Otago Glaucoma Surgery Outcome Study: Cytology and Immunohistochemical Staining of Bleb Capsules around Molteno Implants

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PuRPOSE. To describe the cytology and immunohistochemistry of Molteno implant capsules from cases of primary and secondary glaucoma.

METHODS. Histologic features of capsules including cell cytology, the distribution of activated (proliferating) cells, apoptosing cells, and membrane bound vesicles (presumed death messengers) were assessed by light microscopy and correlated with clinicopathological features in 10 noninflamed eyes with good intraocular pressure control (nine autopsy and one enucleation) obtained from 2 months to 16.8 years after insertion of Molteno implants.

RESULTS. All bleb capsules demonstrated two distinct layers. The thin external layer was cellular with fairly numerous small blood vessels coursing through normally staining, regularly arranged collagen fibers. The thicker, deeper layer was avascular, relatively acellular, and characterized by regularly arranged swollen and fragmented collagen fibers. Most cells in the external layer appeared normal; however, between 5% (in recently formed blebs) and =50% (in well established blebs) showed cytological and/or immunohistochemical changes characteristic of metabolic activation and/or apoptosis. All cells in the deeper layer, regardless of time after surgery, also demonstrated cytological and/or immunohistochemical staining characteristic of metabolic activation and/or apoptosis. In addition, the deeper layer evidenced large numbers of minute membrane-bound vesicles (presumed death messengers).

CoNCWSIONS. The balance between activation and apoptosis regulates the thickness and permeability of bleb capsules, and the normal lifecycle of bleb capsules includes continual inner surface degeneration and external surface renewal. (Invest ophthalmol Vis Sci. 2006;47:1975-1981)
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The success of filtering operations for glaucoma depends on aqueous induction of two opposing responses: an early inflammatory response to heal the conjunctival incision and lay down sufficient collagen to raise the intraocular pressure (IOP) to normal levels followed by a fibrodegenerative response that inhibits the fibroproliferative response, breaks down collagen, and maintains a "draining bleb." 

Histologic and immunohistochemical examinations of eyes with Molteno implants have been reported. Rubin et al. reported three eyes in which the IOP had been successfully controlled for 2 to 6 years and concluded that, in the outer layers of the bleb walls, few, mostly degenerated inflammatory cells were present, whereas Classen et al., describing three unsuccessful implants (two Molteno implants and one von Denffer implant), identified myofibroblasts suggesting longstanding scar modulation and noted that macrophages may invade the filtration bleb. Molteno et al. found that capsules around 75 functioning implants evolved through histologic stages and concluded that the final thickness and permeability of the capsule depended on the relative intensity of opposing aqueous induced fibroproliferative and fibrodegenerative processes and observed that the inner layers of the bleb capsule showed progressive swelling, fragmentation, and disappearance of collagen with lowering of the IOP.

Initially, it was thought that the postoperative period of inflammation was due to proinflammatory chemical factors in glaucomatous aqueous and that inflammation ceased when a period of drainage had removed these factors from the eye. This hypothesis guided our initial attempts to develop methods of preventing bleb capsule inflammation.

Our first attempt at controlling bleb inflammation involved the oral administration of a synergistic combination of antiinflammatory drugs (prednisone 5-10 mg three times daily; diclofenac 50-75 mg three times daily, and colchicine 0.3 mg three times daily for a 70-kg adult) for the first 6 weeks after surgery. This regimen was (and is) very effective but required close supervision of the patient's compliance.

The second approach involved modifying the episcleral plate so that aqueous escaped through a secondary tube when the IOP was more than 6 to 12 mm Hg. This implant was placed on the sclera without connecting the tubes to the anterior chamber or conjunctiva. After 6 to 8 weeks, the primary tube was inserted into the anterior chamber and the free end of the secondary tube exposed in the superior fornix. It was thought that this technique would drastically reduce capsule inflammation and that when the secondary tubes were cut and allowed to retract into the tissues, the aqueous from eyes that had been normotensive for 8 to 11 weeks would not cause inflammation and produce thin permeable bleb capsules. Cutting of the secondary tubes resulted in gross elevation of IOPs within 2 to 3 weeks, and surgical exploration demonstrated thick, heavily fibrosed bleb capsules that resulted from exposing the extraocular tissues to reduced amounts of aqueous at low IOP. This behavior did not support the "proinflammatory factor" hypothesis and at the time (1975) no alternative hypothesis seemed plausible.
Subsequently, it was found that delaying the onset of drainage by tying off the tube of the implant with an absorbable ligature and allowing the tissues to form a thin, fibrous capsule around the Molteno implant before drainage of aqueous into the bleb capsule effectively prevents postoperative hypotony. Furthermore, this method results in a thinner bleb capsule and a lower final IOP demonstrating that draining increased volumes of aqueous into the episcleral tissues at higher pressures somehow reduces bleb capsule fibrosis.8,9

On the basis of these clinical and histologic observations we hypothesized the following:

1. Drainage of aqueous into well-vascularized subconjunctival connective tissue (mesodermal tissue) dilutes the interstitial tissue fluid and acts as a noxious stimulus causing vasodilation and activating mesodermal cells. Activated fibroblasts synthesize collagen, producing a barrier to the passage of aqueous. This process continues for as long as cells in close proximity to patent blood vessels (≤50 µm) are exposed to the aqueous.10

2. Once sufficient fibrosis has occurred (to resist the passage of aqueous) the IOP increases and exceeds the capillary pressure (15-25 mm Hg) in the deeper part of the bleb capsule. This zone effectively becomes avascular (>50 µm from the nearest patent blood vessel), changing the tissue environment, as aqueous displaces the interstitial tissue fluid. Under these conditions, cells deplete nutrients and are exposed to proapoptotic factors that include low protein concentration and hypoxia. The local effects of apoptosis include breakdown of the deeper layers of the collagen barrier and transport of death messengers with the flow of aqueous to the fibroproliferative outer portion of the capsule where they destroy activated cells and inhibit the fibroproliferative response. A decreasing number of cells migrate into the capsule where they are activated and undergo apoptosis for the rest of the patient's life.

These hypotheses explain the exaggerated fibrosis produced by modified implants that diverted aqueous to the conjunctiva, as follows. The IOP and tissue pressure around the implant cannot increase above 12 mm Hg, so that the capillary vessels in the deep layers of the capsule remain patent and aqueous continues to drain into vascular connective tissue so that the hypoxic apoptotic response does not occur. Thus, reduced volumes of aqueous at low pressures produce a long-lasting vascular fibroproliferative response.10

We used recently developed cell markers for "activation" and apoptosis of macrophages and fibroblasts to demonstrate cellular events in capsules around 10 Molteno implants, to evaluate our hypothesis that a fibroproliferative response occurs when aqueous permeates well-oxygenated tissue <50 µm from a patent capillary, whereas an apoptotic fibrodegenerative response occurs when aqueous permeates less-well-oxygenated tissue >50 µm from a patent capillary.

METHODS Pathologic Material

Ten capsules with overlying and adjacent connective tissue, of which nine postmortem cases included conjunctiva, from functioning Molteno implants in noninflamed eyes obtained between 2 months and 16.8 years after operation were subjected to histologic, cytologic, and immunohistochemical studies. Specimens were obtained at intervals of between 30 minutes and 5 hours after death (nine cases) or enucleation...
(one case). Three specimens were part of our previous series that reported the histologic features' and seven additional specimens obtained since May 2002 were included. The underlying etiology and implant type are described in Table 1. No eyes had received either antimetabolites or the synergistic combination of anti-inflammatory drugs of prednisone, diclofenac, and colchicine.5-7 All eyes had good IOP control before death or enucleation (mean, 11.2 mm Hg; range, 6-18).

Informed consent for donation of eyes for research purposes was obtained before enucleation or death. This study adhered to the tenets of the Declaration of Helsinki.

Surgical Technique

The surgical techniques used for immediate and delayed drainage of aqueous have been described.4,B,9.11-13

Fixation and Processing of Tissue

Capsules and whole eyes were fixed in 10% neutral buffered formalin for 3 hours, microwaved for 20 minutes at 50°C, and placed in 70% alcohol to harden further. The lateral half of the bleb capsule and adjacent tissue were excised to allow removal of the episcleral plate of the implant before standard paraffin processing. The excised half of the bleb capsule was embedded separately and oriented to allow for the cutting of serial sections that were initially tangential to the surface of the bleb capsule but subsequently became less oblique as the plane of section extended to the margin of the capsule where they were almost perpendicular to the wall of the bleb. The portion of capsule remaining on the eye was oriented to allow the cutting of sections perpendicular to the capsule. After embedding in paraffin, 5-µm serial sections were cut and mounted on glass slides.

Histologic and Immunohistochemical Staining of Tissue

Stains included hematoxylin and eosin, Gomori trichrome using Harrison's hematoxylin, anti-CD68, anti-proliferating cell nuclear antigen (PCNA), terminal deoxynucleotidyl transferase-mediated deoxyuridinetriphosphate [dUTP], nick-end labeling (FUNEL), and anti-active caspase-3 reagents.

CD68 and PCNA Immunohistochemistry

All sections were dewaxed in xylene and rehydrated through a graded series of ethanol.

CD68 is a lysosomal membrane protein expressed strongly in cytoplasmic granules and weakly on the surface of macrophages, monocytes, neutrophils, basophils, and NK cells.4 Dewaxed sections were placed in 3% hydrogen peroxide solution for 10 minutes followed by a 100-minute retrieval in proteinase K (S3030; DakoCytomation, Glostlup, Denmark). Sections were then placed in Tris buffer, after which protein blocking agent (407501; Thermo Electron Corp., Runcom, Cheshire, UK) was added. This was followed by addition of monoclonal mouse anti-human CD68 serum (clone PG-M1 [reference 0876]; DakoCytomation). The visualization system consisted of LSAB2-HRP (Dako) and diaminobenzidine chromogen (K3466; DakoCytomation) counterstained by Mayer's hematoxylin. The negative control was provided by omitting the anti-CD68 serum and substituting normal serum in the same dilution. Positive controls consisted of human gastric mucosa, skin, and lymph node.
Proliferating cell nuclear antigen (PCNA) is strongly expressed by cells undergoing replication at the end of the G1 phase and beginning of S-phase of the cell cycle and is involved in a range of cell activities, including cell cycle arrest, DNA repair, and replication. For PCNA staining, sections were exposed to antigen unmasking by heating in 0.01 M citrate buffer (pH 6) for 20 minutes in a 1000-W microwave oven (Sharp, Osaka, Japan) before they were allowed to cool in solution for a further 15 minutes. Nonspecific binding was blocked with 5% (v/v) goat serum before applying 1:150 dilution of anti-PCNA (Novacastra, Newcastle-upon-Tyne, UK) and incubating overnight at 4°C. Biotinylated anti-rabbit IgG was applied and amplified with streptavidin-biotinylated horseradish peroxidase complex. Signals were developed for visualization with AEC (Sigma-Aldrich, Poole, UK), counterstained with Gill's hematoxylin, and mounted with glycerol. Negative control sections included omitting the primary body from the dilution buffer, and positive controls included granulosa cells of healthy follicles in mouse ovary.

TUNEL Staining and Active Caspase-3

All sections were dewaxed in xylene and rehydrated through a graded series of ethanol.

TUNEL is used for in situ labeling of DNA strand breaks that form in individual nuclei of apoptotic cells. TUNEL is sensitive and fairly specific for apoptosis, although TUNEL reactivity lasts for only =4 hours in an apoptotic cell. Fluorescein-dUTP with appropriate filters was used to photograph bleb wall cell labeling. Antigen unmasking was performed by incubating sections in 20 µg/mL proteinase K (Sigma-Aldrich) for 20 minutes, followed by permeabilization for 2 minutes at 4°C in a solution consisting of 0.1% (w/v) Triton-X-100 and 0.1% (v/v) trisodium citrate. The TUNEL label (fluorescein-dUTP and dNTP mix; Roche, Mannheim, Germany) was combined with 10% (v/v) TUNEL enzyme (terminal deoxynucleotidyl transferase; Roche) and applied to each section for 60 minutes. Sections were mounted with Vectashield (Vector Laboratories, Burlingame, CA) and coverslipped. Imaging was performed on a laser scanning confocal microscope (510 Axioplan 2; Carl Zeiss Meditec, GmbH, Jena, Germany). The fluorescein label was excited at 488 nm with an argon laser, and the emission wavelength was collected with a band-pass filter in the range of 505 to 530 nm. A negative control was prepared on a single section within the assay by excluding the TUNEL enzyme. A positive control section was assessed by applying 1 U/mL DNase 1 (Promega) and incubating for 30 minutes at 37°C before the addition of the TUNEL enzyme-label mix.

Active Caspase-3 Immunohistochemistry

Active caspase-3 labeling is specific for cells undergoing irreversible apoptosis (typical noninflammatory cell death). After dewaxing and rehydration, the sections were exposed to antigen-unmasking in 0.1 M Tris-HCl buffer 1-" (pH 10) with 5% (w/v) urea by heating for 20 minutes in a 1000-Wt microwave oven (Sharp). Peroxidase activity was quenched with 0.3% (v/v) H2O2. Sections were blocked with a 1:200 dilution of donkey serum and incubated at 37°C for 1 hour with 2 Jg/mL rabbit antiactive caspase-3 monoclonal antibody (Research Diagnostics Inc., Flanders, NJ) followed by biotinylated anti-rabbit IgG (GE Healthcare, Little Chalfont, UK). Signals were amplified with streptavidin-biotinylated horseradish peroxidase complex (GE Healthcare), developed with 3-amino-9-ethylcarbazole (AEC; Sigma-Aldrich), and counterstained with hematoxylin. The negative control included substitution of the primary antibody with an equivalent amount of Ig
isotype serum. The positive control was granulosa cells of atretic follicles in mouse ovary.

Examination of Slides
Stained sections were examined and photographed by light microscopy using bright-field, dark-background, polarized-light, phase-contrast, and ultraviolet fluorescent techniques (Orthoplan with Zernike positive phase-contrast condenser; Leitz, Wetzlar, Germany; laser scanning confocal microscope, 510 Axioplan 2; Carl Zeiss Meditec, Inc.).

Cell density was measured by averaging the counts of cells per high-power field area as calculated using the correction figures supplied with the microscope (Leitz) for area dimension and confirmed with an eye piece micrometer.

RESULTS

Histologic Structure of Connective Tissue and Conjunctiva
The overlying connective tissue was edematous but otherwise normal, and the conjunctiva was histologically normal.

Histologic Structure of Bleb walls
The wall of the bleb capsule when cut perpendicular to its surface consisted of an outer, relatively cellular and moderately vascular connective tissue layer (the fibroproliferative layer), merging more deeply with an avascular, relatively acellular layer of bleb wall adjacent to the aqueous cavity (the fibrodegenerative layer; Fig. 1). The panel labels in all figures are the same as described in Figure 1.

Oblique and tangential planes of sectioning showed details of cellular morphology within the bleb capsule (Figs. 2-9).

Extracellular Matrix
The outer fibroproliferative layer displayed the typical morphology staining and birefringence of normal collagen and elastin fibers (Fig. 3). The inner fibrodegenerative layer occupied from 43% to 77% of the total thickness and exhibited the altered staining typical of hyaline degeneration of collagen, with loss of birefringence (Fig. 3). In some specimens, degeneration and disappearance of collagen left isolated elastic fibers projecting into the aqueous cavity.

Cell Distribution

Fibroproliferative Layer. This layer consisted of small blood vessels surrounded by cells identified morphologically as mesenchymal cells, "resident" and "wandering" macrophages, fibroblasts, and indeterminate cells within a collagenous matrix.20 Cell density as measured on tangential sections of the bleb apex, just deep to the vessel layer was 20 to 133 cells/0.055 mm2 (5-µm section; Table 1; Figs. 1-5).

Fibrodegenerative Layer. In this layer, the number of cells decreased to 1 to 39 cells/0.055 mm2 (5-µm section) in the deepest layer of tangential sections from the bleb apex (table 1, Fig. 3). Tangential and oblique sections enabled observation of flattened nuclei and cytoplasm in plan rather than in profile as in perpendicular sections (Figs. 1-9). The cell population consisted of fibroblasts and macrophages, with 10%
FIGURE 1. Vertical section of a 4.1-year-old bleb capsule showing cells in profile (case 3 in Table 1; same bleb capsule as Figs. 2-5, 7-10, 16-18; stain: hematoxylin and eosin). (A) Tenon's tissue, (B) outer fibroproliferative layer of the capsule, (C) the fibrodegenerative layer, and (D) the bleb cavity. All figures are oriented and labeled in the same way. Original magnification, X250.
FIGURE 2. Oblique section of a 4.1-year-old bleb capsule showing cells in plan (case 3 in Table 1; same bleb capsule as in Figs. 1, 3-5, 7-10, 16-18). Stain, hematoxylin and eosin; original magnification, X250.

FIGURE 3. Tangential section of a 4.1-year-old bleb capsule in polarized light showing the distribution of birefringent collagen fibers (case 3 in Table 1; same bleb capsule as in Figs. 1, 2, 4, 5, 7-10, 16-18). Stain, hematoxylin and eosin; original magnification, X63.

FIGURE 4. Oblique section of the outer half of a 4.1-year-old bleb capsule showing enlarged, vacuolated, and fragmented fibroblasts and condensed, basophilic, irregular macrophages (case 3 in Table 1; same bleb capsule as in Figs. 1-3, 7-10, 16-18). Stain, hematoxylin and eosin; phase contrast; original magnification, X400.

FIGURE 5. Oblique section of the inner half of a 4.1-year-old bleb capsule showing enlarged, vacuolated, and fragmented fibroblasts and condensed basophilic irregular macrophages. Compare the sizes of the cells on the inner surface with those on the outer surface of Figure 4 (case 3 in Table 1; same bleb capsule as in Figs. 1-4, 7-10, 16-18). Stain, hematoxylin and eosin; phase contrast, original magnification, X400.

FIGURE 6. Oblique section of a 13.5-year-old bleb capsule showing a fibroblast with a mitotic figure (case 9 in Table 1). Stain, hematoxylin and eosin; original magnification, X1000.

FIGURE 7. Oblique section of a 4.1-year-old bleb capsule (case 3 in Table 1; same bleb capsule as in Figs. 1-5, 8-10, 16-18) showing presumed apoptotic macrophage. Note chromatin condensation and blebbing of nuclear membrane. Stain, hematoxylin and eosin; phase contrast, original magnification, X1000.

FIGURE 8. Oblique section of a 4.1-year-old bleb capsule (case 3 in Table 1; same bleb capsule as in Figs. 1-5, 7, 9, 10, 16-18) showing enlarged vacuolated cell with bleb on inner surface and broken down cell remnant. Stain, hematoxylin and eosin; phase contrast, original magnification, X1000.

FIGURE 9. Oblique section of a 4.1-year-old bleb capsule showing membrane bound vesicles released by disintegration of an apoptotic cell (case 3 in Table 1; same bleb capsule as in Figs. 1-5, 7, 8, 10, 16-18). Stain, hematoxylin and eosin; original magnification, X1000.

FIGURE 10. Oblique section of a 4.1-year-old bleb capsule showing phagocytosis of a double-stained caspase-3 (pink cytoplasm)- and PCNA (dark brown staining nuclear remnants)-positive cell by a faintly PCNA-positive phagocyte in the perivascular space of a blood vessel (case 3 in Table 1; same bleb capsule as in Figs. 1-5, 7-9, 16-18). Stains, anti-caspase-3 and anti-PCNA; original magnification, X1000.

FIGURE 11. Vertical section of the outer layer of a 12.6-year-old bleb capsule showing two apoptotic cells (one close to the capillary and the other midway between (A) and (B); case 8 in Table 1; same bleb capsule as in Fig. 12). Stain, Gomori trichrome using Harrison's hematoxylin; original magnification, X400.

FIGURE 12. Vertical section of the outer layer of a 12.6-year-old bleb capsule showing phagocytosis of an apoptotic cell by a macrophage. Note the melanin granule in the macrophage (same bleb capsule as in Fig.
11) Stain, Gomori trichrome using Harrison's hematoxylin; original magnification, X 1000.

FIGURE 13. Oblique section of an 8.1-year-old bleb capsule showing the superficial distribution of CD-positive macrophages (brown) and fibroblasts (blue) in subconjunctival connective tissue (case 5 in Table 1; same bleb capsule as in Figs. 14, 15). Stain, anti-CD68; original magnification, x400.

FIGURE 14. Oblique section of an 8.1-year-old bleb capsule showing distribution and density of CD68-positive macrophages (brown) and fibroblasts (blue; case 5 in Table 1; same bleb capsule as in Figs. 13, 15). Stain, anti-CD68; original magnification, X250.

FIGURE 15. Oblique section of 8.1-year-old bleb capsule showing dark brown-stained activated cells expressing proliferating cell nuclear antigen (pCNA; case 5 in Table 1; same bleb capsule as in Figs. 13, 14). Stain, anti-pCNA; original magnification, X250.

FIGURE 16. Ultraviolet oblique section of a 4.1-year-old bleb capsule showing distribution of fluorescent TUNEL-positive, presumed apoptotic cells (case 3 in Table 1; same bleb capsule as in Figs. 1-5, 7-10, 17, 18). Stain, TUNEL; original magnification, x400.

FIGURE 17. Oblique section of a 4.1-year-old bleb capsule showing the absence of caspase-positive cells in loose subconjunctival tissue and three caspase-positive cells (pink) on the external surface of the bleb capsule (case 3 in Table 1; same bleb capsule as in Figs. 1-5, 7-10, 16, 18). Stain, anti-active caspase-3; original magnification, x400.

FIGURE 18. Oblique section of a 4.1-year-old bleb capsule showing distribution of pink-staining caspase-3-positive apoptotic cells (case 3 in Table 1; same bleb capsule as in Figs. 1-5, 7-10, 16, 17). Stain, anti-active caspase-3; original magnification, X250 to 80% of the cell population identified morphologically as macrophages in different blebs.

Morphology of Cells

Fibroblasts exhibited an altered appearance in the deeper aspects of the bleb walls toward the aqueous cavity. Fibroblast nuclei and cytoplasm became enlarged, vacuolated and fragmented, with occasional replacement by clumps of cellular fragments. These cells were often surrounded by numerous tiny membrane-bound vesicles and minute basophilic particles. Macrophages were identified by their condensed, relatively basophilic, elongated, branching or irregular nuclei. This typical appearance of macrophages was also altered toward the aqueous cavity with typical apoptotic bodies, nuclear chromatin condensation, vacuolization, shrinkage and fragmentation (Figs. 4-9). These cellular changes could not easily be recognized in perpendicular sections but were well seen in oblique or tangential sections using phase contrast microscopy.

Mitotic Figures

Only two definite mitotic figures were found on examination of all tissue sections (>800 slides; Fig. 6). These two mitoses occurred at the junction of the fibroproliferative and fibrodegenerative layers of bleb capsules.

Membrane-Bound Vesicles
Disintegration of cells in the fibrodegenerative inner portion of the bleb capsule produced a large number of variably basophilic membrane bound vesicles ranging in size from a diameter of 4 µm to the limit of optical resolution (0.4 µm). These vesicles were most numerous in the deeper layers of maximum cellular degeneration, with a smaller number in the more superficial layers of the capsule and occasional vesicles in perivascular spaces of capillaries on the capsule surface (Fig. 9).

Phagocytosis of Apoptotic Cells

Most (=85%) apoptotic cells in the outer fibroproliferative layer were actively phagocytosed before releasing membrane bound vesicles (Figs. 10-12).

Cellular Immunohistochemistry Distribution of Labeled Cells

Anti-CD68 labeled macrophages that were widely distributed in the loose subconjunctival connective tissue with an increased number close to the outer surface of the bleb capsule (Figs. 13, 14). In the bleb capsule itself, CD68-positive cells were most numerous around capillary blood vessels on and in the outer fibroproliferative zone with progressively decreasing numbers of cells in the deeper layers of the fibrodegenerative zone.

Anti-PCNA labeled moderate numbers of activated cells in the loose conjunctival connective tissue close to the outer surface of the bleb capsule. In the bleb capsule itself PCNA-positive cells were most numerous on and in the outer fibroproliferative zone with a progressively decreasing number of cells in the deeper layers of the fibrodegenerative zone (Fig. 15).

TUNEL stained very few weakly fluorescent cells scattered in the subconjunctival connective tissue with moderate numbers of more strongly fluorescent cells close to the outer surface of the bleb capsule. Fluorescent labeling showed numerous cells around blood vessels in the fibroproliferative zone, with the most intense labeling at the junction between fibroproliferative and fibrodegenerative layers and with a progressively decreasing number of cells in the deeper layers of the fibrodegenerative zone (Fig. 16).

Anti-caspase-3 did not show any positive cells scattered in the subconjunctival connective tissue; however, occasional positive cells were observed close to the outer surface of the bleb capsule (Fig. 17). Anti-caspase-3 showed numerous cells around blood vessels in the fibroproliferative zone, with the most intense labeling at the junction between fibroproliferative and fibrodegenerative layers and with a progressively decreasing number of cells in the deeper layers of the fibrodegenerative zone (Fig. 18). A comparison between immunostained and conventionally stained sections showed that =20% of all cells in the bleb capsules were caspase-3 positive.

DISCUSSION

This study demonstrated that a cycle of cell death and replacement occurred within bleb capsules. We suspect similar processes characterize the lateral and deep boundary tissues of filtering blebs after trabeculectomy. We believe that the processes occurring within bleb capsules in quiet eyes are not primarily dependent on alterations in chemical factors within aqueous, but constitute two fundamental responses of mesodermal tissue cells to any stimulus that alters their local environment (including the low protein and oxygen levels of aqueous
compared with interstitial tissue fluid), z1 A fibroproliferative response occurred when aqueous permeated well-oxygenated tissue less than 50 µm from a patent capillary, whereas an apoptotic fibrodegenerative response occurred when aqueous permeated less well oxygenated tissue more than 50 µm from a patent capillary.1

Apoptosis of liver cells in mice induced by an intraperitoneal injection of 10 µg Fas ligand killed 50% of animals within 8 hours.22 This implies that apoptosis is complete by =12 hours. If cells in human bleb capsules complete apoptosis in the same time, then the cells that had migrated into the capsules were destroyed within =30 to =60 hours. This in turn implies that cells that traversed the full thickness of the capsule before completing apoptosis migrated vertically at =6 to =12 µm per hour, which is consistent with the observed migration rate of fibroblasts in tissue culture of 40 to 50 µm per hour.23 It seems likely that the flattened cells would have negotiated the regularly arranged collagen fibers of the bleb capsule by moving along an irregular pathway for 40 to 50 µm, to advance 6 to 12 µm deeper into the capsule. These arguments depend on the assumption that apoptosis occurs as rapidly in the bleb capsule as it does in liver. Although the overall metabolic activity of human bleb capsules is clearly less than that of mouse liver, apoptosis is an energy-dependent cellular process, so it is very probable that cells were turned over at relatively short intervals, of days to weeks. Tonographic measurements of improvement in facility of outflow, after drainage by Molteno implants,24 combined with the known area of bleb capsules in individual cases, indicated that aqueous flowed through established bleb capsules at =7 to =14 µm a minute. This surprisingly rapid flow, together with the finding of membrane-bound vesicles in decreasing numbers in the more superficial layers of the capsules suggested that they were continuously produced in large numbers in the deeper layer from where they were carried by the current of aqueous toward the superficial vascular layer where they triggered apoptosis in activated cells with which they came into contact. We believe that most cells, undergoing apoptosis in the well-oxygenated superficial vascular layer, are rapidly phagocytosed by nearby cells before release of death messengers thereby preventing further apoptosis in the loose subconjunctival connective tissue.

Recent research has shown that cells undergoing apoptosis exert an anti-inflammatory reaction in most tissues of the body and furthermore play an important role in maintaining the immunologic privilege of ocular tissues.25 The findings of this study demonstrate that cellular events including activation of fibroblasts and macrophages and apoptosis of fibroblasts and macrophages play an important role in the regulation of bleb capsule fibrosis. These insights help clarify basic cell responses within implant bleb capsules and may contribute to increasingly rational clinical manipulations favoring improved outcomes of filtering surgery.

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References


Bleb Capsules around Molteno Implants 1981


