Electron Microscopy Observation of Human Pulmonary Ultrastructure in Two Patients with High-Altitude Pulmonary Edema

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Running Head: Electron microscopy study of HAPE

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ABSTRACT

We examined the pulmonary ultrastructure in tissue from two patients with high altitude pulmonary edema (HAPE) by electron microscopy. In one case, we found that neutrophils were trapped in pulmonary capillary lumen of alveolar-capillary wall and part of the cytoplasm of a neutrophil protruded and adhered to the capillary endothelium. There were several degranulated vacuoles in the cytoplasm of the neutrophil. The pulmonary capillary wall was deformed, thickened, and swollen and there was evidence of degeneration. In another case, infiltration of neutrophils and macrophages, proliferation of type II pneumocytes, and numerous red blood cells were also observed in alveolar air space. These electron microscopic ultrastructural observations illustrate for the first time damage to the pulmonary alveolar-capillary barrier in lung tissue of humans with advanced HAPE.

Keywords: Electron microscopy, High altitude pulmonary edema, Pulmonary alveolar-capillary barrier

INTRODUCTION

High altitude pulmonary edema (HAPE) is a non-cardiogenic pulmonary edema. It is life-threatening and usually develops in susceptible individuals following their rapid exposure to high altitudes over 2,500 meters (m) above sea level (Bärtsch and Swenson, 2013). The pathogenesis involves hypobaric hypoxia-induced pulmonary vasoconstriction that causes pressures to be regionally inhomogeneously elevated in pulmonary arteries, arterioles, and capillaries. This leads first to non-injurious dynamic alterations in alveolar-capillary permeability that can ultimately lead with time and higher pressures to stress failure of pulmonary capillaries eventually results in a non-inflammatory hemorrhagic pulmonary edema which may evolve with time with features of secondary inflammation (West et al., 1995; Swenson et al. 2002). The pathological features of HAPE by light microscopy are characterized by marked spreading of alveolar edema, pulmonary capillary dilatation and alveolar septal wall thickening (Droma et al., 2001). However, these histological findings have not yet been verified by electron microscopy at the ultrastructural level because human lung tissue from HAPE patients are not easily available due to matters of safety and informed consent. Two studies of a HAPE-like illness in Sprague-Dawley rats demonstrated stress failure of pulmonary capillaries at the ultrastructural level (West et al., 1995; Bai et al., 2010). Here, we report electron microscopy observations on cellular ultrastructure in human lung tissue of two patients with HAPE, focusing on the evidence of alteration and damage to the pulmonary alveolar-capillary barrier.

CASE DESCRIPTION

The current study was approved by the Ethics Committee of Shinshu University

School of Medicine. Written informed consent for bronchoscopy and lung tissue biopsy was obtained from both patients.

Case 1: A 67-year-old man arrived and camped at the foot (600 m) of the Japan Alps in the summer season. He coughed while climbing up to 3,000 m on the next day. His cough worsened with the development of pink sputum while trekking higher to 3,180 m. Symptoms of fatigue, anorexia, sleep difficulty, dyspnea, and cyanosis soon followed. He was rescued by helicopter three days after beginning his ascent and admitted to our hospital at an altitude of 600 m. On admission, he was fully conscious. Cyanosis on the lips and extremities were observed. Coarse crackles were audible at the bases of both lungs. Chest x-ray and computed tomography (CT) examinations showed patchy infiltrative shadows in the lower fields of both lungs. Arterial blood gas analysis showed a partial pressure of oxygen (PaO_2) of 42.9 Torr on ambient air. By right heart catheterization, the pulmonary artery pressure (PAP) was 40/23 mmHg (systolic/diastolic pressure) with mean PAP of 29 mmHg and pulmonary capillary wedge pressure (PCWP) was 12 mmHg. HAPE was diagnosed according to diagnostic criteria proposed at the 1991 International Hypoxia Symposium (Hackett and Oelz, 1992). Supplemental oxygen and bed rest were promptly provided. His symptoms and abnormal chest x-ray shadows completely resolved after one week of treatment with oxygen therapy and bed rest.

Case 2: A 49-year-old man arrived at the foot of the Japan Alps in the summer season. After climbing to 3,180 m and staying overnight in a lodge at 3,000 m, he developed symptoms of coughing, difficulty breathing, and cyanosis on the third day of his ascent. He was rescued by helicopter and admitted to our hospital on the next day. On admission, he was fully conscious. Cyanosis was presented on his lips and extremities. Coarse

crackles were audible over both lungs. Chest x-ray and CT examinations showed a pattern of bilateral patchy alveolar filling indicative of pulmonary edema. The PaO_2 was 35.6 Torr on ambient air. Under condition of oxygen supplement, his pulmonary artery pressure was 31/13 mmHg with mean PAP of 19 mmHg and PCWP was 10 mmHg by right heart catheterization. He was diagnosed with HAPE as above and treated with supplemental oxygen and bed rest. The symptoms and abnormal lung shadows diminished with treatment of oxygen therapy and bed rest and he was fully recovered after one week.

BRONCHOSCOPY

Flexible bronchoscopy was carried out on the second day of hospital admission in both cases after obtaining informed consent. In addition, bronchoscopy was repeated after 7 days of treatment in case 2 with his informed consent in order to observe lung structure in the recovery stage of HAPE. Bronchial alveolar lavage (BAL) was performed and the BAL fluid (BALF) was obtained for total cell count as well as multinucleated cells with May–Grunwald–Giemsa staining. Bacteriological examination was performed as our previous study (Kubo et al., 1998). Two to three lung tissue specimens were obtained from the lower lobe of edematous segments by transbronchial forceps biopsy. Specimens were then placed in 2.5% glutaraldehyde for fixation and then specifically prepared for electron microscopy. Experts in electron microscopy in the Pathology & Cytology Laboratories of Japan in Tokyo blinded to the diagnosis of these patients examined these specimens.

RESULTS

Multinucleated cells in bronchial-alveolar lavage fluid

Table 1 shows the data regarding multinucleated cells in the BALF of the two patients and they are consistent with previous BALF findings in patients with HAPE (Kubo et al., 1998, Schoene et al., 1988). The number of total cells was elevated and included increased amounts of neutrophils in both of patients (Table 1) indicative of increased pulmonary capillary permeability. In addition, total cell and neutrophil count were greatly diminished in second lavage seven days later of case 2 (Table 1). Bacteriological cultures of the BALF were negative in both of the patients.

Electron microscopy observation

We obtained 29 electron micrographs of different magnification of the lung tissue in case 1 and 20 in case 2. The most typical cellular findings were the presence of infiltrated neutrophils as well as the numerous red cells, macrophages and proliferation of type II pneumocytes in the alveolar air space. The noteworthy findings by electron microscopy were that of a neutrophil trapped in the capillary lumen with a part of its cytoplasm protruding and adherent to the basement membrane of the capillary endothelium (Figure 1, case 1). There were several degranulated vacuoles in the cytoplasm of this neutrophil. The wall of this pulmonary capillary was deformed, thickened, and swollen (Figure 1, case 1). Figure 2 shows a slightly thickened alveolar-capillary barrier at ultrastructural level in the lung tissue of case 2. A type II pneumocyte with numerous mature lamellar bodies is observed in alveolar space (Figure 2). Figure 3 shows a neutrophil infiltrating in the pulmonary parenchyma (Figure 3, left) and numerous red blood cells in the alveolar space in case 2 (Figure 3, right); the latter indicating disruption of the alveolar-capillary barrier.

DISCUSSION

Our electron microscopy findings in two cases of advanced HAPE (4-5 days after the onset of HAPE) demonstrate that the ultrastructure of pulmonary alveolar-capillary wall can be impaired in HAPE patients as evidenced by deformation and thickening of the pulmonary endothelium, infiltration of neutrophils into the pulmonary parenchyma, and red blood cells in the alveolar space.

This is a novel pathological observation by electron microscopy of the ultrastructure of human lung tissue in patients with HAPE showing evidence of impairment of the pulmonary alveolar-capillary barrier. This is the first direct evidence of capillary stress failure in humans caused by hypoxia-induced pulmonary vasoconstriction. Previous electron microscopy studies in rat models of HAPE (West et al., 1995; Bai et al., 2010) support this supposition in demonstrating disruption of capillary endothelial layer, swelling of alveolar epithelial layer, and red blood cells in alveolar spaces. All these changes suggest a role of stress failure of pulmonary capillaries in advanced HAPE of several days duration.

The increased total cell count with large numbers of neutrophils in the BALF of two patients with HAPE (Table 1) indicates increased pulmonary capillary permeability at the moment of tissue sampling. The increased multinucleated cells in the BALF of these two patients could be explained as the consequence of stress failure in pulmonary capillaries caused by hypoxia-induced pulmonary vasoconstriction. It is likely that the highly exposed basement membrane of the damaged pulmonary capillaries undergoing stress failure can attract neutrophils that become activated and degranulated to cause further destruction of alveolar-capillary barrier. Supporting cellular evidence by electron microscopy is that the exposed basement membrane of the damaged pulmonary

capillaries also attracts platelets in the HAPE-like model of Sprague–Dawley rats (West et al., 1995; Bai et al., 2010).

The major limitations of this small study are that the data analysis was not quantified and that the control lung tissue was not obtained. The highest level of quantitative data analysis of electron microscopy requires specific sample preparation with sophisticated techniques and control lung specimens from subjects of similar age, smoking history and underlying lung disease, and in the case of HAPE, samples obtained from subjects without pulmonary edema after equivalent altitude exposure and duration. Nevertheless, our present electron microscopic observations on human pulmonary ultrastructure in advanced HAPE is novel and adds to our knowledge of the pathophysiology of HAPE in humans.

Using electron microscopy, we can observe a small portion of the lung ultrastructure in HAPE at an incredible level of cellular detail. We identified features of several typical cells in the lungs of HAPE patients, which heretofore have not been seen in the lavage fluid from patients with HAPE. It is expected that these novel electron microscopy observations of the human pulmonary ultrastructure in subjects with HAPE may contribute to a deeper understanding of its pathophysiology.

Competing Interests

The authors state that we have no conflict of interest in the present study.

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TABLE 1. Multinucleated cells in the bronchial-alveolar lavage fluid

Multinucleate cells	Case 1 (on admission)	Case 2 (on admission)	Case 2* (after 7 days)
Total cells (x 10^3/ml)	130	319	92
Macrophages (%)	59.2	46.2	79.4
Neutrophils (%)	35.0	42.3	3.2
Lymphocytes (%)	5.8	10.9	17.0
Eosinophils (%)	0	0.6	0.4

*The data was obtained after 7 days of treatment in the second time of bronchoscopy. (A second bronchoscopy in Case 1 was not performed).

FIGURE LEGENDS

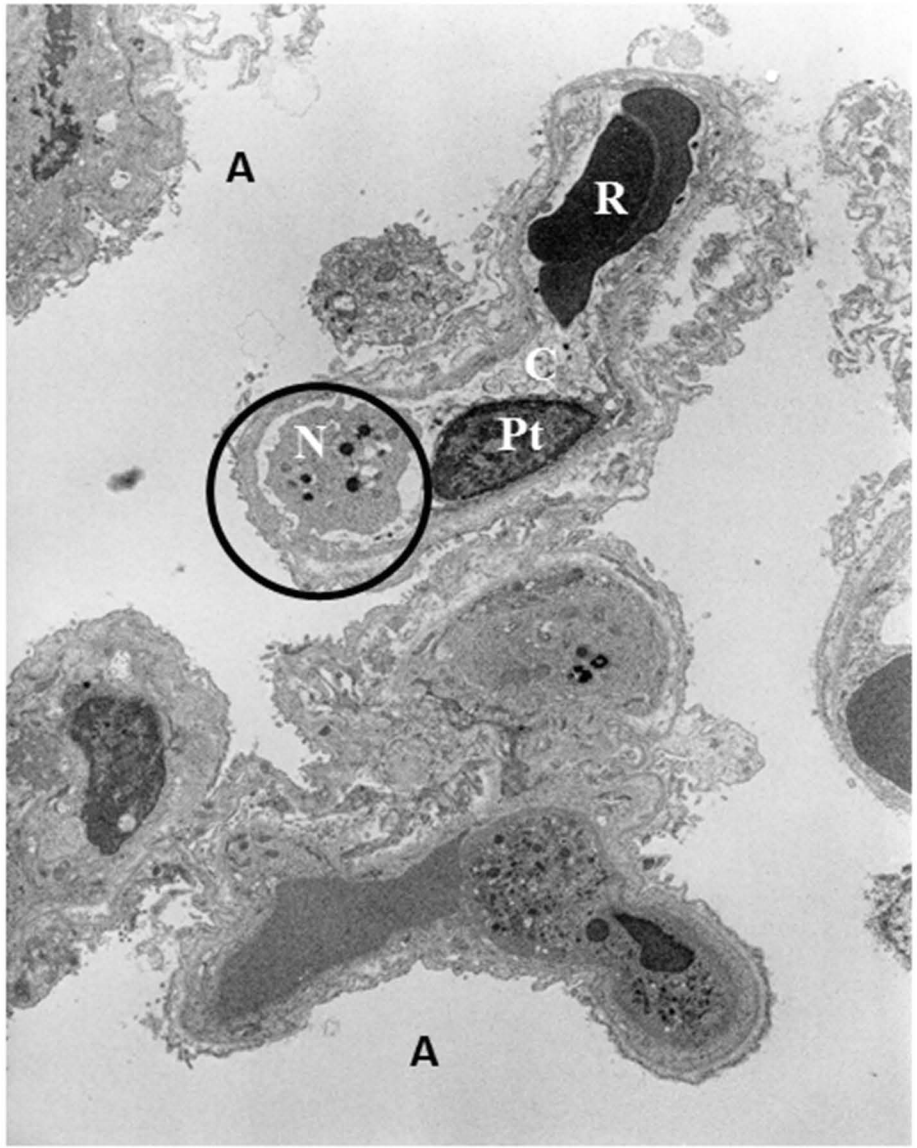
Figure 1. Electron micrograph showing a neutrophil in a pulmonary capillary lumen in case 1 of high-altitude pulmonary edema (HAPE). Left: A neutrophil (N), a red blood cell (R), and a platelet (Pt) were observed in the pulmonary capillary lumen (C). The part marked with black circle was further magnified and presented in the right figure. Magnification x 4,500. Right: A neutrophil (N) was trapped and a part of the cytoplasm of the neutrophil protruded and adhered to the basement membrane of the capillary endothelium (black arrow). There were several degranulated vacuoles (D) in the cytoplasm of the neutrophil. The endothelial layer was swollen (SW) and thickened (TH). Magnification x 23,000. A: alveolar space; C: pulmonary capillary lumen; White arrows: segments of the nucleus.

Figure 2. Electron micrograph showing the typical ultrastructure of alveolar-capillary barrier in case 2. A neutrophil (N), a red blood cell (R), and a platelet (Pt) were observed in the pulmonary capillary lumen (C). The capillary wall was slightly thickened. A type II pneumocyte (Type II cell) with numerous mature lamellar bodies (LB) was observed in the alveolar space, explaining the proliferation of type II pneumocytes for the rapidly recovery of HAPE. A red blood cell (R) was observed in the alveolar space, indicating disruption of the alveolar-capillary barrier. Magnification x 4,600. A: alveolar space.

Figure 3. Electron micrograph showing a neutrophil (N) infiltrating in pulmonary parenchyma (Left, white arrows: segments of the nucleus; magnification x 4,500) and several red blood cells (R) in the alveolar space in case 2 (Right, M: Macrophage;

magnification x 3,240). A: alveolar space.

Figure 1



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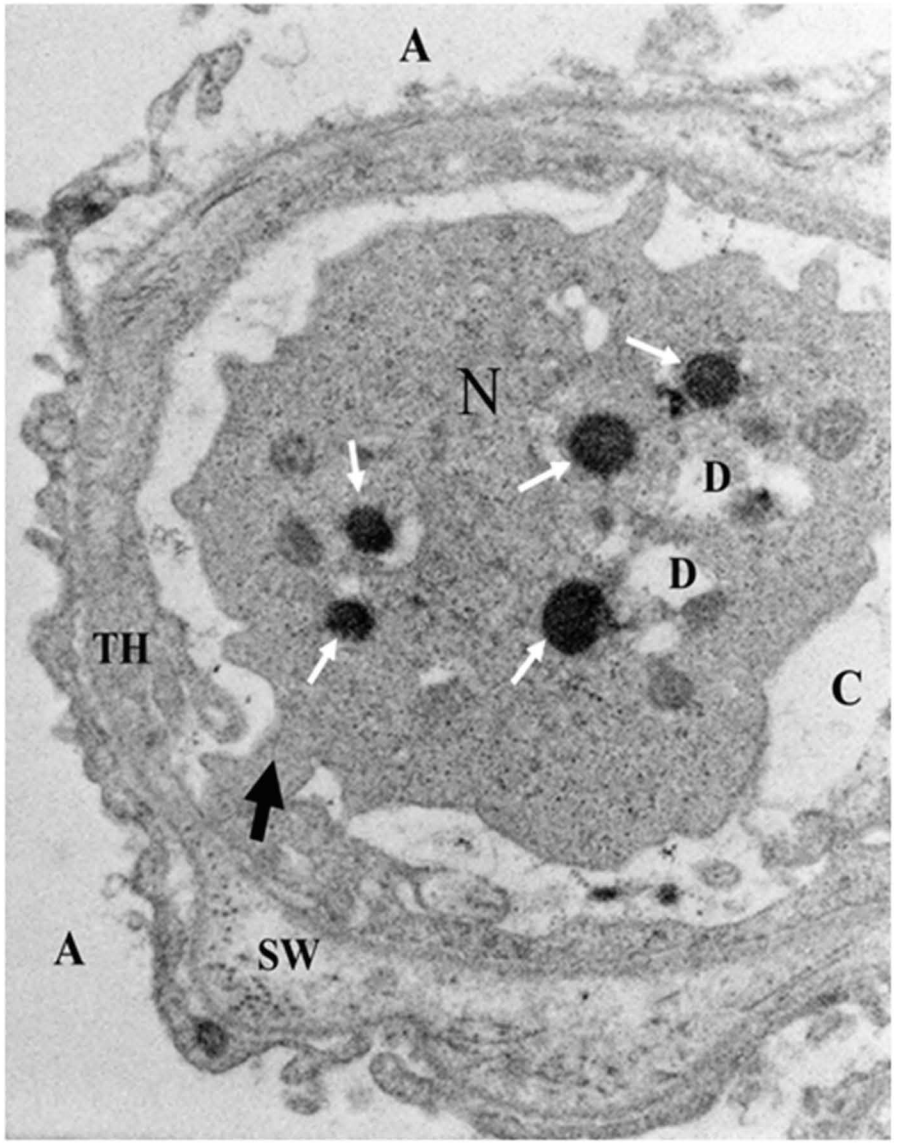

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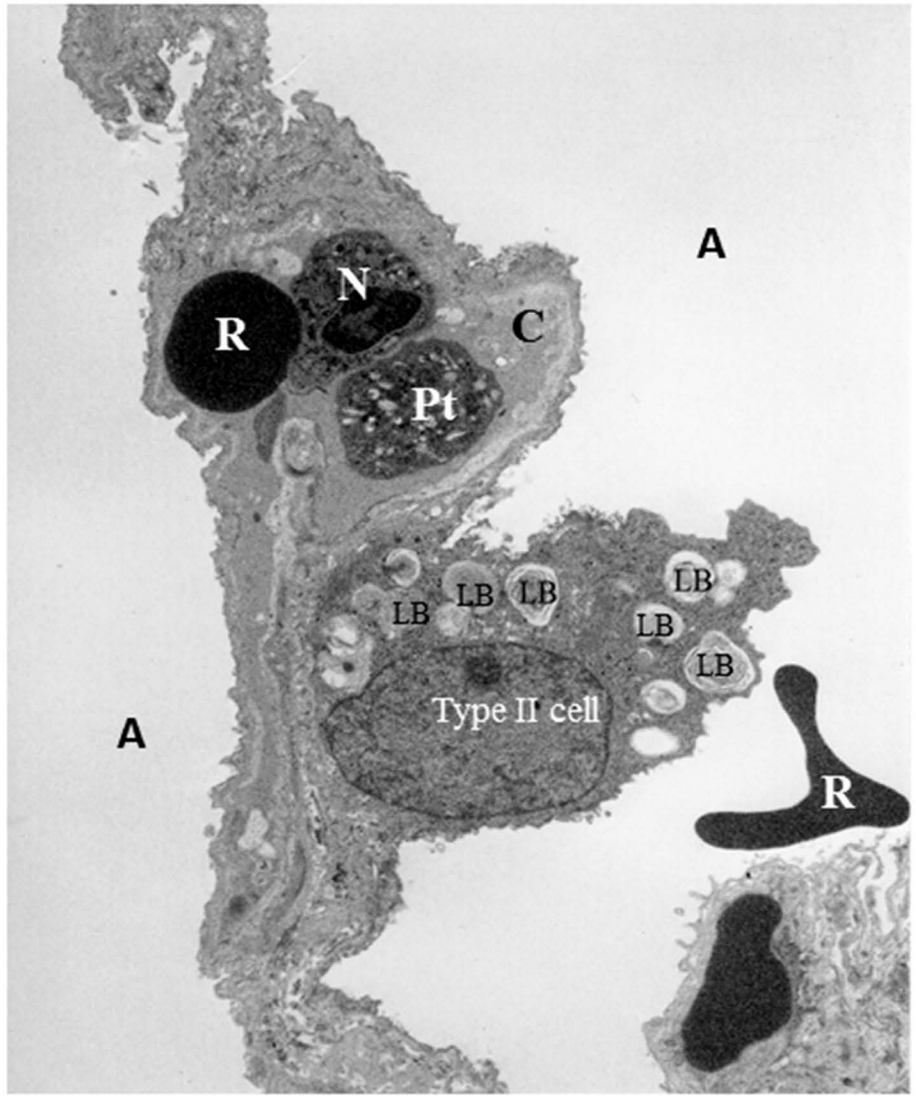
Figure 2.



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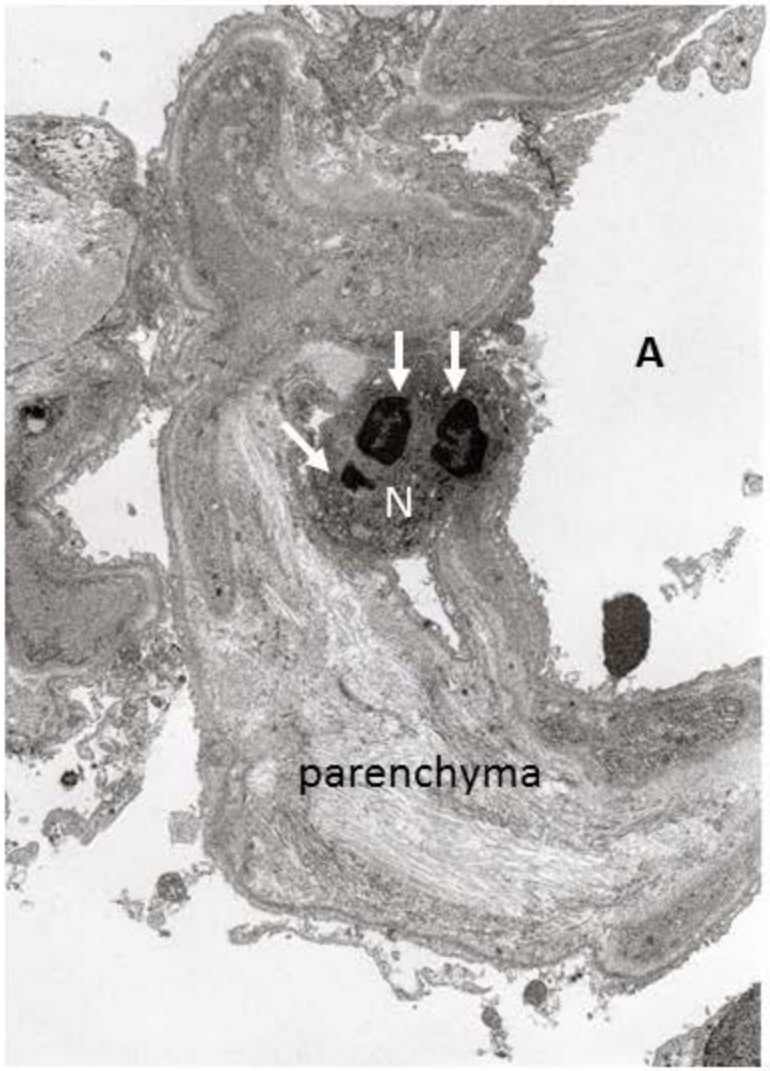

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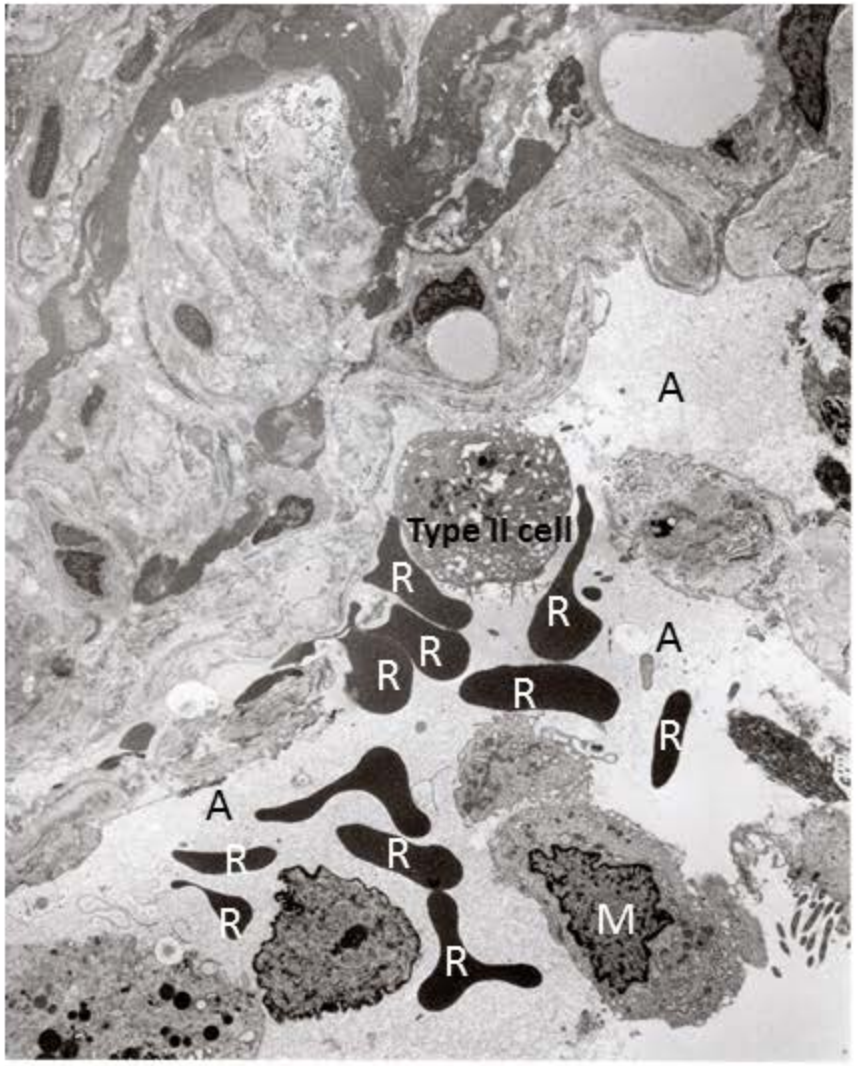

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