Scanning Electron Microscopic Study of Hyalocytes in the Guinea Pig Eye

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Summary. The ultrastructure and distribution of hyalocytes were examined in guinea pig eyes by scanning electron microscopy (SEM) and transmission electron microscopy (TEM).

Hyalocytes were distributed randomly on the vitreous surface of the retinal inner limiting membrane, where they were elongated in shape with a spherical perikaryon and a few stout processes. On the epithelial surface of the ciliary body, however, the cells were stellate with some short processes. The cells of both regions included typical dense hyalocyte granules in the cytoplasm.

The surface morphology of hyalocytes indicates that the cells are wandering macrophages. The abundance of free cells in the ciliary body epithelium suggests that the area is a site for the emigration of hyalocytes or their precursors from the ciliary stroma.

The homogeneous population of hyalocytes in the posterior part of the eyeball may be useful for experimental studies of the cell *in vivo* or their isolation for study *in vitro*.

A homogeneous population of cells known as "hyalocytes" is found in the cortical layer of the vitreous body of various animal species.

The morphological characteristics of the hyalocytes have been described using light microscopy (SZIRMAI and BALAZS, 1958; HAMBURG, 1959; WOLTER, 1960; BALAZS et al., 1964), and electron microscopy (SEAMAN and STORM, 1965; BLOOM and BALAZS, 1965; TENG, 1969; HOGAN et al., 1971; SAGA et al., 1984;

SALU et al., 1985). Their phagocytic activity has been confirmed experimentally by the injection of foreign substances into the vitreous (HAMBURG, 1959; O'STEEN and KARCIOGLU, 1974; FRANÇOIS et al. 1976; BURKE and FOSTER, 1982; UEHARA et al., 1996) and in cultured hyalocytes (BALAZS et al., 1964; GRABNER et al., 1980). Immunocytochemical studies showed hyalocytes belong to the monocyte/macrophage lineage (LAZARUS and HAGEMAN, 1994; ZHU et al., 1999). Although the functions of hyalocytes have not been completely clarified, the consensus is that they are macrophages.

In the present study, we examined the three-dimensional structure and the distribution of hyalocytes in the guinea-pig eye by SEM to provide further evidence concerning the nature of this cell type from the viewpoint of their surface morphology.

MATERIALS AND METHODS

Adult guinea pigs of both sexes, weighing about 200 g, were used in this study. Under deep anaesthesia by intraperitoneal injection of Nembutal, the animals were perfused with physiological saline, and then with 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) from the aorta. Enucleated eyeballs were cut into halves along an equatorial plane. Specimens were kept in the same fixative for 2-3 days.

For SEM studies, fixed specimens were immersed in 1% tannic acid solution for 1 h after removal of the gelatinous portion of the vitreous body. After rinsing several times in a buffer, they were postfixed with buffered 1% OsO₄ for 1 h, and then dehydrated in an ascending series of acetone and transferred to isoamyl acetate. The specimens were dried by the critical point method using CO₂, sputter-coated with gold, and observed with Hitachi S-450 SEM.

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Fig. 1. Low magnification micrograph of the posterior part of the eye. Elongated free cells are distributed on the inner limiting membrane of the retina. Wheel-mark like furrows radiate from the optic disc (OD). $\times 200$

For the correlative TEM studies, small pieces of the glutaraldehyde-fixed retina and ciliary body were excised and postfixed in buffered 1.3% OsO_4 for 2 h. The specimens were then dehydrated by ethanol and embedded in Epoxy resins. Thin sections were cut with a diamond knife, stained with uranyl acetate and lead citrate, and examined with Hitachi H-500 TEM.

RESULTS

Inner limiting membrane surface

In a survey view of the posterior part of the eyeball (Fig. 1), free cells were found scattered on the inner limiting membrane of the retina (about 100 cells/ mm^2). Numerous wheel-mark like furrows were noted on the inner limiting membrane surface radiating from the optic disc. These undulations may reflect the arrangement of nerve fibers in the retina converging to the optic nerve.

Most of the free cells were elongated (about $50\ \mu\text{m}$) with a centrally located spherical perikaryon of $5\ \mu\text{m}$ in diameter, and two major stout cytoplasmic processes extending in opposite directions (Fig. 2). In some cells, a perikaryon was located at one end of the cell. In both types of cells, branchings were observed in the distal portions of the processes. The cell surface was relatively smooth, except for a few short cytoplasmic projections.

These cells were entangled with a fine fibrillar network of the vitreous cortex. Each fibril was uniform in size (about $0.1\ \mu\text{m}$ in diameter) in this area.

Epithelial surface of the ciliary body

In the ciliary body, irregularly shaped free cells were observed on the vitreous surface of the epithelium (Fig. 3).

Most free cells were ovoidal or stellate in contour, having a spherical perikaryon (about $5\ \mu\text{m}$ in diameter) and some short processes. Small globular struc-

Fig. 2. Typical hyalocytes on the inner limiting membrane surface. Bi-directional stout processes arise from centrally located perikaryon. The cell surface is relatively smooth except for some tiny microvilli. The cells are entangled with a fine fibrillar network. $\times 3,600$

Fig. 3. Epithelial surface of the pars plana of the ciliary body. Free cells are ovoidal with some short cylindrical or ruffled processes. The epithelial surface is characterized by meridionally oriented spindle-shaped ridges. $\times 2,600$

Fig. 4. TEM micrograph of the epithelium of the ciliary body. A migrating cell (*arrow*) is seen between epithelial cells. A free cell is also seen in the vitreous cavity (*double arrows*). $\times 4,100$

Fig. 5. TEM micrographs of a hyalocyte on the epithelial surface of the ciliary process. The cell is irregular in shape and equipped with numerous cytoplasmic processes. G hyalocyte granule. $\times 9,400$

tures were often seen attached to the ruffled-bordered processes. The cells were smaller (about 20 μm in length) than the cells of the retinal inner limiting membrane. The density of free cells was, however, higher in the ciliary body (about 500 cells/ mm^2). In the grooves between ciliary processes, free cells were observed in the dense fibrillar network adjacent to the epithelial surface. In TEM micrographs, free cells were often encountered between epithelial cells, suggesting cell migration between the vitreous space and the ciliary body stroma (Fig. 4). Cells on the ciliary epithelial surface had an irregularly indented outline because of the presence of numerous cytoplasmic projections. The cells contained specific large dense inclusions known as hyalocyte granules (Fig. 5).

DISCUSSION

Judging from their cellular morphology and localization, the free cells described in the present study correspond to the hyalocytes of the vitreous body which have been described for other species. Although our observations were restricted to the cortical layer of the vitreous body adjacent to the retina and ciliary body, these free cells were considered to be typical hyalocytes because most are known to be located in the cortical layer in this species (HAMBURG, 1959). In general (WOLTER, 1960; BALAZS et al., 1964), the hyalocytes on the inner limiting membrane were elongated cells with a few stout processes. The cells were extremely large as compared with the cells on the ciliary body epithelium.

In cow (SZIRMAI and BALAZS, 1958), ox (HAMBURG, 1959) and human (LEE and GRIERSON, 1977) eyes, the cells of the vitreous surface were described to be accumulated linearly along the underlying blood vessels of the retina. The free cells observed in this study were scattered throughout the inner surface of the receptive part of the retina. This difference may be due to the fact that guinea pigs lack blood vessels in the retina (COOPER and SCHILLER, 1975). In the guinea pig eye, hyalocytes may enter the vitreous cavity across the ciliary body epithelium, and move posteriorly to the retinal surface. On the other hand, HADDAD and ANDRÉ (1998) suggested a cell division for hyalocytes in the posterior chamber of the rabbit eye. The possibility of cell proliferation in this region cannot be ruled out.

As an example of macrophages on the free tissue surface, we can mention intraventricular macrophages (known as Kolmer cells). The surface morphology of this cell type has been described by many

authors (HOSOYA and FUJITA, 1973; COATES, 1975; MESTRES and BREIPOHL, 1976; KRSTIC, 1981). The free cells observed in our study have morphological features similar to the type II suprapendymal cells, which are cells with some pseudopod-like processes that are suggested to be macrophages.

Although several SEM studies have described the three dimensional structure of the vitreous fibers and cells (HANSSON, 1969; LEE and GRIERSON, 1977; OHSAWA and MIKI, 1982), the distribution of the cells was difficult to show clearly in the animal species used. In guinea pig vitreous body, the vitreous fiber network is so sparse in the coat layer that the hyalocytes are well visualized with SEM. Recently, UEHARA et al. (1996) also observed the hyalocytes in the chicken eye by SEM. However, the morphology and the distribution of hyalocytes were different from those of mammals; the cells were quite numerous on the pecten oculi, suggesting that the cells are functioning in the active portion of the avian eye.

In conclusion, the guinea pig vitreous body is particularly suitable for observation of the morphology and distribution of hyalocytes. The uniform population of cells may also be useful for the experimental studies of hyalocytes, either *in vivo* or *in vitro*.

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