fibrosis and expression of matrix metalloproteinase-9 in asthmatic airway inflammation. J Allergy Clin Immunol 1998;102:783-8.
21. McFarden ER Jr. Hypothesis: exercise-induced asthma as a vascular phenomenon. Lancet 1990;335:880-3.
22. Orsida BE, Li X, Hickey B, Thien F, Wilson JW, Walters EH. Vascularity in asthmatic airways: relation to inhaled steroid dose. Thorax 1999;54:289-95.
23. Folkman J. Angiogenesis in cancer, vascular, rheumatoid and other diseases. Nat Med 1995;1:27-30.
24. Gospodarowicz D, Abraham JA, Schilling J. Isolation and characterization of a vascular endothelial cell mitogen produced by pituitary-derived folliculo stellate cells. Proc Natl Acad Sci USA 1989;86:7311-5.
25. Connolly DT, Heuvelman DM, Nelson R, Olander JV, Eppley BL, Delfino JJ, et al. Tumor vascular permeability factor stimulates endothelial cell growth and angiogenesis. J Clin Invest 1989;84:1470-8.
26. Millauer B, Wizigmann-Voss S, Schnurch H, Martinez R, Moller NPH, Risau W, et al. High affinity VEGF binding and developmental expression suggest Flk-1 as a major regulator of vasculogenesis and angiogenesis. Cell 1993;72:835-46.
27. DeVries C, Escobedo JA, Ueno H, Houck K, Ferrara N, Williams LT. The fms-like tyrosin kinase, a receptor for vascular endothelial growth factor. Science 1991;255:989-91.
28. Fava RA, Olsen NJ, Spencer-Green G, Yeo K-T, Yeo T-K, Berse B, et al. Vascular permeability factor/endothelial growth factor (VPF/VEGF): Accumulation and expression in human synovial fluids and rheumatoid synovial tissue. J Exp Med 1994;180:341-6.
29. Detmar M, Brown LF, Claffey KP, Yeo K-T, Kocher O, Jackman RW, et al. Overexpression of vascular permeability factor/vascular endothelial growth factor and its receptors in psoriasis. J Exp Med 1994;180:1141-6.
30. Broadley KN, Aquino AM, Woodward SC, Buckley-Sturrock A, Sato Y, Rifkin DB, et al. Monospecific antibodies implicate basic fibroblasts growth factor in normal wound repair. Lab Invest 1989;61:571-5.
31. Henke C, Marineili W, Jessurum J, Fox J, Harms D, Peterson M, et al. Macrophage production of basic fibroblast growth factor in the fibroproliferative disorder of alveolar fibrosis after lung injury. Am J Pathol 1993;143:1189-99.
32. Bashkin P, Doctrow S, Klagsbrun M, Svahn CM, Folkman J, Vlodavsky I. Basic fibroblast growth factor binds to subendothelial extracellular matrix and is released by heparitinase and heparin-like molecules. Biochemistry 1989;28:1737-43.
33. Muthukrishnan L, Warder E, McNeil PL. Basic fibroblast growth factor is efficiently released from a cytolsolic storage site through plasma membrane disruptions of endothelial cells. J Cell Physiol 1991;148:1-16.
34. Fett JW, Strydom DJ, Lobb RR, Alderman EM, Bethune JL, Riordan JF, et al. Isolation and characterization of angiogenin, an angiogenic protein from human carcinoma cells. Biochemistry 1985;24:5480-6.
35. Takahashi Y, Kitadai Y, Bucana CD, Cleary KR, Ellis LM. Expression of vascular endothelial growth factor and its receptor, KDR, correlates with vascularity, metastasis, and proliferation of human colon cancer. Cancer Res 1995;55:3964-8.
36. Tolonen J, Gronblad M, Virri J, Seitsalo S, Rytomaa T, Karaharju EO. Platelet-derived growth factor and vascular endothelial growth factor expression in disc herniation tissue: an immunohistochemical study. Eur Spine J 1997;6:63-9.
37. Tolonen J, Gronblad M, Virri J, Seitsalo S, Rytomaa T, Karaharju EO. Basic fibroblast growth factor immunoreactivity in blood vessels and cells of disc herniations. Spine 1995;20:271-6.
38. Haley KJ, Drazen JM. Inflammation and airway function in asthma. What you see is not necessarily what you get. Am J Respir Crit Care Med 1998;157:1-3.
39. Strauss LC, Rowley SD, La Russa VF, Sharkis SJ, Stuart RK, Civin CI. Antigenic analysis of hematopoiesis. V. Characterization of My-10 antigen expression by normal lymphohematopoietic progenitor cells. Exp Hematol 1986;14:878-86.
40. Bender JG, Unverzagt KL, Walker DE, Lee W, Van Epps DE, Smith DH, et al. Identification and comparison of CD34-positive cells and their subpopulations from normal peripheral blood and bone marrow using multicolor flow cytometry. Blood 1991;77:2591-6.
41. Ferrara N, Houck K, Jakeman L, Leung DW. Molecular and biological properties of the vascular endothelial growth factor family of proteins. Endocr Rev 1992;13:18-32.