Trabecular cells are intimately related to the process of aqueous humor outflow. The cells have various functions, including synthesis and degradation of collagen, proteoglycans, and other extracellular matrix components, and the phagocytosis of extracellular debris. Failure of the cells to perform these functions, or a lack of enough cells to perform them adequately, may be factors in the pathogenesis of glaucoma. Animal studies of trabecular phagocytosis found that trabecular cells may leave their position on the trabecular lamellae and migrate from the eye after ingesting foreign material. If these cells are not replaced, such phagocytosis-derived cell loss could have detrimental long-term effects, especially in view of the decrease of trabecular cellularity with age and with primary open-angle glaucoma.

Although there are many animal studies, limited work has been done on phagocytosis and its potential consequences in intact human meshwork. Most reports are limited to descriptive studies of eyes enucleated after severe trauma or to studies done on trabeculectomy specimens. It is not known if phagocytosis by human trabecular cells causes cell loss as suggested in animal studies, or if ingestion of foreign particles has detrimental effects on trabecular cells.

We used an organ culture system of the trabecular meshwork to study phagocytosis by intact human trabecular meshwork. Two areas were examined: (1) the phagocytic ability of trabecular cells and their response to foreign particles and (2) the potential for human cells to migrate from the lamellae as suggested in other animal studies. The effect of the organ culture technique itself on meshwork phagocytosis was examined in a preliminary study using an in vivo-in vitro correlation in cats.

Materials and Methods

Human Eyes

Fifty-two human eyebank eyes were placed in culture within 18 hrs postmortem. Eyes with glaucoma, uveitis, or other active conditions were not studied. Average age of donor eyes was 73 ± 7 yr (range, 60-92 yr). Eyes were bisected at the equator, and the iris, lens, and vitreous were removed. The anterior segment was clamped in a modified petri dish, and the eye was perfused with culture medium. The culture medium was Dulbecco’s modified Eagle’s medium with high glucose (4500 mg/l) and the addition of antibiotics (penicillin, streptomycin, and amphotericin B—1 ml/100 ml, A9909; gentamicin—1.7 mg/100 ml, G 0889, Sigma, St. Louis, MO). Eyes were perfused at the normal human aqueous flow rate, 2.5 µl/min, and incubated at 37°C with 5% CO₂.

At the appropriate time during culture eyes received either 0.6 ml of blood, 0.6 ml of 0.5 µm latex...
microspheres (2.04 × 10^6 particles/mm^3), or 0.6 ml of zymosan particles (approximately 2.7 × 10^6 particles/mm^3; particle diameter approximately 3.0 μm diameter). A second cannula connected to an open reservoir maintained intraocular pressure at 17 mm Hg during the infusion. The blood used was freshly drawn whole human heparinized human blood, type O negative. No attempt was made to cross-match tissue and blood types. Zymosan was prepared according to the method of Sherwood et al.14 Eyes were then cultured for periods ranging from 1 hr to 7 days after which they were perfused with fixative at 17 mm Hg.

Two series of experiments were conducted. In the first series, eyes were perfused with the phagocytosis challenge 24 hr after being placed in culture and then cultured for 1 hr, 1 day, 3 days, or 7 days. In the second series, to study the effect of culture on the phagocytic process, eyes were cultured for 1 week before being perfused with the phagocytic challenge, after which they were cultured for an additional 1 hour, 1 day, 3 days, or 7 days. At least two eyes were used for each time in each series.

After the designated time in culture, the eyes were fixed by perfusion at 17 mm Hg, divided into four quadrants, and again fixed in 2% paraformaldehyde/1% glutaraldehyde in 0.067 M cacodylate buffer, pH 7.4. Tissue sections were dehydrated in a graded series of ethanol and embedded in Araldite 502 (Polysciences, Warrington, PA). Tissue sections containing latex beads were dehydrated in a graded series of ethanol processed with acetone rather than propylene oxide, to prevent dissolution of beads. Thick sections of 1 μm were prepared and stained with toluidine blue for light microscopy (LM). Thin sections from representative blocks were prepared for transmission electron microscopy (TEM), stained with lead citrate and uranyl acetate, and examined in a Phillips CM-12 electron microscope (Eindhoven, Holland).

Quantitation of the number of the trabecular cells involved in phagocytosis was counting the number of cells with ingested particles and performed by the total number of trabecular cells in each section. Obvious inflammatory cells and macrophages were excluded. Blood and zymosan particles were visible with LM, and the latex microspheres were small enough that TEM was needed to confirm ingestion by trabecular cells.

Cat Eyes

The effect of organ culture on meshwork phagocytic activity was studied in two adult cats. The anterior chamber of one eye was perfused with 0.6 ml of zymosan at 17 mm Hg, using a vent system consisting of a second needle in the anterior chamber connected to an open reservoir of Dulbecco's saline 22.4 cm (17 mm Hg) above the eye. After 3 days, the animal was sacrificed with an overdose of sodium pentobarbital, the zymosan-perfused eye fixed at 17 mm Hg, and the undisturbed fellow eye placed in organ culture. The cultured eye was perfused immediately with zymosan, cultured 3 days, fixed at 17 mm Hg, and processed for histology. All experiments conformed to the ARVO Resolution on the Use of Animals in Research.

Results

Cat Eyes

In vivo, phagocytic activity was abundant, with numerous zymosan-laden rounded cells. Most trabecular cells appeared in their usual horizontally elongated position on the lamellae, but a smaller number of trabecular cells were rounded-up or out of position from the lamellae. An inflammatory response also ensued, with numerous polymorphonuclear cells and macrophages present throughout the meshwork (Fig. 1).

In vitro, the cultured meshworks did not show the inflammatory cell response to the zymosan. Less phagocytic activity was present, although rounded, zymosan-laden cells were again found in all regions of the meshwork. Despite the abundant presence of free zymosan particles, most trabecular cells did not contain ingested zymosan but rather remained horizontally elongated and in position on the trabecular lamellae (Fig. 1). Overall, more trabecular cells were involved in phagocytosis in the in vivo situation compared with the cultured eye; approximately 50% of trabecular cells on the lamellae contained zymosan in vivo as opposed to 10% of cells in vitro.

Human Eyes

Phagocytosis occurred with all three types of particles (Figs. 2–4). Latex microspheres were ingested more frequently than other particles, with most eyes having an average of 15% of trabecular cells containing ingested microspheres (range, 0%-65%). Zymosan was ingested less frequently, with only about 2% of cells containing ingested zymosan (range, 0%-4%; nine of 20 eyes demonstrated phagocytosis of zymosan). Erythrocytes were also ingested infrequently; nine of 16 eyes showed phagocytosis of erythrocytes; with approximately only 2% of cells containing erythrocytes. Trabecular cell uptake of latex microspheres and erythrocytes occurred in all three regions of the meshwork: uveal, corneoscleral, and juxtacanalicular. Microspheres were also found in the lining cells of Schlemm's canal (Fig. 5). In contrast, zymosan uptake appeared predominantly in the uveal and corneoscleral regions. More ingested particles per trabecular cell were noted in the latex microsphere eyes.
than the zymosan eyes (> eight microspheres/cell versus two zymosan particles/cell). None of the ingested particles appeared toxic to trabecular cells; the mitochondria, rough endoplasmic reticulum, and other organelles remained normal. Free uningested particles of all three types were found in Schlemm's canal. Latex microspheres were also found in giant vacuoles (Fig. 5B). Duration of time in culture before receiving the phagocytic challenge did not affect the phagocytic activity of the trabecular cells; eyes cultured for 24 hr and eyes cultured for 7 days before receiving the phagocytic challenges reacted similarly. Likewise, donor age and length of postmortem interval before culture did not affect phagocytic activity.
Latex Microspheres

Most trabecular cells remained in position on the trabecular lamellae after ingesting microspheres. Some rounded cells containing beads were noted approaching Schlemm's canal (Fig. 5B). These were believed to be trabecular cells although they could also have been microsphere-laden endogenous tissue macrophages. Ingested microspheres did not appear to cause much reaction in the trabecular cells but remained either as discrete particles or in clumps in the cytoplasm. No whorled membrane structures or other phagolysosome complexes were seen (Fig. 3).

By TEM, the initial stages of phagocytosis of latex microspheres were observed. Latex microspheres are shown within the cytoplasm of trabecular cells (Fig. 3). These microspheres can be seen within intertrabecular spaces (open arrows). Early stages of contact between the microspheres and trabecular cells are also evident (long arrows). (×7,500).
Fig. 4. Zymosan within trabecular cells, uveal meshwork area (arrows). Zymosan particles characterized by coffee bean appearance: inner elongate core surrounded by outer, less dense cortex. Early contact between trabecular cell and zymosan particle visible in lower right (open arrow). Asterisks: trabecular beams (X7,500).

microspheres involved the formation of short, blunt cytoplasmic processes which eventually enclosed the entire particle. The cytoplasm in these areas contained abundant microfilaments. A modification of the cytoplasmic membrane immediately adjacent to the latex microsphere occurred during the initial stages, involving the formation of a “coated pit,” a thickened densification of the membrane at the point of contact between the microsphere and the cell (Figs. 3, 6).

Zymosan

In contrast to latex microspheres, zymosan did not appear to be associated with changes in the trabecular cell cytoplasmic membrane. Even in close apposition, the cytoplasmic membrane did not develop coated pits or contain an increased number of pinocytic vesicles. Microtubules were not as prominent a feature in the cell cytoplasm adjacent to zymosan particles as in the latex bead eyes.

Most trabecular cells containing ingested zymosan remained on the trabecular lamellae and did not round up or appear to migrate. Although some cells in the uveal region appeared rounded, this was probably a culture-induced effect; it has been seen in other cultured eyes. Trabecular cells from eyes exposed to zymosan for 3 days and longer contained zymosan which was in the process of being degraded. The zymosan core appeared indistinct and enlarged, and the zymosan cortex was often reduced and thinned in size. In addition, these eyes contained zymosan breakdown products in the intertrabecular spaces. This material could have been present in the initial zymosan infusion, however, it appeared in noticeable quantities only in the later culture times. It is not known if these fragments formed spontaneously from intact zymosan in the intertrabecular spaces or were exocytosed fragments of digested particles. Trabecular cells in the area of the breakdown products remained elongated in shape and in position on the lamellae. The cells did not appear to ingest these fragments in preference to whole particles.

Blood

Erythrocytes were found in the intertrabecular spaces, Schlemm’s canal, and in giant vacuoles. Phagocytosis was rare; often the intertrabecular spaces could be full of erythrocytes and yet trabecular cells would remain undisturbed, in position on the lamellae. Those trabecular cells showing phagocytosis also remained on the lamellae. Erythrocyte–trabecular cell interaction appeared to involve physical contact of cells followed by indentation of the trabecular cell profile. Most ingested erythrocyte material appeared as fragments of cells; few intact erythrocytes were found in the trabecular cells. Ingested fragments appeared in several stages of degradation, from relatively intact red cells, to red cells with denatured hemoglobin, to empty red cells with loss of intracellular hemoglobin (Fig. 2). Small round electron-dense spherules were found in the intertrabecular spaces (Fig. 7). These minute (0.1-μm) particles were also found in the juxtacanalicular tissue and occasionally in trabecular lamellae in portions lacking a covering of trabecular cells. The particles resembled the extracellular fragments of erythrocytes described by Gadially, although they were also similar to type I matrix vesicles.

Discussion

Human trabecular cells are phagocytic and capable of ingesting various particles. The presence of a foreign particle does not necessarily induce a phagocytic response, however, free particles were seen in the intertrabecular spaces and in Schlemm’s canal even in
eyes with less than 10% of trabecular cells engaged in phagocytosis. Undoubtedly the culture process affects the human cell's capacity for phagocytosis just as it affected the feline trabecular cell phagocytic response. The proportions of trabecular cells involved in phagocytosis is probably higher in humans in vivo, as it was higher in the cat (50% in vivo versus 10% in vitro). The recruitment of additional trabecular cells in the phagocytic process probably occurs in vivo as an accompaniment to inflammation. The lack of inflammation in the culture situation, however, allows a unique opportunity for observation of trabecular cell phagocytosis and separation of the effects of inflammation from the potential effects of phagocytosis.
itself. Such phagocytosis without inflammation may occur, for example, in pigmentary glaucoma or in exfoliation syndrome.

The trabecular cell response to foreign particles may vary with the type or size of the particle. A difference was noted in the cellular ingestion mechanisms; latex microspheres were associated with extensions of cellular processes, prominent cytoplasmic microfilaments, and cytoplasmic membrane formation of coated pits. Coated pits have been found to be associated with clathrin, a structural protein thought to form a scaffold for the invagination of the pit and
also facilitate recycling of ingested membrane.\textsuperscript{16,17} By contrast, zymosan and blood did not appear to be associated with these events.

Limited trabecular cell loss, if any, accompanied the phagocytic process in these single-exposure experiments. A few microsphere-laden rounded cells were found in the intertrabecular spaces and near Schlemm's canal. The lack of inflammatory response and an accompanying macrophage infiltration makes it likely that these cells were trabecular cells. Most trabecular cells remained in position, however, indicating that the process of phagocytosis per se does not cause trabecular cell loss. In addition, the finding of zymosan or microspheres in the cytoplasm of trabecular cells in position on the lamellae indicates that trabecular cells can handle various particles without leaving the trabecular beams or undergoing necrosis. Evidence for the intracellular digestion of zymosan also supports the idea of trabecular cell processing of ingested material while remaining in situ. Clinically the tendency for trabecular cells with ingested material to remain on the trabecular lamellae is consistent with the heavy pigmentation of the meshwork seen in pigment dispersion syndrome and would also be desirable phylogenetically. With heavier phagocytic loads, or repeated challenges, the trabecular cells may react differently, both in vivo and in vitro.

Bulk flow of particular debris is probably a major mechanism of removal of foreign material, rather than phagocytosis and digestion. Although this study was designed to examine phagocytosis and not quantitate the mechanisms of removal of foreign particles, the finding of free erythrocytes, zymosan particles, and latex microspheres in Schlemm's canal and the relatively low proportion of cells involved in phagocytosis support the concept of bulk flow removal of debris. This idea is also similar to findings in several in vivo human studies of hyphema.\textsuperscript{18,19} Use of unmatched blood and serum, which probably contained antigens and antibodies foreign to the cells in culture, did not appear to increase uptake of erythrocytes. Part of this negligible effect could be due to the fact the blood was type O.

In conclusion, trabecular cells retain their phagocytic ability in organ culture. Phagocytosis does not necessarily cause trabecular cell loss or cell migration as has been suggested in animal studies. Trabecular cells appear capable of digesting foreign particles while remaining in situ on the lamellae. Although these studies apply only to a one-time challenge of the meshwork with foreign particles, they may serve as the basis for future investigations of chronic, repeated challenges as may occur in pigmentary glaucoma.

Key words: trabecular meshwork, phagocytosis, organ culture, tissue culture, zymosan

References