Vascular endothelial growth factor in patients with high-altitude pulmonary edema

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HIGH-ALTITUDE PULMONARY EDEMA (HAPE) is a life-threatening condition defined as noncardiogenic pulmonary edema (11). It affects healthy people after rapid ascent to altitudes >2,500 m (10, 13). Although exaggerated pulmonary hypertension was suggested as important in the pathogenesis of HAPE (9), it is not sufficient to trigger pulmonary edema (24). Accumulating evidence concerning inflammation and increased vascular permeability has been clearly revealed by bronchoalveolar lavage (BAL) studies in patients with HAPE, suggesting that additional mechanisms play a role in this condition (14, 15, 25, 26, 30).

VEGF is a potent endothelial cell-specific mitogen and permeability factor, known to be involved in vascular basement membrane destruction and angiogenesis (6, 8, 16). Overexpression of VEGF in the lung induces an increased pulmonary vascular permeability, resulting in pulmonary edema (12). In addition, VEGF has been shown to be markedly upregulated in the hypoxic condition (5, 17, 20, 31). Recent studies suggested that VEGF production in hypoxia might be involved in high-altitude cerebral edema (HACE) (27, 34). HAPE sometimes occurs in conjunction with HACE. The increased capillary permeability is likely to be a crucial mechanism in both disorders (15, 25, 26).

We hypothesized that VEGF might also play some pathophysiological part in the development of HAPE.

In the present study, we measured the concentrations of VEGF in venous serum and BAL fluid (BALF) in patients with HAPE at admission and during the recovery stage, respectively, which were compared with those in healthy young volunteers.

METHODS

Subjects. The nine patients with HAPE enrolled in the study were all nonsmoking male climbers, with an average age of 36.9 yr. They had been born and resided at low altitude. They had been rescued while climbing in the Japan Alps and transported to our institution, Shinshu University Hospital (610 m above sea level). The altitude at the onset of HAPE ranged from 2,350 to 3,190 m above sea level. The average duration of the HAPE victims at high altitude was 3–4 days. We diagnosed HAPE based on the following criteria (10): onset at high altitude of the typical symptoms, including cough and dyspnea at rest; absence of infection; presence of pulmonary rales and cyanosis; disappearance of symptoms and signs within 3 days of the start of treatment with bed rest and supplemental oxygen; and chest roentgenographic infiltrates consistent with pulmonary edema. All subjects met the criteria at the onset of the disorder, and two subjects were also comatose and showed the condition of HACE. All recovered promptly during hospitalization without the intervention of mechanical ventilation.

We recruited five nonsmoking men, with an average age of 21.0 yr, as a control group. They had all been born and resided at low altitude. They had no history of cardiopulmonary problems and were taking no other medications at the time of this study. All subjects gave informed consent, and the study protocol was approved by the Institutional Committee on Human Research of our School of Medicine.

Peripheral blood. Venous blood samples from the patients with HAPE were drawn into serum tubes containing beads and clot activator at the time of admission before oxygen administration and at the moment of discharge (on the 6th to 10th day after admission). Concomitantly, the blood samples...
were also obtained from the five controls. The samples were kept at room temperature for 30 min before centrifugation at 3,000 rpm for 10 min, and then the supernatants were kept frozen at −70°C until assayed.

The serum VEGF was measured by using a quantitative sandwich ELISA kit (Human VEGF Quantikine, R&D Systems, Minneapolis, MN), according to the manufacturer’s instructions. This assay had an intra-assay precision of 4.5% and an interassay precision of 7.0%. This assay measured biological active VEGF121 and VEGF165. The expected mean serum value was 220 pg/ml, according to the manufacturer’s instructions.

In addition, the circulating white blood cell (WBC) count and C-reactive protein (CRP) level of the patients were measured at admission as well as at discharge.

**BALF.** BAL was performed in the patients with HAPE within 12 h after admission and at the time of discharge (on the 6th to 10th day after admission). After subcutaneous injection of atropine (0.5 mg) and pethidine hydrochloride (0.5 mg/kg), 2% lidocaine solution was sprayed in the oral pharynx and upper airway for local anesthesia. A sterile fiberoptic bronchoscope (Olympus BF 1T, Olympus, Tokyo, Japan) was wedged in the B4 or B5 segmental bronchus. Three 50-ml aliquots of sterile normal saline warmed to 37°C were instilled successively into the lobe, and each was in turn removed by gentle suction. The mean percent retrieval of the instilled saline was 57.5 ± 5.0% at admission and 59.0 ± 2.3% at recovery. The lavage fluid was filtered through gauze. One aliquot was set aside for counting the number of total cells. Another aliquot was spun in a cytometer at 500 rpm for 5 min and stained by the May-Grunwald-Giemsa method to identify cells in a population of 200 cells. The remaining aliquot was centrifuged to remove cellular elements and was stored frozen at −70°C for biological analysis at a later time. The same method was performed in the control subjects. The mean percent retrieval of the instilled saline was 60.3 ± 2.9%.

VEGF was measured with the ELISA method in the supernatant samples of the BALF, as described above. We also measured the concentrations of total protein and albumin in the BALF of the patients and controls using the pyrogallol red method and the immunoturbidimetric assay, respectively (14, 15).

**Statistical analysis.** Values are expressed as means ± SE. The two-tailed Student’s *t*-test was used for the comparisons of the measurements between the admission and recovery within the patients, as well as between the HAPE patients and controls within all of the subjects. A *P* value < 0.05 was considered statistically significant.

**RESULTS**

**Measurements in peripheral blood.** The circulating WBC counts (13,319 ± 710 cells/μl) and CRP level (3.64 ± 0.83 mg/dl) were elevated in all patients with HAPE at admission, whereas these values were returned to the normal range at discharge. Figure 1 shows the concentrations of VEGF in serum in both groups. The VEGF of the HAPE patients at recovery (423.7 ± 44.7 pg/ml) was significantly increased compared with that at admission (260.7 ± 38.7 pg/ml, *P* < 0.05), although there was no significant difference in VEGF between the HAPE at admission and the controls (260.7 ± 38.7 vs. 228.8 ± 50.5 pg/ml, *P* > 0.05).

**Measurements in BALF.** For patients with HAPE, significantly elevated values were total cell count (348.1 ± 71.0 vs. 42.6 ± 5.4 × 10^3 cells/ml, *P* < 0.05), alveolar macrophages (123.8 ± 19.0 vs. 36.9 ± 5.3 × 10^3 cells/ml, *P* < 0.01), and neutrophils (119.9 ± 19.2 vs. 0.6 ± 0.2 × 10^3 cells/ml, *P* < 0.001), compared with those in control subjects. In addition, the total protein (342.6 ± 62.5 vs. 5.4 ± 0.7 mg/dl, *P* < 0.005) and albumin (2,267.2 ± 298.1 vs. 29.6 ± 1.4 μg/ml, *P* < 0.00005) levels were significantly higher in patients with HAPE than in the controls. At recovery, all of these elevated values in HAPE were normalized as in the controls. The bacteriological examinations of the BALF yielded negative results in all samples.

Figure 2 shows the concentrations of VEGF in the BALF in both groups. VEGF was detectable in all samples. The VEGF of the patients at admission (42.8 ± 9.9 pg/ml) was markedly deprived comparing with that of controls (265.2 ± 34.9 pg/ml, *P* < 0.00005). Subsequently, the deprived VEGF was gradually restored at the recovery stage (79.8 ± 5.6 pg/ml), which, however, could not reach significance compared with that at admission.

**DISCUSSION**

The elevated values of circulating WBC count and CRP in the peripheral blood, accompanied by the increased levels of total cell count and protein in the BALF in the patients with HAPE, were similar to those in previous studies (14, 15, 25, 26), which revealed a transient inflammatory process during the early stage of HAPE. The most noteworthy finding in the present study was that the concentration of VEGF in the BALF of patients was markedly deprived compared with that in controls, indicating that the production of VEGF is insulated in the lung of the patients. In addition, the deprived VEGF in the BALF of the patients was improved gradually, following a similar VEGF dynamics in venous serum during the stage of recovery.
VEGF is a potent mitogenic and permeability factor predominantly targeting endothelial cells (6, 16). VEGF expression is upregulated by hypoxia (5, 17, 20, 31), and its role in hypoxia-induced angiogenesis has been extensively studied in a variety of disease entities. Xu and Severinghaus (34) showed that the transcription of VEGF and the production of VEGF protein in the rat brain were upregulated during the first week of hypoxia. They hypothesized that the angiogenesis process induced by VEGF might be involved in the development of HACE. Overexpression of VEGF was also proposed to contribute to the pathogenesis of hypoxic pulmonary hypertension (5, 32) and increased pulmonary vascular permeability (6, 12), both of which are principal mechanisms in HAPE (9, 11). We measured the VEGF in peripheral blood and BALF in the HAPE patients at the early stage and showed that the levels of VEGF were unexpectedly depressed in BALF, although they remained unchanged in peripheral blood. Because all blood samples were obtained before oxygen administration, all BAL studies were performed within 12 h after admission, and no patient received mechanical ventilatory intervention that could interfere with the biological functions of the lung, so the present findings appear to reflect the lung condition of HAPE at the early stage.

Recently, several investigations were conducted for studying the pathophysiological role of VEGF in HAPE and found that the VEGF at high altitude was not elevated in association with acute mountain sickness, HAPE, or hypoxia, concluding that VEGF might not mediate critical pulmonary permeability during the earliest time at high altitude (18, 22, 33). However, except for one measurement of VEGF in the pulmonary capillaries (33), all other measurements were the VEGF concentration in the systemic circulation. Therefore, the pathophysiological role of VEGF in the HAPE lung remains to be clarified. Despite being characterized by endothelial cell specificity, VEGF is expressed and released by epithelial cells that are in close proximity to the microvasculature in the highly vascularized lung tissues (3). Studies elsewhere showed that the VEGF mRNA and protein were abundant in the distal airway epithelial cells of the development and adult lungs and important in driving the development of the pulmonary capillary bed and ultimately the air-blood barrier (4). The high concentration of VEGF in BALF in the lung of controls revealed in this study implied that VEGF might play an essential role in the maintenance of physiological function of lung. Based on those above, we suspect that the deprivation of VEGF in the lung of HAPE at admission might disturb the air-blood barrier function, resulting in increased pulmonary vascular permeability, whereas the restoration of VEGF in the lung during recovery could repair the dysfunctional air-blood barrier and protect against increased permeability.

The highlighted point of the present study was to focus on the pathophysiological role of VEGF in the lung, the insulted organ of HAPE. Among normal tissues, the lung has a relatively high-VEGF message abundance (2). The VEGF concentration in BALF of nonsmoking normal subjects, detected by ELISA, was reported to be between 67 and 289 pg/ml (mean = 141 pg/ml) by Boussat et al. (3) and 265 ± 34.9 pg/ml by the present study. The role of VEGF in the normal lung is unknown. The abundant expression demonstrated by immunohistochemical staining throughout the pulmonary parenchyma suggested an essential role of VEGF in the maintenance of the unique balance of microvascular permeability and endothelial cell function (5). VEGF is constitutively expressed by human bronchial and alveolar epithelial cells and can be further induced by hypoxia and other vascular mediators, probably by autocrine or paracrine mechanisms (3–5). Brown et al. (4) observed that VEGF might be an important autocrine growth factor for distal airway epithelial cells in the developing human lung, including the development of the pulmonary capillary bed and air-blood barrier. Boussat et al. suggested that VEGF might play an important role in alveolar and bronchial vessels under either physiological or pathological conditions. Patorvian et al. (21) further found that, in addition to the well-known angiogenic property, the VEGF overexpression in lung tissue did not affect the pulmonary circulation in normoxic condition but attenuated the development of pulmonary hypertension and right ventricular hypertrophy in rats exposed to chronic hypoxia, indicating a protective function of VEGF to the endothelium under hypoxic condition. This experiment also identified that such protective function might be realized by enhancing the release of endothelial nitric oxide formation that then contributed to attenuation of hypoxia-induced pulmonary hypertension and pulmonary vascular remodeling (21). All of these experiments provide confidential evidences to support our hypothesis concerning the physical protective and repairing function of VEGF in the recovery stage of HAPE described above.

![Graph showing VEGF concentration in BALF](image)
The marked deprivation of the VEGF level in BALF of HAPE patients at admission is most probably a result of the attenuated release of it due to the injured type II pneumocytes rather than a course to induce hyperpermeability in HAPE. Pathological studies have demonstrated that the type II pneumocytes and capillary endothelial cells were damaged in the HAPE lung (7, 29). These findings suggest that VEGF is less likely to be involved in the pathogenesis of pulmonary edema formation as a permeability factor. However, supported by the present evidence of the increased VEGF in both the systemic and pulmonary circulations during the recovery stage of HAPE, it is suggested that VEGF is probably related to the repair of the impaired cellular layer. Because the findings derived from animal study suggested that alveolar epithelium was the primary site of increased VEGF mRNA abundance in the recovering lung injured by acute hyperoxia, the type II cells might regulate the proliferation of subjacent endothelium and contribute to microvascular endothelial repair after the damage (19). Endothelial cells are involved in several key processes in restitution of the air-blood barier in alveoli (1). The regulations of angiogenesis and endothelium repair in the lung are probably crucial to satisfactory healing (3). The dramatic response to oxygen therapy in HAPE patients at an early stage suggests that the cellular biological impairment in lung of HAPE is reversible (23). Taken together, it is suggested that this re-released VEGF in the BALF and systemic circulation plays a certain role in the marked recovery of HAPE.

In summary, a marked deprivation of VEGF in BALF of the HAPE patients at admission after its gradual restoration at recovery, accompanied by similar dynamics in peripheral blood, was observed. It is suggested that VEGF appears less likely to have a critical part in the pathogenesis of HAPE, but rather an important role in the repair process.

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REFERENCES


