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BULGING MESOTHELIAL CELLS OF THE VISCERAL PLEURA OF THE RAT MIMIC THE NETWORK OF SUBPLEURAL LYMPHATICS

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ABSTRACT

The mesothelial surface of the visceral pleura of the Wistar rat was viewed at high resolution by scanning electron microscopy (SEM). The pleural surface showed exquisite linear arrangements made up of bulging mesothelial cells. They were organized in irregular circles that often presented anastomotic junctures. This arrangement of pleural mesothelial cells mimics the organization of subpleural lymphatics of the lung. A low density of microvilli was seen inside the irregular circles, contrasting with the microvilli-rich mesothelial cells seen on or outside these arrangements. These SEM features of the mesothelium may be related with the formation of microdomains for fluid absorption across the visceral pleura into subpleural lymphatics.

The topography of lymphatics in the lung is heterogeneous: the vessels are numerous underneath the visceral pleura, scarce in the alveolar domain of the organ, and abundant again at its hilar region (1-4). The lymph that is collected by the superficial lymphatics is drained into the hilar vessels and then into the bronchial lymph nodes (5,6). The subpleural lymphatics are located immediately underneath the mesothelial cells of the visceral pleura of the lung. In fact, only a basement membrane and a thin layer of connective tissue separate these vessels from the mesothelium.

On using scanning electron microscopy (SEM) to investigate the detailed surface structure of the visceral pleura of the rat, we have found linear arrangements of bulging mesothelial cells. Comparison of these mesothelial arrangements with those of subpleural lymphatic vessels prompted us to propose here that they correspond to the underneath network of subpleural lymphatics, and we conclude that they may be related with fluid absorption through the visceral pleura.

MATERIALS AND METHODS

Animals

10 adult male Wistar rats were purchased from a Spanish breeder (Charles River Laboratories España, SA, Spain). All animals had unrestricted access to food (commercial chow) and water and were treated in accordance with the European Union laws on animal protection (directive 86/609/EC). Standard housing conditions were used that involved keeping two rats for a plastic cage (42 x 27 x 16 cm) with a steel lid.

Scanning Electron Microscopy (SEM) of the Lung Surface

The rats were sacrificed by a lethal intravenous injection of sodium pentobarbital (40 mg/kg), the middle lobe of the right lung excised, and processed for SEM. The samples

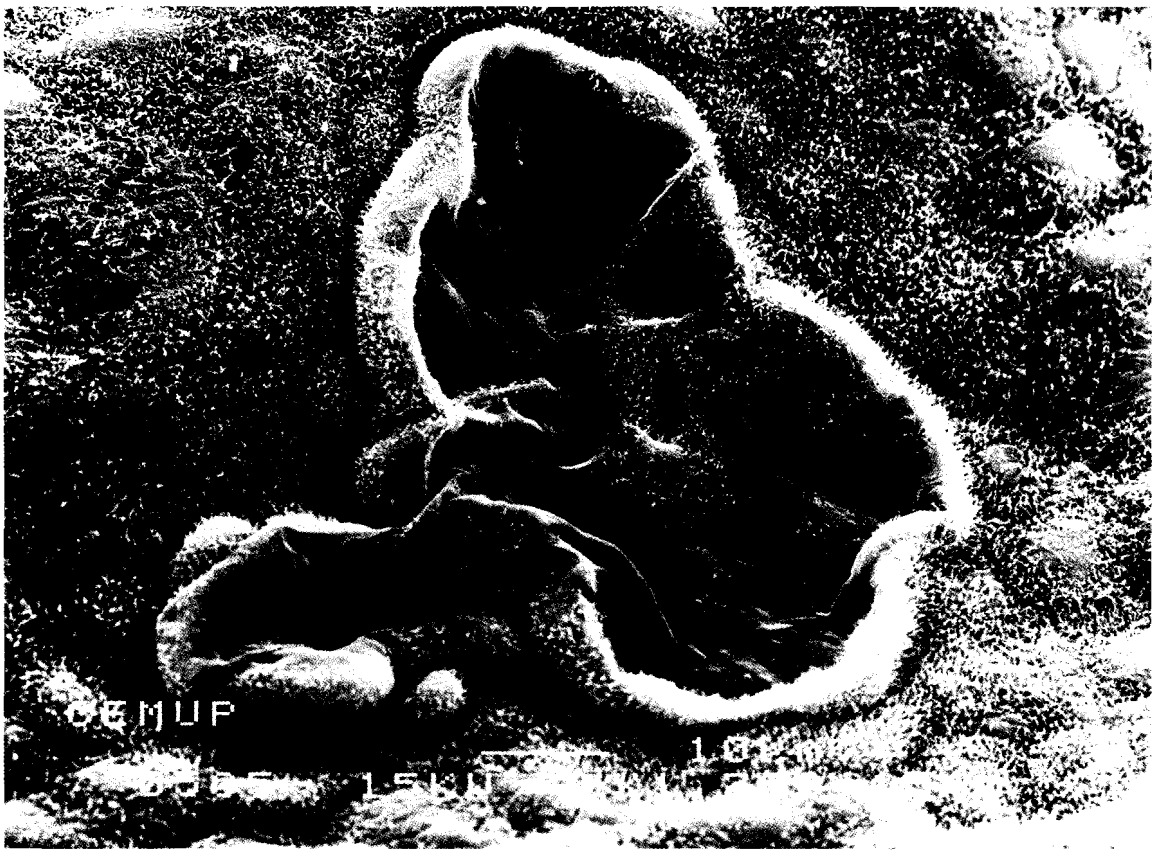

Fig. 1: SEM micrograph of the visceral mesothelial surface of Wistar rat pleura showing an irregular circle formed by focal elevation of mesothelial cells. The area inside the bulging mesothelium is poor in microvilli (1300x).

were fixed in toto in a solution of 3% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2, washed in several changes of 5% sucrose in 0.1 M phosphate buffer, dehydrated, critical point-dried, and coated with gold-palladium. Observations of the samples by SEM (JEOL JSM-3106F, Japan) were performed at an accelerating voltage of 10 kV.

Vascular Injection of Subpleural Lymphatics

Toluidine blue was injected with a Hamilton syringe into the subpleural lymphatic network of Wistar rat lungs. The injected vessels were photographed with a digital camera.

RESULTS

SEM scrutiny of the pleural mesothelium that covered the middle lobe of the lung of adult Wistar rats documented the high density of microvilli that are observed on the apical surface of the mesothelial cells that line the visceral pleura. In addition, we have found that there was focal bulging of mesothelial cells on the surface of the same pleural leaflet of the lung. These elevated mesothelial cells formed linear arrangements that were organized into irregular circles *(Fig. 1)*. Large units of bulging mesothelial cells were also seen; they showed anastomoses among the individual irregular circles *(Fig. 2)*.

A low density of microvilli was often detected inside the circle of bulging mesothelial cells. This was in contrast with their high density on mesothelial cells located

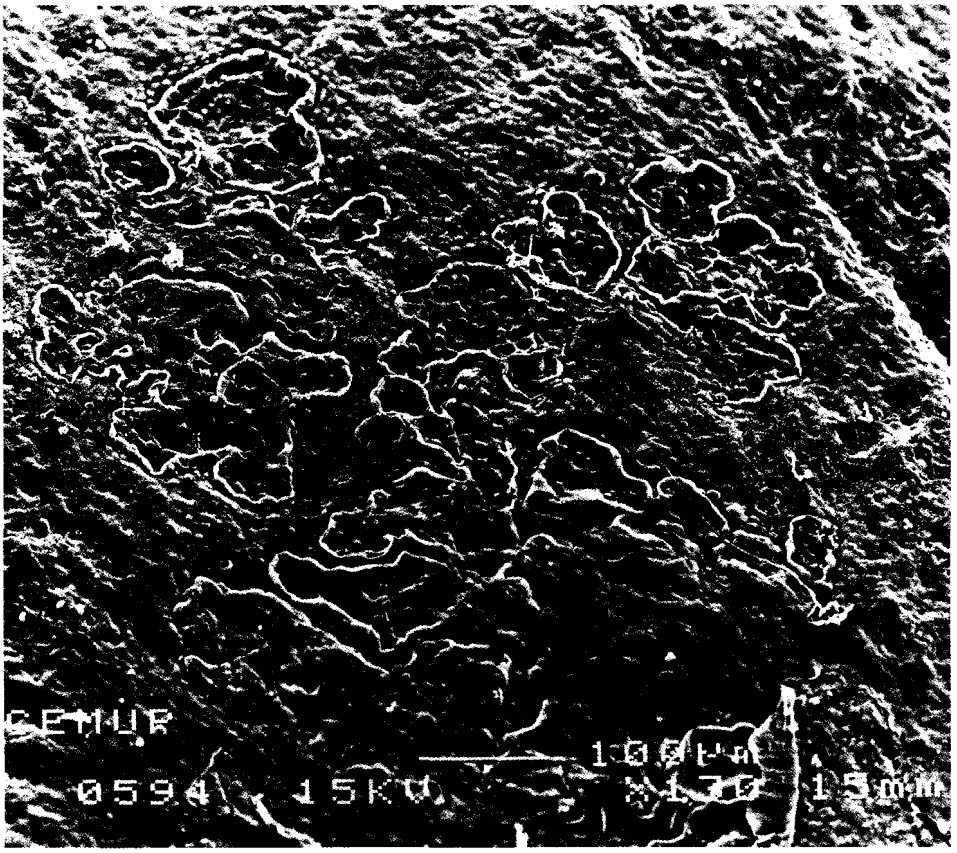

Fig. 2: SEM micrograph of the visceral mesothelial surface of Wistar rat pleura, showing anastomosis between several irregular circles of bulging mesothelial cells (170x).

on or outside of the elevated circles *(Fig. 3)*. The linear arrangements on the mesothelium closely resembled the linear organization of subpleural lymphatics of the lung that were visualized by vascular injection with toluidine blue *(Fig. 4)*. This suggests that mesothelial cells are bulged out by underneath lymphatic vessels.

DISCUSSION

The major structural difference between the visceral and parietal leaflets of the pleura is the presence of stomas in the parietal pleura and absence of any holes on the visceral pleura (7-9). Stomas are focal openings of the serosal surface that continue directly into lymphatic capillaries of the parietal pleura. The absence of stomas on the visceral pleura makes fluid absorption through this leaflet much slower than through the parietal leaflet, since the fluid has to go through the mesothelial layer and, therefore, its flow is limited by the rate of transcellular transport across the mesothelium (9-11).

We document here by SEM that the Wistar rat visceral pleura presents irregular circles of bulging mesothelial cells. We propose that they correspond to lymphatic capillaries located underneath the elevated mesothelium. This interpretation is derived from the close geometrical similarity of these arrangements with those of subpleural lymphatics that were visualized by micro-injection. In fact, our own observations and previous studies have established that

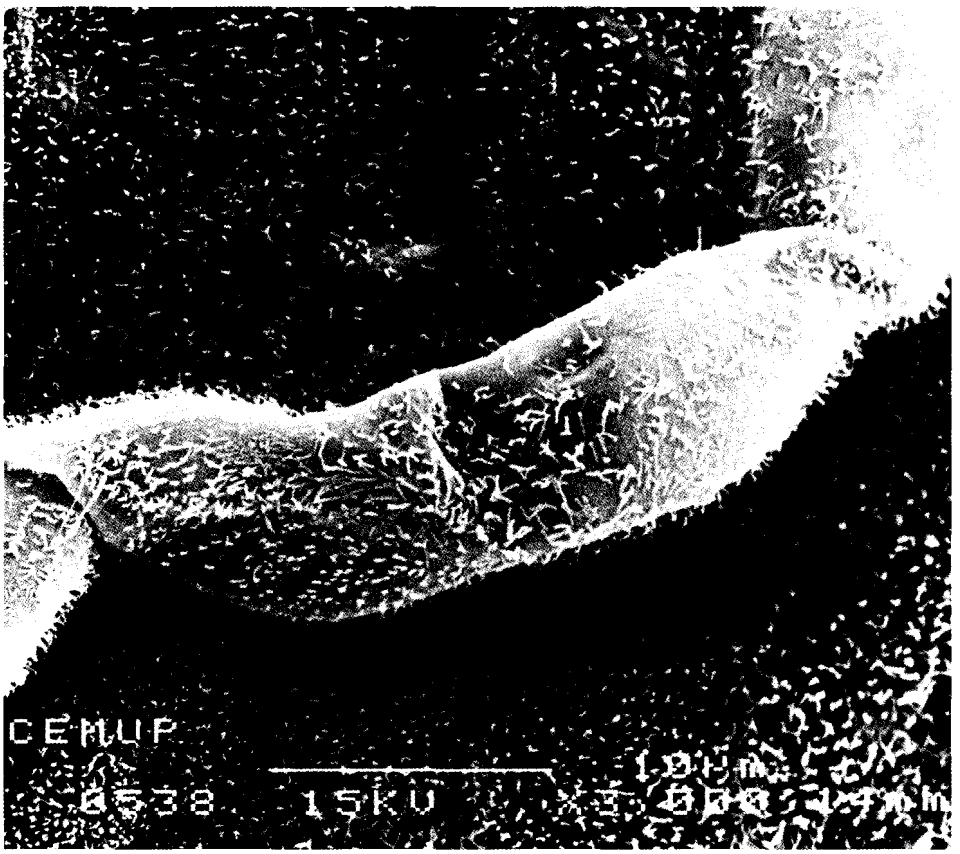

Fig. 3: SEM micrograph of the visceral mesothelial surface of Wistar rat pleura depicting different densities of microvilli inside (low density, top in the figure) and on or outside (high density, bottom in the figure) an irregular circle of bulging mesothelial cells (3000x).

Fig. 4: Wistar rat lung with the subpleural lymphatic network visualized by injection with toluidine blue.

subpleural lymphatic capillaries are organized into rich anastomotic circles, the same arrangement of bulging mesothelial cells that we have found on the pleural surface of the rat by SEM (1,2,5).

We also suggest that the distinct density of microvilli inside and outside the arrangements of elevated mesothelium, that we have herein illustrated, may reflect different ability of the two areas with regards to fluid absorption across the visceral pleura.

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