A Scanning Electron Microscopic Study of Macrophages in the Lens Cavity of the Mouse Embryo.

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ABSTRACT. During early lens formation in mouse embryonic eyes, macrophages appear not only around the lens primordium but also inside the lens. At the lens vesicle formation stage, macrophages were randomly scattered within the cavity but, shortly after that stage, all of them specifically attached only to the inner surface of the anterior wall of the lens vesicle. At the primary lens fiber elongation stage, numerous small epithelial cell fragments were expelled from the lens epithelium into the cavity, and macrophages showed active phagocytosis and removed them from the cavity. In the early lens formation stage, macrophages move into the lens cavity, where they play an essential role in removing cell debris derived from the lens epithelium.

Key words ① **macrophage** ② **lens cavity** ③ **SEM** ④ **lens formation** ⑤ **lens epithelium**

Macrophages are known to play an important role in eye development in the mouse embryo[1)~6)]. By an immunohistochemical study using F4/80 monoclonal antibody, we previously showed that numerous macrophages appear and do not distribute uniformly in the lens cavity during early development of the mouse lens[7)]. To determine the precise localization of macrophages in the lens cavity, an SEM study is more informative than either immunohistochemical or TEM studies. The aim of our present SEM study was to confirm the localization, surface morphology and phagocytosis of macrophages in the lens cavity at the three-dimensional level.

MATERIALS AND METHODS

Animals

A total of 57 ICR mouse embryos were studied. An adult male and female were caged together overnight, and the next morning was taken as day 0 of pregnancy. Pregnant mice were killed by cervical dislocation at 10.5, 11, 11.5, 12, 12.5 and 13 days of gestation. At each gestational day, at least seven embryos were used for scanning electron microscopic observation.

Scanning Electron Microscopy

Fetal heads were midsaggitally cut with a razor blade, and then were immersed in phosphate buffered

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subgroups was difficult due to the presence of an intermediate type, the majority of the macrophages within either spherical or convexo-concaval cavities were S1- and S2-type, and S3-type macrophages increased in number within narrowing convexo-concave cavities. Both S1- and S2-type macrophages appeared to express active phagocytosis to epithelial cell fragments.

DISCUSSION

Our present SEM study on early lens formation showed that, inside the lens vesicle, numerous macrophages and small cell fragments from the lens epithelium were observed on the inner surface of the lens epithelium. Spherical cell fragments appeared early in the lens placode invagiation stage, and macrophages showed active phagocytosis to remove them from the cavity at the primary lens fiber elongation stage.

Macrophages as professional phagocytes have a scavenging function of removing apoptotic bodies formed during the degenerative process in apoptosis[8]. In the developing eyes in mammals, apoptotic cell death has been recognized in the neuroepithelium of the optic cup and lens epithelium[9~12], and, during lens morphogenesis, apoptosis occurs not only in the epithelium at the border of the lens placode but also in the lens stalk epithelium. The apoptotic processes in the developing lens are known to involve a caspase-3 dependent pathway[12], and apoptotic cell fragments have been observed in the lens pit and lens cavity. Present SEM observation revealed small spherical bodies of the cell fragments not only on the surface of the lens pits but on the inner surface of the anterior wall of the lens vesicle, and quite numerous free macrophages, which had a scavenging function to eliminate them, were seen inside the lens vesicle. By removing cell fragments from the lens cavity, macrophages play an essential role in early lens formation.

Regarding the surface morphology of the macrophages, the majority were of S-type, and these were subdivided into S1, S2 and S3 subgroups. The macrophages in the cavity predominantly consisted of both S1-and S2-types at the primary lens fiber elongation stage. Thereafter the proportion of S3-type macrophages showed an increase. As is well known, macrophages showing active phagocytosis have numerous lamelopodia and filopodia, forming small pits on the cell surface for phagocytosis[13~15]. Our present observations showed that, in lens formation, S1- and S2-type macrophages having the characteristic morphology of active phagocytosis changed to S3-type cells as the cavity became narrower. Due to the surface morphology, F-type macrophages might be moving to the inner surface of the anterior wall of the vesicle to become S-type cells. Macrophages changed their surface morphology inside the lens cavity with development of the lens epithelium and primary lens fiber probably due to their phagocytotic activities. Between the lens vesicle formation stage and the lens cavity closure stage, 11-13 days of gestation, most of circulating cells in the peripheral circulation of mouse embryo are primitive erythroblasts from yolk sac[16], and various embryonic tissues including fetal liver contain numerous scavenger macrophages to remove degenerating primitive erythroblasts and their debris[17,18]. Since these scavengers are categorized as fetal macrophages, macrophages in the lens cavity also appear to the same fetal macrophage group.

Since the lens vesicle was formed by invagination of surface ectoderm, the cavity originally was connected with the amniotic cavity. In human fetuses, amniotic fluid physiologically contains macrophages[19,20], so that it may be possible for macrophages inside the lens cavity to move directly from the amniotic cavity at the lens placode invagination stage. However, as shown in our previous report[7], macrophages were distributed not only in the mesenchyme around the lens primordium but also within the

lens epithelial layer, and their mitotic figures could be identified in the cavity. Therefore, it appears to be reasonable that macrophages migrate into the lens cavity from outside mesoderm around the lens primordium to proliferate inside the vesicle. In apoptosis, dying cells themselves secrete chemotactic factors, i.e."eat-me" signals, on the cell surface[21], and neighboring cells that engulf apoptotic cells have also revealed several chemokines for attraction of macrophages for apoptotic cell clearance[22,23]. In addition to the variety of macrophage surface morphology, their migration and emigration in the lens, and, their localization and proliferation within the cavity, all might be considered to be under the influence of chemokines, although cells responsible for chemokine production in developing eyes should be the subject of further investigation.

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