The Structural Differentiation of the Alveolar Lining Cells

II. On the Structural Components of the Alveolar Wall during the Process of Development

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I. Introduction

To date, various researchers (Low 1953, Low and Sampaio 1957, Itagi 1956, Karrer 1956 & 1958, Policard et al. 1956, Schulz 1959, Yasuda 1958, Woodside & Dalton 1958) have established that structural components of the alveolar wall are as follows: the cellular elements consist of the alveolar epithelium, alveolar septal cell (stroma cell of the alveolar wall) and the alveolar macrophage; the extra cellular components are composed of the basement membrane, collagen fibrils and the elastic fiber.

In order to investigate what kind of relationship these components have towards each other during the process of differentiation through growth stages, the author has made electron microscopic observations of the lung tissues of rats' embryos of late stages of gestation to the time immediately before delivery. This is the time when the growth of the alveolar wall proceeds most rapidly. Of the above components, the author has already reported on the structural differentiation of the alveolar epithelium (Suzuki 1966), and therefore this will be omitted in this report. However, the fact that during the process of structural differentiation of the alveolar wall just before delivery almost no detaching or desquamation of the alveolar epithelium could be observed, may be said to be an important observation in understanding the morphogenesis of the alveolar wall. During the last stages of gestation, as the alveolar wall begins to

resemble the appearance of the mature alveolar wall, the stroma decreases the space which occupies in the lung tissue of the embryo. This fact signifies the fact that the growth of the capillary in the stroma has become more active, and the cellular components within the stroma is actively participating in the formation of the alveolar wall. This period may be understood to be the most important period in deciding the localization of the structural components of the prospective alveolar wall.

Alveolar macrophage is another component of the alveolar wall. There have been many theories concerning the origin of this alveolar macrophage, as the author will describe later, but roughly speaking the theories may be divided into those of epithelial cell origin and mesenchymal cell origin. The author would like to probe whether the localization of the alveolar macrophage of resting stage within the alveolar wall is already settled from the late stages of gestation or not, and also whether migration of the alveolar macrophage from the stroma can already be seen at the same stage as was suggested by Low and Sampaio (1957).

II. Materials and Methods

Materials used were embryos of Wistar strain rats, i. e. embryos of 18 days, 19 days, 20 days and 21 days gestation. These embryos were taken out from anaesthetized mothers' uterus and after opening the chests, the lung tissues of the embryos were cut with a blade knife to less than 1 mm³. These materials were fixed for one hour in 1% phosphate buffered osmium tetroxide.

Dehydration was done by acetone or alcohol, gradually upgrading the percentage to 70%, 80%, 95% and 100%. The time of dehydration was 20 minutes altogether. Luft's epoxy resin was used as embedding material. The specimens were sliced by a Porter-Blum type ultramicrotome, stained in 1% uranum acetate for ten minutes, and in lead staining for another five minutes. These specimens were then observed by T-6 type and HU-10 type electron microscopes.

Specimens of about 1 μ thickness were always sliced by an ultramicrotome and were previously observed through a phase microscope before making electron microscopic observations.

III. Observations

Light microscopically, rat's embryos of 18 to 19 days gestation are at the stage where the alveolar epithelium and the stroma do not yet mix with each other in a complicated manner.

Fig. 1 is part of the lung tissue of a 19 days old fetus and the distance between the alveolar epithelium and the vessels is quite large. Between the endothelium of the vessel and the epithelium, mesenchymal cells containing lipid granules in its cytoplasm may be seen. At this stage, a continuous basement membrane has already been formed immediately beneath the alveolar epithelium as is clearly seen in the highly magnified photograph, Fig. 8. Beneath the capillary endothelium, basement membrane although not complete, has already been formed, as is seen in Fig. 10. Figs. 6 and 7 show that mitotic figures are frequently observed in the alveolar epithelium and mesenchymal cells, which proves that proliferation of cells is occurring. Findings that the quantity of glycogen granules are small in the mesenchymal cells, that the endoplasmic reticulum is better developed in the cytoplasm, and that there is a large quantity of lipid granules in the cells show the difference of the fine structure from the alveolar epithelial cells. Also the shape of the cells is irregular, and there are frequently projections of cytoplasm beneath the epithelium (Fig. 8) and these facts are considered to be structural characteristics of the mesenchymal cell.

Fig. 2 is the lung tissue of a 21 days old fetus. Here, in comparison to Fig. 1, the capillary lumen are more close to an attenuated alveolar epithelium. It is to be noted that the localization of the mesenchymal cells containing lipid granules is still between the alveolar epithelium and the capillary endothelium, even in an alveolar wall which has taken such a complicated structure. It is also interesting to note that lipid granules that are characteristically seen in the mesenchymal cells are almost never seen in the cytoplasm of the cells regarded as capillary endothelium.

Fig. 3 is also the lung tissue of a 21 days old fetus. The capillary endothelium (Ed) is seen immediately beneath the epithelial cells which closely resemble in shape a mature B type alveolar epithelial cell. The portion seen in Fig. 3 is considered to be the place that will become the prospective alveolar niche. Although the alveolar wall seen in Fig. 3 is still thicker than a mature one, the relationship between the alveolar epithelium and the other alveolar wall structural components i.e., the capillary endothelium

and mesenchymal cell, is identical with the relationship between the alveolar epithelial cells and alveolar septal cells, or between the epithelial cells and the endothelial cells of a mature alveolar wall.

Alveolar macrophage at resting stage which is said to be observed attached on an A type alveolar epithelial cell, could not be seen in a developing alveolar wall of late stages of gestation. The author also did not observe any mesenchymal cells breaking through the basement membrane and lining in the layers of the epithelial cells.

Another cellular element in the stroma is the endothelium of vessels. Figs. 9 and 10 show the capillary endothelium (Ed) and Fig. 11 shows the endothelium of the small artery (Ed). Fig. 9 and Fig. 10 is of a 20 days old fetus, and already attenuation of the endothelium can be seen. Pinocytotic vesicles of the endothelium can also be seen in Fig. 10, and tight junction (Tj) is seen in the junctional region of the endothelial cells. The structures of the endothelium of the small artery, of the intracytoplasmic organelles, of the junctional region, and further of the lamina elastica interna and the manner of alignment of the smooth muscle cells in the tunica media, all seen in Fig. 11, show that differentiation has occurred to the same extent as a mature small artery. However, basement membrane cannot yet be observed clearly immediately beneath the capillary endothelium in Fig. 9, and in Fig. 10, although a basement membrane-like structure can be observed in part of the outer side of the endothelium, this structure is not yet continuous.

Beneath the epithelium of a mature bronchiole smooth muscle cells can be seen, but beneath the epithelium that may be the bronchioles of the lung tissue of a 19 days old fetus, smooth muscle cells may be observed through the basement membrane (Fig. 5). In other words, in the latter half of the gestation stage, differentiation of cells closely resembling mature smooth muscle cells can be seen. Elastic fiber and collagen fibrils have been observed in mice fetus by Woodside and Dalton (1958), but these were also observed in the lung tissues of a 19 days old fetus of rat as is shown in Fig. 4.

IV. Discussion

Lung tissues of rats in late gestation show rapid progress in the structural differentiation of the alveolar wall, and change into a very complicated structure within a short time. The author has made electron microscopic studies of these lung tissues and came to

the following conclusion in regard to the origin of the alveolar epithelium, the basement membrane, and the septal cells which compose the alveolar wall.

Through embryological studies of the alveolar epithelium, it has been established by Yasuda (1958), Woodside et al. (1958) and Suzuki (1966) that the two types of epithelial cells are of entodermal origin. Among the basement membranes, the basement membrane seen beneath the epithelium is a continuous membrane which marks the boundary between the epithelium and the stroma. The arrangement order of epithelium, basement membrane and stroma (capillary endothelium and mesenchymal cells) does not change throughout the process of growth. It is thought that as growth proceeds, among the cellular elements of the stroma which faces the epithelium through the basement membrane, the capillary endothelium increases and becomes larger in quantity than the mesenchymal cells. Mesenchymal cells contain lipid granules which are observed in large quantities in the cytoplasm of the stroma, are rich in rough-surfaced endoplasmic reticulum, and have irregular projections of the cytoplasm. As the structural differentiation of the alveolar wall progresses and the alveolar wall begins to show a complicated pattern, these mesenchymal cells, by facing the epithelium through the basement membrane, or by facing the capillary endothelium through the basement membrane, begin to show the localization and characteristics of septal cells. In other words, alveolar septal cells are thought to be cells of mesenchymal origin.

The positional relationship between the alveolar septal cells and the basement membrane system, the fact that the alveolar septal cells hold the position of supporting the capillary endothelium in the alveolar wall, and further the fact that these cells have complicated cellular projections, all show that the characteristics of these cells closely resemble those of the mesangial cells in the renal glomerulus as reported by Suzuki (1963). Whether the cellular function and the response to stimuli of these septal cells show the same characteristics as the mesangial cells which also are of the mesenchymal cell origin is an interesting speculation, but must await future research.

The structure of the capillary endothelial cytoplasmic organelles, the structure of the junctional region of the endothelium, and the shape of the attenuated endothelium, observed in the lung tissue stroma in late stages of gestation, resemble those of the mature alveolar capillary endothelium. Such findings of growth of the al-

veolar capillary in late stages of gestation have already been observed in mice by Woodside-Dalton (1958). The growth of the basement membrane beneath the capillary endothelium seems to be a little retarded compared to the basement membrane beneath the alveolar epithelium.

The fact that almost no lipid granules were observed within the cytoplasm of the endothelium forming the capillary lumen is an important finding to distinguish them from the prospective septal cells. However, it is not clear whether the lipid granules in the mesenchymal cells disappeared during the process of transformation into endothelium, or whether there were no lipid granules from the start in the mesenchymal cells which are to differentiate into endothelium.

Collagen fibril and elastic fiber are extra cellular structural components. Both could be seen in the materials used at this time. The materials were of late stages of gestation. This finding agrees with Woodside-Dalton (1958)'s findings in mice.

There are two conflicting theories on the embryological origin of the alveolar macrophage. Seemann (1931), Bargmann (1935, 1936), Clara (1936), Akazaki (1943), Macklin (1951), Hayek (1935) and Ito (1965) take the epithelial cell origin theory, while Lang (1926, 1929), Mathis (1931), Brodersen (1933) Shimada (1963) Yamori (1963) etc. take the mesenchymal cell origin.

Low and Sampaio (1957) who succeeded to observe the alveolar macrophage for the first time under the electron microscope, reported that these cells are free in the alveoli or rest on an uninterrupted layer of epithelium. As for their origin, they supposed these cells had migrated from the mesodermal area of the alveolar wall. The author could not find cells that could be regarded as alveolar macrophage in the alveolar wall of late stages of gestation in his present study, and therefore could not reach a definite conclusion. However the author can place his opinion on this point as follows:

If the alveolar macrophage are cells of mesenchymal origin, the theory put forth by Policard, Collet and Pregernain (1957) that the alveolar macrophage transformed from alveolar cells localized on the basement membrane goes not mean the mesenchymal origin theory. Therefore, the author's embryologic observation concluded that what they call alveolar cells are nothing but epithelial cells of entodermal origin. And if we assume with Low and Sampaio (1957) that migration occurs from the stromal region,

this phenomenon must occur after delivery, and at that time the migrating mesenchymal cells must burst through the basement membrane, wait for an opening in the plasma membrane consisting of adjacent epithelial cells joined by "tight junction" and finally must reach the epithelial cell layer. In order to fix the origin of the alveolar macrophage, we must await observations of post-delivery growth of the alveolar wall.

V. Conclusion

The author made observations of lung tissues of rat's embryos of 18 days, 19 days, 20 days and 21 days gestation, considered what structural differentiation occurs in the structural components of the alveolar wall, what locational relationship these components show towards each other in the process of growth, and reached the following conclusions.

1) From the immature lung tissues where the epithelium and the stroma are clearly separated, to the lung tissues of late gestation where the structure of the alveolar wall becomes as complicated as that of a mature alveolar wall, the order of the arrangement of elements composing the future alveolar wall is consistent. In other words, the order of the elements is decided in the late stages of gestation.

2) Alveolar septal cells are of mesenchymal origin and in the late stages of gestation, their structural characteristics can already be seen. Large quantities of lipid granules are seen, and the cellular projections are complicated. The author reported on the similarity of the structure of this cell to the mesengial cells of the renal glomerulus.

3) The author could not reach a conviction on the origin of the alveolar macrophage. The study of the origin of this cell must await observations on post-delivery materials.

4) As already reported, collagen fibrils and elastic fibers could be observed in the stroma or late stages of gestation. Fine structure of the capillary endothelium also resembled a mature capillary endothelium.

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Explanation of Photographs

E. p.	prospective epithelial cell	C. L.	capillary lumen
M. C.	mesenchymal cell	Cf.	collagen fibril
Ed.	endothelial cell of alveolar capillary	El.	elastic fiber
Lg.	lipid granules	Sm.	smooth muscle cell
A. L.	alveolar lumen	B. M.	basement membrane

All the scales indicate 1 μ .

- Fig. 1. Low power view of the lung tissue of a 19 days old rat embryo. No close interrelationship between the epithelial cells and vessels can be observed yet. In the cytoplasm of the mesenchymal cells between them, a large amount of lipid granules is seen.
- Fig. 2. Alveolar wall of 21 days gestation. Compared to Fig. 1, the relationship between the alveolar epithelium, the capillary endothelium and the mesenchymal cells have become complicated.
- Fig. 3. A portion of the alveolar wall of the same period as Fig. 2. The relationship between the epithelium, mesenchymal cells (alveolar septal cells), and the capillary endothelium is equivalent to those seen in a mature alveolar wall.
- Fig. 4. Collagen fibrils and elastic fibers may be seen in the stroma beneath the alveolar epithelium of 19 days gestation.
- Fig. 5. Through the basement membrane of the prospective bronchiole of 19 days gestation, smooth muscle cells may be observed.
- Figs. 6 and 7. show mitotic figures which are seen in the latter half of gestation (19 and 20 days). Fig. 6 shows epithelial cells, and Fig. 7 shows mesenchymal cells.
- Fig. 8. The lung tissue of 19 days gestation. The relationship between the epithelial cells, basement membrane and mesenchymal cells are shown. A large quantity of lipid granules are seen in the mesenchymal cells, and the endoplasmic reticulum has developed. Between the basement membrane and the main part of the cells there are several cytoplasmic projections that may be projections of these cells. This closely resembles findings on alveolar septal cells in a mature alveolar wall.
- Figs. 9 and 10. Show part of the capillary seen in the alveolar wall of 20 days gestation. In Fig. 9, the basement membrane is still obscure. In Fig. 10, a discontinuous basement membrane can be seen beneath the endothelium.
- Fig. 11. Endothelium of the small artery and part of the smooth muscle cells in the media is observed in the lung tissues of 20 days gestation.