—**Original**—

Heterogeneous Distribution of Thrombomodulin and von Willebrand Factor in Endothelial Cells in the Human Pulmonary Microvessels

Oichi Kawanami[1], Enjing Jin[1], Mohammad Ghazizadeh[1], Masakazu Fujiwara[1], Li Jiang[1], Mikio Nagashima[1], Hajime Shimizu[1], Tamiko Takemura[2], Yoshiharu Ohaki[3], Satoru Arai[3], Makoto Gomibuchi[4], Kazuyo Takeda[5], Zu-Xi Yu[5] and Victor J. Ferrans[5]

[1]Department of Molecular Pathology, Institute of Gerontology, Nippon Medical School, Japan
[2]Department of Pathology, Nippon Red Cross Hospital Center, Japan
[3]Surgical Pathology, and [4]Surgery Sections, Hokusoh Hospital, Nippon Medical School, Japan
[5]Pathology Section, National Heart, Lung and Blood Institute, National Institutes of Health, USA

Abstract

Laser scanning confocal fluorescence microscopy techniques were used to study the localization of von Willebrand factor (vWf; Factor VIII-related antigen) and thrombomodulin (transmembrane receptor for thrombin) in the microvascular endothelial cells in the normal human lung. Tissues were obtained from lobectomy specimens resected for solitary nodules (7 adenocarcinomas and 4 hamartomas) from 11 patients. The plasma membranes of the capillary endothelial cells in the alveolar zones (A-zones) showed red linear fluorescence for thrombomodulin. However, their cytoplasm was mostly unreactive for vWf. The microvessels which were located in the connective tissue (C-zones), including peribronchial, and subpleural areas and large vascular walls, consistently demonstrated band-like green fluorescence for vWf in their cytoplasm, and their plasma membranes usually lacked reactivity for thrombomodulin. Only a limited number of peribronchial capillaries measuring < 10μm in diameter showed a mosaic-like appearance, in which red fluorescence along the plasma membranes was found together with green fluorescence in the subjacent cytoplasm. In the juxtaalveolar (J-zones) microvessels located along the borders between A- and C-zones, and measuring up to 40 μm in diameter, the endothelial cells showed a mosaic-like pattern of distribution of the two antigens. However, the localization of thrombomodulin in the J-zone microvessels was separate and independent from that of vWf. The thrombomodulin-reactive cells were directly connected to the alveolar capillary endothelial cells. Heterogeneous patterns of distribution of thrombomodulin and vWf suggest that topographic differences of endothelial function occur to maintain a balance of coagulation and anticoagulation in the normal human lung. (J Nippon Med Sch 2000; 67: 118—125)

Key words: pulmonary microvessel, endothelial cells, confocal microscopy, thrombomodulin, von Willebrand factor

Address Correspondence to: Oichi Kawanami, MD, PhD, The Department of Molecular Pathology, Institute of Gerontology, Nippon Medical School, Kosugi-cho 1–396, Nakahara-ku, Kawasaki Japan, 211–8533, E-mail : kawanami/ig@nms.ac.jp

Journal Website (http://www.nms.ac.jp/jnms/)

Introduction

Vascular endothelial cells express multiple antigenic determinants, which vary according to the size of the vessel[1]. Capillary endothelial cells strongly express class I and class II MHC and ICAM, although the expression of these components is mostly undetectable in large vessels[2]. The expression of endothelial cell antigens is influenced by the organ and the microenvironment in which these cells are found[3,4]. Microvascular endothelial cells of human lung form cobblestone-like monolayers when grown on gelatin substrate, but become organized into capillary-like tubes when grown on Matrigel[5]. The growth rate and the response to agonists vary in endothelial cells derived from different organs[6].

In transgenic mice expressing a fragment of the gene for von Willebrand factor (vWf), Aird et al.[7] showed upregulation of vWf in cardiac microvascular endothelial cells in the presence of cardiac myocytes. In contrast, the expression of vWf in these endothelial cells was not influenced by the addition of 3T3 fibroblasts or mouse hepatocytes. Von Willebrand factor is localized in the cytoplasmic inclusions (Weibel-Palade bodies) of endothelial cells and is widely accepted as one of the biological markers of endothelial cells. However, normal alveolar capillary endothelial cells often lack immunohistochemical reactivity for vWf[8-10]. Nevertheless, the expression of vWf was found to become prominent in the capillary endothelial cells of alveolar walls that underwent fibrotic changes in humans[8,9] and animals[10]. Vascular endothelial cells are exposed to procoagulant and anticoagulant factors, the function of which is balanced under normal circumstances. Thrombomodulin[11-14], which is a transmembrane receptor for thrombin, has been demonstrated in endothelial cells of pulmonary alveolar capillaries in normal rabbit[15] and mouse[16] as well as endothelial cells of both arteries and veins in normal mouse lung[16]. Immunofluorescence and electron microscopic observations have been made on cultured endothelial cells of rabbits[17]. However, details of the distribution of thrombomodulin and vWf have not been reported in normal pulmonary microvessels. We have applied conventional immunofluorescence and confocal laser scanning microscopic techniques to map the distribution of these components. The results obtained demonstrate the heterogeneous localization of these antigens, which showed a mosaic-like pattern of distribution in the juxtaalveolar zones of microvessels. This pattern is suggestive of sites of transition between two types of endothelial cells, which correspond to the pulmonary circulation (thrombomodulin-dominant) and the bronchial circulation (vWf-dominant). A detailed study of this problem forms the basis of this report.

Materials and Methods

(1) *Tissues*

Histologically normal human lung tissues were taken at areas distant from isolated solitary nodules in lobectomy specimens from 11 patients (7 peripherally located adenocarcinomas and 4 hamartomas). They were 6 men and 5 women, ranging in age from 39 to 73 years (mean age, 63 years). Six patients (4 men and 2 women) were current smokers (mean, 24.5 pack-years). This study was approved by the Ethical Review Committee of Nippon Medical School. The tissues were used for unfixed frozen sections, or frozen sections fixed for 1 hr at 4℃ with buffered 4% p-formaldehyde. For the immunofluorescence studies described below, the sections were reacted with antibodies against thrombomodulin and vWf. The frozen sections showed excellent reactivity with these antibodies.

(2) *Dual immunolabeling for fluorescence microscopy*

Dual labeling for fluorescence microscopy was performed according to the double indirect immunofluorescence method[18]. The sections were incubated overnight at 4℃ with a combination of a 1: 50 dilution of a mouse monoclonal IgG antibody against thrombomodulin (TM 1009; DAKO, Carpinteria, CA, USA) and a 1: 400 dilution of a rabbit polyclonal antibody against vWf (DAKO) as the two primary antibodies. After thorough washing, the sections were incubated with a mixture of two secondary antibodies, which consisted of a 1: 100 dilution each of Texas red-labeled horse anti-mouse IgG and FITC-labeled goat anti-rabbit IgG (Vector Laboratories, Burlingame, CA, USA), for 40 min at room temperature in the dark. In some of the

samples, these reactions were followed by nuclear counterstaining for 15 min at room temperature with 0.01%4', 6-diamidino-2-phenylindole (DAPI; Sigma Chemical, St Louis, MO, USA). The sections were viewed with a fluorescence microscope (model BX 50 Olympus, Tokyo, Japan) equipped with a BX-FLA system and a multiple bandpass filter, and were also examined with a laser scanning confocal fluorescence microscope (Model TCS-SP, Leica, Heidelberg, Germany) equipped with argon and argon-krypton laser sources. Images were also scanned at different levels of the thickness of the section, at intervals of 0.2 to 1.0 μm. In the resulting preparations, red fluorescence represented thrombomodulin, green fluorescence depicted vWf, and nuclei showed a blue fluorescence.

(3) *Immunohistochemical control procedures*

Immunofluorescence negative control preparations consisted of: 1) omission of the primary antibody from

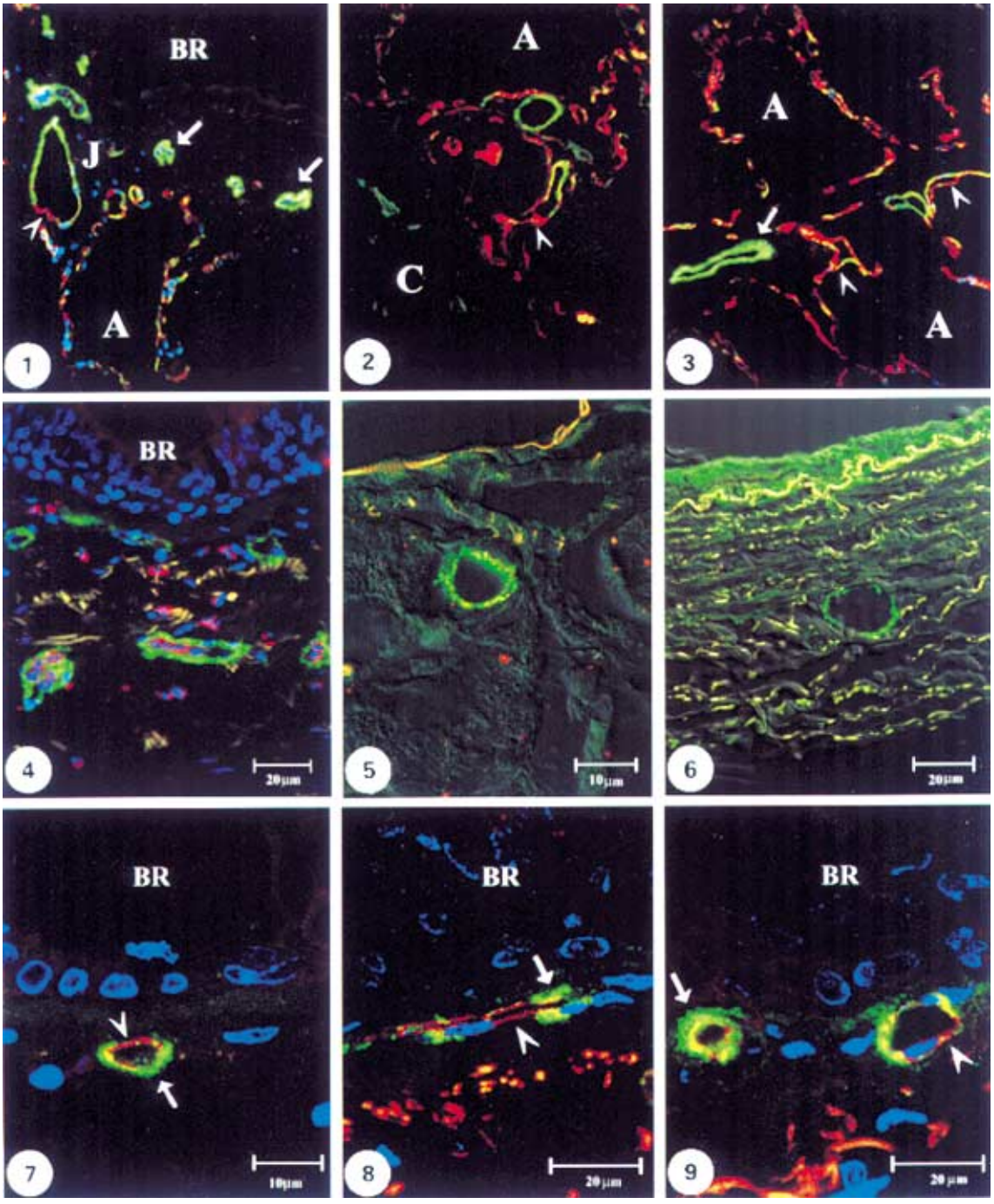

Fig. 1

the staining procedure, and 2) substitution of corresponding amounts of normal immunoglobulin for the primary antibodies. Both of these control procedures consistently gave negative results.

Results

In this study, emphasis was placed on the examination of microvessels that had an average luminal diameter no larger than 40 μm. In order to recognize the topographical distribution of the pulmonary microvessels, we classified them as follows: 1) alveolar capillaries (herein referred to as A-zone capillaries), which are located in the alveolar walls (**Fig. 1–1 through 1–3**); 2) connective tissue zone (C-zone) microvessels, which are located in the connective tissue beneath the epithelial basement membrane of the airway (**Fig. 1–1**), among bronchial glands (**Fig. 1–2**) and smooth muscle cells of the airways, the walls of large vessels, interlobular septa (**Fig. 1–3**) and subpleural regions, and 3) juxtaalveolar zone (J-zone) microvessels, which are located in the junctional areas along the borders between A-zones and C-zones (**Fig. 1–1 through 1–3**).

1. Conventional dual immunofluorescence study

The endothelial cells lining the A-zone capillaries showed only red linear fluorescence for thrombomodulin (**Fig. 1–1 to 1–3**). The endothelial cell loops of microvessels located in the C-zones (**Fig. 1–1 to 1–3**) were reactive for vWf. These vessels were mostly unreactive for thrombomodulin. The J-zone microvessels adjacent to alveolar capillaries showed mosaic-like patterns of distribution of thrombomodulin and vWf. The expression of these two antigens appeared in separate cells along the vascular loops in peribronchial (**Fig. 1–1 and 1–2**) and interlobular venules (**Fig. 1–3**). The endothelial cells of microvessels that were in direct continuity with alveolar capillaries tended to express thrombomodulin.

2. Confocal microscopic study

(1) *C-zone microvessels*

The cytoplasm of endothelial cells of microvessels larger than 10 μm in diameter reacted for vWf, as demonstrated in areas such as subepithelial layers (**Fig. 1–4**), bronchial glands (**Fig. 1–5**) and the walls of large vessels (**Fig. 1–6**). Reactivity for thrombomodulin was undetectable in most of these microvessels. However, capillaries that measured less than 10

Fig. 1 Dual immunofluorescent staining for vWf (green) and thrombomodulin (red). DAPI-stained nuclei appear blue. Nomarski differential interference contrast optics were used for the images shown in 5 and 6.

1. Pulmonary microvessels in an alveolar zone (referred to as A in the figure) show red linear fluorescence along the alveolar capillary loops. The microvessels in connective tissue (C-zone) below the bronchial epithelial layers (BR) uniformly show green fluorescence (arrows). In the juxtaalveolar (J)-zone (areas bordering A-zone and peribronchial C-zone), the endothelial cell lining of a dilated microvessel demonstrate mosaic-like combinations of red and green fluorescence. The endothelial cell segments connected to adjacent alveolar capillaries (arrowhead) show red fluorescence.

2 and 3. A juxtaalveolar microvessel in the borders between A- and C-zones shows a mosaic-like distribution of the two antigens. Note the presence of thrombomodulin in the endothelial cell segment which faces the alveolar lumen (arrowhead). Microvessels scattered in the C-zone in 2 and an interlobular venule (arrow) in 3 are characterized by vWf-dominant (green fluorescence) endothelial cells. Original magnification ×200.

4 to 6. connective tissue microvessels > 10 μm in diameter similarly contain endothelial cells with vWf-dominant pattern, and are located in submucosal tissue beneath the bronchial epithelial layer (4) (Scattered spots of pink color in 4 are related to autofluorescence.), among the bronchial glands (5) and in vasa vasorum of large pulmonary arteries (6).

7 to 9. The endothelial cells of C-zone capillaries＜10μm in diameter show a mosaic-like distribution of vWf and thrombomodulin. Thrombomodulin (arrowhead) is clearly visualized at the sites of the cytoplasmic segments showing extreme attenuation. The remaining endothelial cells occasionally have plump segments which show reactivity for vWf (arrow) in association with the surface plasma membranes reactive for thrombomodulin.

μm in diameter, were part of the bronchial circulation, and were located just beneath the bronchial epithelial layer, occasionally showed areas of marked cytoplasmic attenuation. These attenuated segments showed red linear fluorescence for thrombomodulin (**Fig. 1–7 through 1–9**). Thick portions of cytoplasmic segments contained vWf-reactive material and were surrounded by plasma membranes that were reactive for thrombomodulin.

(2) *A-zone capillaries*

The plasma membranes of the alveolar capillary endothelial cells showed red linear fluorescence for thrombomodulin (**Fig. 2–1**). This linear fluorescence measured approximately 2 μm thick along the air-blood barrier, but split into two lines at the nuclear area and extended alongside the luminal and the abluminal plasma membranes (**Fig. 2–1**). The reaction for vWf was hardly detectable in any of the segments of normal A-zones. A positive reaction for vWf in the cytoplasm of alveolar capillaries either coincided with larger deposits of collagen in the alveolar walls, or was localized at a transition to pulmonary venules (**Fig. 2–2**).

(3) *J-zone microvessels*

Many alveolar capillaries merged into pulmonary venules at the edges of the juxtaalveolar zone (**Fig. 2–2**). The latter vessels were connected to pulmonary veins of larger sizes (**Fig. 2–3 and 2–4**), as found in the interlobular C-zones (**Fig. 1–3**). The endothelial cells in the venules of the J-zones had relatively thin cytoplasmic segments that were reactive for both thrombomodulin and vWf. The segments reactive for thrombomodulin were occasionally interrupted by thicker cytoplasmic segments that showed variable degrees of reactivity for vWf (**Fig. 2–2 through 2–4**). The areas of plasma membranes that were reactive for thrombomodulin were in direct continuity with endothelial lining of the alveolar capillaries (**Fig. 2–4**). The other side of the endothelial cells was reactive for vWf and connected with C-zone microvessels which were of the vWf-dominant type. These two fluorescence colors were rather sharply bordered, but it was impossible to distinguish between the cytoplasmic extensions of the two cells. The mosaic-like pattern found in the venules of the interlobular J-zones (**Fig. 2–2 through 2–4**) was similar to that seen in microvessels of other J-zones, including those in peribronchial (**Fig. 1–1, 1–2**) and subpleural areas. The lumina of the microvessels of the J-zones often were widely distended. The shorter diameters of the microvessels averaged 19.9 ± 15.0 μm (mean ± S.D.), and the average longer diameter was 39.3 ± 24.0 μm. The walls of these vessels appeared quite thin, averaging 2.52 ± 1.25 μm in thickness. The endothelial cells of the intrapulmonary arteries measuring<40 μm were characterized by full reactivity for vWf.

Discussion

The present study demonstrates the specific patterns of distribution of three different types of immunoreactivity in the pulmonary microvessels: 1) thrombomodulin-dominant; 2) vWf-dominant, and 3) mosaic-like pattern of thrombomodulin and vWf.

1. Thrombomodulin-dominant pattern

We found thrombomodulin to be localized to the plasma membranes of microvascular endothelial cells in the A-,C- and J-zones. In particular, the plasma membranes of the capillary endothelial cells in the A-zones expressed thrombomodulin alone, and this pattern is herein referred to as thrombomodulin-dominant. This localization of thrombomodulin is in accord with previous reports indicating its presence in vascular endothelial cells[15–17,22]. Thrombomodulin would effectively restrain the induction of the coagulation cascade[11,14]. The widespread distribution of thrombomodulin in the pulmonary microvessels is in agreement with biochemical and immunofluorescence data showing that the lung has the richest expression of this agent among highly vascularized organs including liver, spleen and kidney[14].

2. von Willebrand factor-dominant pattern

The C-zone microvessels, including capillaries and other small vessels with a diameter larger than 10 μm, were composed of relatively thick endothelial cells, which consistently showed reactivity for vWf. These endothelial cells usually lacked reactivity for thrombomodulin. Thus, they are characterized as being of the vWf-dominant type.

In contrast to thrombomodulin, the alveolar capil-

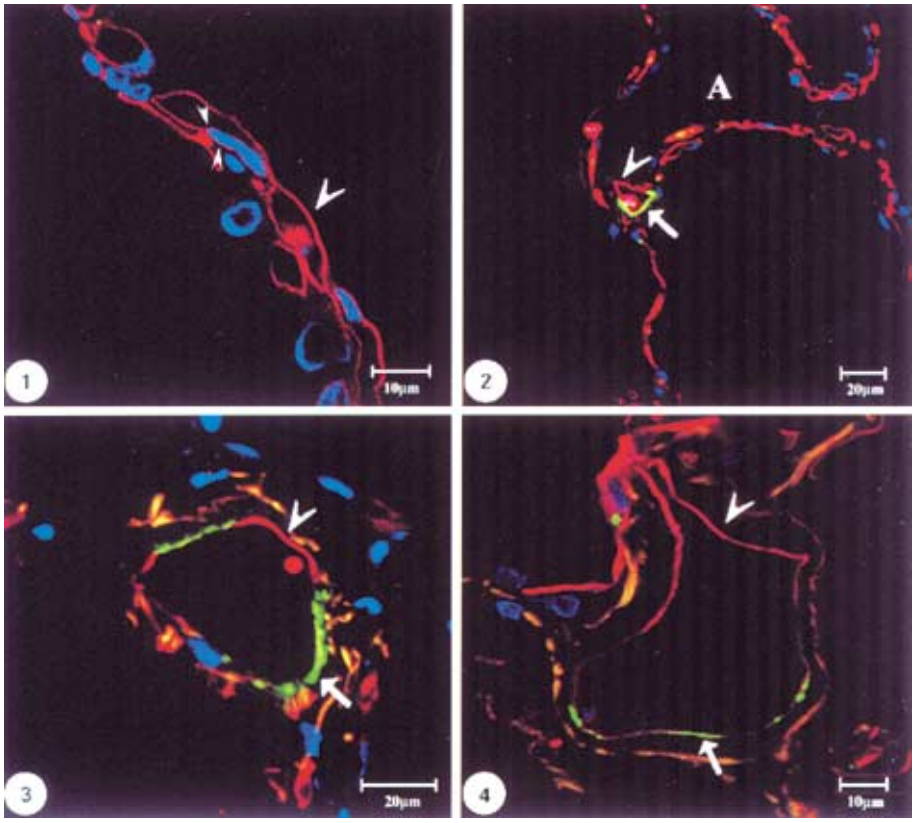

Fig. 2 Dual immunofluorescent stainings for vWf (green) and thrombomodulin (red) as observed by confocal fluorescence microscopy. DAPI-stained nuclei appear blue. 1. The plasma membrane of alveolar capillary endothelial cells is exclusively reactive for thrombomodulin. At the nuclear region of the endothelial cells, the luminal and abluminal plasma membranes show finer red fluorescence (small arrowheads) in comparison with the plasma membrane along the air-blood barrier (large arrowhead). 2. 1, 2 and-like appearance of green fluorescence (arrow) occurs in the endothelial cell lining of the pulmonary venule at the junctions of emerging alveolar capillaries. The combination with red linear fluorescence (arrowhead) in the region connected to the A-zone capillaries forms a mosaic-like pattern of distribution of the two antigens. 3. Alternation in the red and green fluorescence colors is evident in a larger venule in interlobular connective tissue. An intravascular leukocyte is also reactive for thrombomodulin. 4. The endothelial cell segments lining a juxtaalveolar microvessel demonstrate a mosaic-like pattern of distribution of vWf and thrombomodulin. The cytoplasmic segments reactive for thrombomodulin (arrowhead) are in direct continuity with alveolar capillary endothelium at the top of the figure. On the other side of the vessel, occasional endothelial cells are exclusively reactive for vWf (arrow).

lary endothelial cells usually lacked reactivity for vWf. The paucity of this expression in normal lung has been previously demonstrated[8,9]. We have shown that the expression of vWf becomes gradually intensified in thicker segments of endothelial cells in proportion to the degree of alveolar fibrosis in humans[8,9] and animals[10,23,24]. The negative reaction for vWf in these areas may be attributed to the concentration of this antigen being below the limit of detection by the method employed in the study.

The levels of vWf mRNA in lung and brain far exceed those in kidney and liver. In general, these levels are significantly higher in endothelial cells of larger vessels than in those of microvessels, and in venous endothelial cells compared with arterial endothelial cells[25].

These findings emphasize the variabilities encountered in the expression of endothelial cell antigens in different regions of the vasculature.

3. Mosaic-like pattern in the C-zone capillaries

A mosaic-like pattern of distribution of thrombo-

modulin and vWf was observed in C-zone capillaries measuring<10 μm in diameter, especially in areas of the bronchial circulation. The plasma membranes of the capillaries were consistently positive for thrombomodulin, whereas the reactivity for vWf was localized in the underlying thicker portions[21] of cytoplasmic segments of these cells. Ultrastructural studies have shown that [9,10,26] capillaries of the bronchial circulation often contain fenestrations. The cytoplasm of cells is greatly attenuated in areas of fenestration. Nonfenestrated capillary endothelial cells in the A-zones[9,10,21] also are positive for thrombomodulin. Thus, the reactivity for thrombomodulin does not necessarily indicate either formation of fenestrations or origin from the bronchial circulation.

4. Mosaic-like pattern in juxtaalveolar microvessels

In contrast to the C-zone capillaries, the mosaic-like pattern in the J-zone microvessels showed mostly the alternate expression of green and red fluorescence along the thin endothelial lining cells. Only in rare instances did we observe the simultaneous expression of thrombomodulin and vWf in the same cytoplasmic segments, such as was seen in the C-zone capillaries. Endothelial cells in the J-zone microvessels extend their cytoplasmic processes into two different directions, i.e., toward alveolar capillaries and C-zone microvessels. In the lungs of rats given intratracheal infusions of silica, endothelial cells of the J-zone microvessels proliferated and migrated into damaged alveolar capillaries[10]. The segments of proliferating endothelial cells were contiguous with alveolar capillary endothelial cells that expressed thrombomodulin. However, reactivity for vWf was present in the segments of endothelial cells extending toward the C-zone vessels. Therefore, the endothelial cells in the J-zone microvessels are located at the sites of transition between A-zone capillaries and C-zone microvessels. These vessels appeared to form connections between the pulmonary circulation and the bronchial circulation, i.e., bronchopulmonary anastomoses, which are known to occur in peribronchial areas[20]. Such connections have been demonstrated by angiography, bismuth infusion[19], Mercox resin injection[27,28] and scanning electron microscopy.

Buck et al.[29] showed that vWf in 11.5 day old embryonic mice was expressed in the presumptive endothelial cells within the mesenchyma, after which the vessels displayed coexpression of thrombomodulin and vWf. In adult lung, the endothelial cells of alveolar capillaries are reactive for thrombomodulin alone. Incubation of endothelial cells with tumor necrosis factor alpha (TNFα) resulted in a time- and dose-dependent suppression of endothelial cell cofactor (thrombomodulin) activity for the anticoagulant protein C pathway[30]. Endotoxin, which promotes the release of cytokines such as TNFα, also causes a decrease in thrombomodulin expression in human vascular endothelium. This would favor intrasvascular coagulation, thereby contributing to the pathogenesis of the disseminated form of this disorder (which can follow the administration of endotoxin)[31]. Local and environmental factors, including blood pressure shear stress and variety of toxic agents[32], may contribute to the development of altered phenotypes in vascular endothelial cells. It will be of great interest to determine how the patterns of reactivity for thrombomodulin and vWf are altered in various diseases, particularly congenital heart disorders associated with increased blood flow through the bronchopulmonary anastomoses.

Acknowledgements: This work was supported in part by grants from the Ministry of Education, Science, Sports and Culture of Japan (in 1999 Grant-in-Aid for Scientific Research (B), Project Number 11470143).

References

1. Kumar S, West DC, Ager A: Heterogeneity in endothelial cells from large vessels and microvessels. Differentiation 1987; 36: 57–70.
2. Page C, Rose M, Yacoub M, Pigott R: Antigenic heterogeneity of vascular endothelium. Am J Pathol 1992; 141: 673–683.
3. Auerbach R, Alby L, Morrissey LW, Tu M, Joseph J: Expression of organ-specific antigens on capillary endothelial cells. Microvasc Res 1985; 29: 401–411.
4. Mutin M, Dignat-George F, Sampol J: Immunologic phenotype of cultured endothelial cells: Quantitative analysis of cell surface molecules. Tissue Antigens 1997; 50: 449–458.
5. Hewett PW, Murray JC: Human lung mivrovessel endothelial cells: Isolation, culture, and characterization.

Microvasc Res 1993; 46: 89–102.
6. Thorin E, Shatos MA, Shreeve M, Walters CL, Bevan JA: Human vascular endothelium heterogeneity: A comparative study of cerebral and peripheral cultured vascular endothelial cells. Stroke 1997; 28: 375–381.
7. Aird WC, Edelberg JM, Weiler-Guettler H, Simmons WW, Smith TW, Rosenberg RD: Vascular bed-specific expression of an endothelial cell gene is programmed by the tissue microenvironment. J Cell Biol 1997; 138: 1117–1124.
8. Yamamoto M, Shimokata K, Nagura H: An immunohistochemical study on phenotypic heterogeneity of human pulmonary vascular endothelial cells. Virchows Arch A Pathol Anat Histopathol 1988; 412: 479–486.
9. Kawanami O, Matsuda K, Yoneyama H, Ferrans VJ, Crystal RG: Endothelial fenestration of the alveolar capillaries in interstitial fibrotic lung diseases. Acta Pathol Jpn 1992; 42: 177–184.
10. Kawanami O, Jiang HX, Mochimaru H, Yoneyama H, Kudoh S, Ohkuni H, Ooami H, Ferrans VJ: Alveolar fibrosis and capillary alteration in experimental pulmonary silicosis in rats. Am J Respir Crit Care Med 1995; 151: 1946–1955.
11. Esmon CT, Owen WG: Identification of an endothelial cell cofactor for thrombin-catalyzed activation of protein C. Proc Natl Acad Sci USA 1981; 78: 2249–2252.
12. Maruyama I, Bell CE, Majerus PW: Thrombomodulin is found on endothelium of arteries, veins, capillaries and lymphatics and on syncytiotrophoblast of human placenta. J Cell Biol 1985; 101: 363–371.
13. Dittman WA, Majerus PW: Structure and function of thrombomodulin: A natural anticoagulant. Blood 1990; 75: 329–336.
14. Fink LM, Eidt J, Johnson K, Cook JM, Cook CD, Morser J, Marlar R, Collins CL, Schaefer R, Xie SS, Hsu SM, Hsu PL: Thrombomodulin activity and localization. Int J Dev Biol 1993; 37: 221–226.
15. DeBault LE, Esmon NL, Olson JR, Esmon DT: Distribution of the thrombomodulin antigen in the rabbit vasculature. Lab Invest 1986; 54: 172–178.
16. Ford VA, Stringer C, Kennel SJ: Thrombomodulin is preferentially expressed in Balb/c lung microvessels. J Biol Chem 1992; 267: 5446–5450.
17. DeBault LE, Esmon NL, Smith GP, Esmon CT: Localization of thrombomodulin antigen in rabbit endothelial cells in culture. An immunofluorescence and immunoelectron microscope study. Lab Invest 1986; 54: 179–187.
18. Nakajima M, Kawanami O, Jin EJ, Ghazizadeh M, Honda M, Asano G, Horiba K, Ferrans VJ: Immunohistochemical and ultrastructural studies of basal cells, Clara cells and bronchiolar cuboidal cells in normal human airways. Pathol International 1998; 48: 944–953.
19. Cudkowicz L: Bronchial arterial circulation in man: Normal anatomy and responses to disease. In: Moser KM, ed. Pulmonary vascular diseases Lung Biology in Health and Disease vol 14. 1979; pp 111–232, Marcel Dekker, Inc. New York, Basel. New York.
20. Charan NB, Turk GM, Dhand R: Gross and subgross anatomy of bronchial circulation in sheep. J Appl Physiol 1984; 57: 658–664.
21. Deffebach MK, Charan NB, Lakshminarayan S, Butler J: The bronchial circulation: Small, but a vital attribute of the lung. Am Rev Respir Dis 1987; 135: 463–481.
22. Tamura A, Matsubara O, Hirokawa K, Aoki N: Detection of thrombomodulin in human lung cancer cells. Am J Pathol 1993; 142: 79–85.
23. Reidy MA, Chopek M, Chao S, McDonald T, Schwartz SM: Injury induces increase of von Willebrand factor in rat endothelial cells. Am J Pathol 1989; 134: 857–864.
24. Kasper M, Schobl R, Haroske G, Fischer R, Neubert F, Dimmer V, Muller M: Distribution of von Willebrand factor in capillary endothelial cells of rat lungs with pulmonary fibrosis. Exp Toxicol Pathol 1996; 48: 283–288.
25. Yamamoto K, de Waard V, Fearns C, Loskutoff DJ: Tissue distribution and regulation of murine von Willebrand factor gene expression in vivo. Blood 1998; 92: 2791–2801.
26. Renkin EM: Cellular and intercellular transport pathways in exchange vessels. Am Rev Respir Dis 1992; 146: S 28–31.
27. Schraufnagel DE: Corrosion casting of the lung for scanning electron microscopy. Electron Microscopy of the Lung. In: Schraufnagel DE, ed. 1990: pp257–297. Marcel Dekker Inc, New York.
28. Peao MND, Aguas AP, de Sa AC, Grande NR: Neoformation of blood vessels in association with rat lung fibrosis induced by bleomycin. Anat Rec 1994; 238: 57–67.
29. Buck CA, Edelman JM, Buck CE, Kennedy G, Baldwin HS: Expression patterns of adhesion receptors in the developing mouse lung: Functional implications. Cell Adhes Commun 1996; 4 : 69–87.
30. Nawroth PP, Stern DM: Modulation of endothelial cell hemostatic properties by tumor necrosis factor. J Exp Med 1986; 163: 740–745.
31. Moore KL, Andreoli SP, Esmon NL, Esmon CT, Bang NU: Endotoxin enhances tissue factor and suppresses thrombomodulin expression of human vascular endothelium in vitro. J Clin Invest 1998; 79: 124–130.
32. Gerritsen ME, Bloor CM: Endothelial cell gene expression in response to injury. FASEB J 1993; 7 : 523–532.

(Received, December 10, 1999)
(Accepted, December 28, 1999)