Immunocytochemical Characterization of Macular Hole Opercula

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Objectives: To immunocytochemically characterize the neural and glial elements of idiopathic full-thickness macular hole (FTMH) opercula excised during vitrectomy, and to correlate them with the outcome of surgery.

Methods: Opercula were collected from eyes undergoing vitrectomy for stage 3 FTMH and processed for transmission electron microscopy, light epifluorescence, and laser scanning confocal microscopy. Glia were identified using anti–glial fibrillary acid protein (GFAP), antivimentin, and anti–cellular retinaldehyde binding protein antibodies. Anti–phosphodiesterase gamma and antirhodopsin were used for cone and rod photoreceptors, and anticytokeratin was used for retinal pigment epithelium. The findings were correlated with the clinical data before and after surgery. For statistical analysis, data were combined with those of a previous study by the authors of 18 opercula.

Results: Opercula from 12 consecutive eyes of 12 patients were studied. In all opercula, GFAP, vimentin, and cellular retinaldehyde binding protein–positive glia were present. Six (50%) of 12 opercula contained more than 5 photoreceptors with somata and internal photoreceptor fibres, but lacking outer segments, demonstrating strong immunoreactivity to anti-phosphodiesterase gamma without antirhodopsin reactivity consistent with cones. Further, 2 (17%) of 12 opercula showed few cones (1-5 cones), and 4 (33%) of 12 contained only glia. Clinopathologic correlation of the 30 opercula from the 2 studies showed that eyes with opercula containing more than 5 photoreceptors were associated with a worse anatomical closure rate after initial surgery, compared with those with fewer than 5 photoreceptors (P = .004). Once closure had been achieved with reoperation, median postoperative vision was similar in both groups (20/40 and 20/60, respectively).

Conclusions: A spectrum of opercula occur in FTMH ranging from those containing only glia to those containing numerous cones. The extent of foveal neuroretinal tissue loss may affect the outcome of surgery.


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PATIENTS AND METHODS

Opercula from patients undergoing vitreous surgery for stage 3 FTMH were collected for immunocytochemical analysis. A stage 3 FTMH was defined, according to Gass’ definition, as a lesion with vitreomacular separation detectable on slitlamp examination by the presence of a free operculum suspended on the separated posterior vitreous cortex, anterior to the preretinal plane. Thus, only eyes with free opercula visible preoperatively were included. Preoperative details of all patients were recorded, and included age, sex, duration of symptoms, previous ocular and medical history, best-corrected Snellen visual acuity, and slit-lamp and fundus examinations with a 78-diopter lens and Goldmann fundus contact lens. The size of the hole was measured preoperatively from red-free photographs and fundus fluorescein angiogram. All opercula included in this study were identified preoperatively as free-floating preretinal tissue or as a lesion with vitreomacular separation detectable on slitlamp examination by the presence of a free operculum visible preoperatively. The operative procedure used in all eyes has been described elsewhere. All operations were performed by 1 surgeon (Z.J.G.). Following a 3-port pars plana vitrectomy, the posterior vitreous cortex was separated from the retinal surface by aspiration using the vitreous cutter. In all cases, the operculum remained attached to the posterior vitreous cortex following its elevation from the retinal surface and was identified while suspended on the detached vitreous cortex in the vitreous cavity. The vitreous cortex temporal to the operculum was then partially excised, and a pair of non-crushing, cupped, intracocular foreign body forceps was used to grasp the operculum. The vitreous cortex adjacent to the forceps was then excised with the cutter under coaxial illumination to allow removal of the forceps from the eye. Thus, all opercula were collected using an atraumatic technique prior to any manipulation or peeling of membranes on the surface of the retina. Specimens were then carefully removed from the forceps and immediately fixed and processed for immunocytochemical analysis.

The retinal surface was then examined with a membrane pick to identify any epiretinal membranes. Membrane peeling was performed only when membranes could be identified. In no case was an attempt made to remove the internal limiting membrane. Following examination of the retinal periphery, air-fluid exchange was performed with aspiration of subretinal fluid using a 34-gauge cannula. The hole was then dried under air for 10 minutes, and an air-perfluoropropane (14%-16% C3F8) exchange was performed to complete the procedure. Reoperations after failed initial surgery were carried out using a standardized technique involving rigorous epiretinal membrane, internal limiting membrane dissection, and C3F8 gas tamponade, as has been described elsewhere.

TRANSMISSION ELECTRON MICROSCOPIC IMMUNOCYTOCHEMISTRY

For this technique, each specimen was fixed immediately in a solution of 4% paraformaldehyde for 20 minutes and then manipulated into small (approximately 2 × 2 × 2 mm) blocks of set 10% agarose gel to allow subsequent handling of the specimen. The blocks containing the specimens were then rinsed in 3 changes of 0.1-mol/L sodium phosphate buffer (pH, 7.4). Subsequent dehydration was achieved by transfer of the agarose block through ascending alcohols (5-minute immersions in 50%, 70%, and 90% ethanol and 4 changes of 100% ethanol), followed by embedding in London Resin white resin (LR White; Ted Pella Inc, Redding, Calif) and overnight curing at 60°C. Semithin (1 µm) and ultrathin (100 nm) sections were then cut using glass and diamond knives, respectively, with an ultramicrotome. The opercula were step sectioned, taking 10 to 20 cuts at 20-µm intervals.

Ultrathin sections were then placed on copper grids and processed for transmission electron microscopic (TEM) immunocytochemistry by the silver enhancement method for immunogold labeling of electron microscopic (EM) sections, using a modification of a previously described technique. Following initial incubation at 20°C for 30 minutes with a blocking buffer (pH, 8.2), containing tris buffered saline (TBS), bovine serum albumin fraction V (BSA) and normal blocking serum, grids were incubated in primary antibody in BSA-TBS overnight at 4°C. This was followed by 3 washes in BSA-TBS with NaCl for 10 minutes. Grids were then incubated with 5 nm colloidal gold secondary antibody in BSA-TBS with NaCl (1:10 dilution) for 2 hours...
CONFOCAL MICROSCOPY

In view of their small size, for this technique, whole opercula were processed without sectioning. Opercula were immediately fixed in 4% paraformaldehyde, followed by rinsing in 4 changes (for 10 minutes; for 60 minutes) of chilled phosphate-buffered saline (PBS) containing 0.5% BSA, 0.1% NaN₃, and Triton x-100 (0.1%) at a pH of 7.3. This was followed by incubation with blocking buffer for 4 hours at 20°C, primary antibodies diluted in PBS-azide at 4°C overnight and 4 further washes in PBS in the same manner as the first washes. Specimens were then placed in fluorescent secondary antibody diluted in PBS overnight at 4°C. Finally, after 4 further washes in PBS, specimens were mounted in 5% N-propyl gallate in glycerol (to retard photobleaching) and coverslipped. Immunofluorescence was analyzed using epifluorescence and confocal microscopy.

ANTIBODIES AND NUCLEAR LABEL

The following primary antibodies were used: rabbit polyclonal anti–phosphodiesterase gamma (PDEG), which was generated against peptide amino acids 73 through 87 at a dilution of 1:1000 for cones and rods; mouse monoclonal antirhodopsin (Rho 4D2) at a dilution of 1:20 for rods; rabbit polyclonal antiglial fibrillary acid protein (anti–GFAP) and mouse monoclonal anti–GFAP (Dako Corp, Carpinteria, Calif), at dilutions of 1:200 and 1:20, respectively; mouse monoclonal antivimentin (Clone V9; Dako Corp), at a dilution of 1:200 for activated glial cells; mouse polyclonal antipodal retinaldehyde binding protein (clone Immunoglobulin 83), at a dilution of 1:200 for Muller glia and retinal pigment epithelium cells; and mouse monoclonal anti-cytokeratin (Clone Cam-52; Becton and Dickinson, Sparks, Md), at a dilution of 1:200, for RPE cells.

For double labeling studies, 2 primary antibodies raised in different species were used, as were the 2 secondary antibodies, which were labeled with fluorescein isothiocyanate (FITC) or indocarbocyanine (Cy3) (Jackson Immuno-Research Laboratories Inc, West Grove, Pa). As a pannuclear label in some preparations, 4',6'-diamidino-2-phenylindole (DAPI; Molecular Probes, Eugene, Ore) was used at 2 µg/mL. Sections were photographed as single, double, or triple exposures for epifluorescence microscopy. For confocal microscopy, “Z series” scans were compiled of the entire specimen, at 1-µm increments per scan, in each fluorescence channel. The images from each channel were then superimposed using the NIH Image software version 1.6 (National Institutes of Health, Bethesda, Md) to produce composite double-labeled or triple-labeled images. The composite “Z series” image of the entire specimen therefore enabled individual cells to be counted.

CONTROLS

Control sections of healthy adult macular and foveal retina were processed as above for immunofluorescence and TEM and used as positive controls. These were also used for negative (no primary antibody) controls for immunofluorescence microscopy, as opercula were used as whole mounts without sectioning and no sections of opercula were available for controls. Ultrathin sections of opercula were used as negative controls for TEM immunocytochemistry.

STATISTICAL ANALYSIS

For data analysis, the Fisher exact or Mantel-Haenszel tests were used for comparison of proportions; the 2-tailed t test for comparison of means; and the Pearson product moment correlation for strength of association between interval data.

RESULTS

Twelve opercula from 12 eyes of 12 patients undergoing surgery for stage 3 FTMH were collected and processed for immunocytochemical characterization. Baseline data analysis revealed that there was a mean age of 66.2 years (range, 61-75 years), a subject sample with 1 (8%) of 12 patients being men, a mean symptom duration of 4.9 months (range, 1-9 months), a mean preoperative hole diameter of 410 µm (range, 310-500 µm), a median preoperative visual acuity of 20/120 (range, 20/60-20/200). Ten opercula were processed for immunofluorescence by light epifluorescence and laser scanning confocal microscopy, and 2 for TEM immunocytochemistry.

In the immunofluorescence study (Figures 1, 2, and 3), 9 were double labeled with anti-PDEG and antirhodopsin antibodies. Of these, 2 of 9 cases showed no reactivity to anti-PDEG (cases 1 and 2). Another 2 of 9 (cases 3 and 4) showed that between 1 and 5 cells had positive reactivity to anti-PDEG (Figure 1A, case 3). Two of nine cases (cases 5 and 6) showed that 5 to 100 cells were reactive to anti-PDEG (Figure 3A and 3B), and 3 of 9 cases (cases 7, 8, and 9) had more than 100 cells positive for PDEG antibodies (Figure 1B and Figure 2). All 9 cases showed negative reactivity to antirhodopsin. One further operculum (case 10) was double labeled with anti-PDEG and anti-GFAP and showed positive reactivity to both antibodies (Figure 3C). Two opercula (cases 11 and 12) were step sectioned and processed for TEM immunocytochemistry. Both showed negative reactivity to anti-PDEG, antirhodopsin and Cam-52, and positive reactivity to anti-GFAP, antipodal retinaldehyde binding protein and antivimentin.

The presence of cells with positive reactivity to anti-PDEG and negative to antirhodopsin in opercula was in-
consistent with tissue undergoing gliosis. Random fashion without discernable retinal architecture, containing more than 5 cones, the cones were distributed in a predictable fashion. In the 6 of 9 opercula containing more than 5 cones, 3 (25%) contained a moderate number of cones (range, 5-50 cones), and 3 (25%) contained more than 50 cones. All opercula were negative for rod photoreceptors, and no correlation was evident between either the duration of symptoms or the size of the operculum and the presence of cones in the operculum. Similarly, there was no correlation between preoperative hole diameter and the presence of cones in the operculum.

Clinically, 8 (75%) of 12 eyes had successful anatomical closure after the first operation. In the group with opercula containing more than 5 cones, 3 (50%) of 6 were closed after the first surgery, compared with 5 (84%) of 6 in the group with fewer than 5 cones. In the cases in which surgery had failed, reoperation was undertaken in 2 of 3 eyes in the group with more than 5 cones and 1 of 1 in the group with opercula containing fewer than 5 cones, with all 3 reoperations resulting in successful anatomical closure. The remaining patient in the first group declined reoperation. Analysis of the postoperative visual acuity following successful closure showed an overall median visual acuity of 20/60 (range, 20/20-20/200), with both groups showing median postoperative acuities of 20/60. Neither the initial anatomical result nor the final visual acuity after successful closure seemed to be affected by the overall size of the operculum or the number of cones within the operculum.

For further statistical analysis, the data were combined with those of our previously published study. This allowed statistical analysis on a total of 30 opercula from 30 eyes of 30 patients, with a mean age of 68 years (range, 60-84 years), a mean duration of symptoms of 4.6 months (range, 1-9 months), and a subject population in which 8 (27%) of 30 patients were males. Of the 30 opercula, 15 (50%) contained photoreceptors, and 15 (50%) contained only glia. Overall, 17 (57%) of 30 had few (2/30 had <5 cones) or no cones (15/30 contained glia only) and 13 (43%) of 30 had more than 5 cones. Anatomical closure was achieved after the first operations in 18 (60%) of 30 patients and in 27 (90%) of 30 after reoperations, with the remaining 3 patients declining a second operation. Although the primary closure rate was significantly better (P = .004) in cases with opercula containing few or no photoreceptors (4/17 [82%]) compared with those with opercula containing photoreceptors (4/13 [30%]), the closure rate after reoperation was similar (16/17 [94%], 11/13 [85%]) in both groups. There was no correlation between anatomical closure and preoperative hole size (mean, 465 µm; range, 310-585 µm) (P = .10). There was also no correlation between preoperative hole size and the presence of cones within opercula.

Analysis of visual outcome showed that overall, the median visual acuity improved from 20/120 preopera-

**Figure 1.** Epifluorescence micrographs of 2 opercula containing cones with positive labeling to anti–phosphodiesterase gamma (PDEG) or fluorescein isothiocyanate (green) and negative labeling to antirhodopsin or indocarbocyanine (red). Nuclei are labeled with 4′,6′-diamidino-2-phenylindole (DAPI) (blue). The anti-PDEG antibody labels the peptide amino acids 73 through 87, which is identical in the rod and cone isoforms of PDEG and therefore labels both rod and cones. In the healthy retina, PDEG is localized throughout the cone outer segments and to the cytoplasm of the cone inner segments, somata, and axons. In rods, PDEG is localized to the outer segments only. Rhodopsin, meanwhile, is localized to the outer segments of rods only. In retinal detachment, PDEG expression declines in both cones and rods, while rhodopsin becomes redistributed to the plasma membrane of the entire rod. Thus, in the opercula described here, positive PDEG labeling (green) confirms the presence of photoreceptors, and negative rhodopsin labeling (red) confirms the photoreceptors to be cones rather than rods. Note that while cones are labeled positively with both anti-PDEG and DAPI (nuclei) (blue), numerous other cells are present that only label positively for nuclear DAPI. A, Case 3. An operculum showing numerous cones and no rods. The patient initially had a stage 2 pericentric (“can opener”) full-thickness macular hole at initial visit, which, by the time of admission for surgery, had evolved into a fully operculated stage 3 hole. B, Case 7. An operculum measuring 67.80 µm showing numerous cones and no rods. The patient had a stage 2 pericentric (“can opener”) stage 2 FTMH was noted at the initial visit, which subsequently progressed to a stage 3 FTMH with vitreofveal separation and a free, mobile operculum by the time she was admitted for surgery. The operculum contained abundant cones.
tively, to 20/60 in the 27 of 30 cases in which successful anatomical closure was achieved. There was a trend for better visual acuity results in the 17 cases with opercula containing fewer than 5 cones (preoperative median, 20/120; postoperative median, 20/40), compared with the 13 cases with opercula containing more than 5 cones (preoperative median, 20/120; postoperative median, 20/60), which did not reach statistical significance ($P = .24$).

Although a worse preoperative visual acuity was associated with a worse final acuity after hole closure ($P < .001$), other preoperative factors such as age, duration of symptoms before initial surgery, and sex did not affect either anatomical or visual outcome. There were also no significant baseline differences in age, sex, duration, and preoperative visual acuity between the 2 groups of opercula, and no correlation between the duration of symptoms before initial surgery and the number of cone photoreceptors in opercula.
Figure 3. Pseudocolor laser scanning confocal micrographs (stereopairs). A, Case 5. Operculum with numerous cones (original magnification × 1000) measuring 100 × 130 µm triple labeled with anti-phosphodiesterase gamma (PDEG) (red), antirhodopsin (green), and 4',6'-diamidino-2-phenylindole (DAPI) for nuclei (blue). A total of 46 cones can be seen with positive reactivity to anti-PDEG (red) only and nuclear DAPI (blue). The nuclei (PDEG-positive and rhodopsin-positive) appear pink, while cone axons (PDEG-positive) appear red. Also seen is a population of cells with positive DAPI nuclear labeling (blue) and negative labeling to both anti-PDEG and antirhodopsin. B, Case 6. Operculum (original magnification × 800) measuring 140 × 100 µm with a total of 41 cones. C, Case 10. Operculum (original magnification × 1000) measuring 95 × 60 µm triple labeled with anti-PDEG (red), anti-gliarial fibrillary acid protein (GFAP) (green), and DAPI (blue) for nuclei. A total of 14 cones are seen with positive reactivity to anti-PDEG and DAPI. Two adjacent glia with typical long processes are seen with strong reactivity to anti-GFAP (green).
Two studies have previously reported on the ultrastructure of macular hole opercula. Madreperla et al\textsuperscript{17} reported on 2 opercula studied by TEM, both of which contained only fibroglial cells and no significant neuroretinal elements. They suggested that these “pseudo-opercula” were formed by the aggregation of epiretinal glia as part of an attempted healing response around the hole. In a more recent study,\textsuperscript{18} we reported on the ultrastructure of 18 opercula studied by TEM, showing 61\% to contain glia only and 39\% to contain glia and neuroretinal tissue, including cone nuclei, axons, and pedicles forming synaptic complexes typical of the external plexiform layer.

The current study describes techniques for processing opercula for immunocytochemistry using TEM, epifluorescence, and confocal microscopy. Confocal microscopy allowed the examination of whole-mounted opercula, which, due to their size and thickness, are ideally suited for this method. This affords much additional information in terms of 3-dimensional structure and qualitative and quantitative analysis of various cellular populations within opercula. Transmission electron microscopic immunocytochemistry was used as a complementary technique to allow the study of immunoreactivity to multiple antibodies in any one specimen, as epifluorescence and confocal microscopy only allowed the study of 2 antibodies on any whole-mounted operculum.

Confocal microscopy of whole-mounted opercula proved to be an extremely sensitive technique, allowing the identification of very small numbers of photoreceptors (<5) within opercula in 2 specimens, which may not have been possible with serial-section EM examination. This probably explains the higher incidence of opercula with any photoreceptors in this study (67\%) compared with our previous study with EM (39\%).\textsuperscript{18} The incidence of photoreceptor-rich opercula (>5) in the current study was 50\%. Thus, for the detection of photoreceptors or other neural elements, confocal epifluorescence seems to be a more sensitive method compared with EM, which necessitates a step-sectioning method.

Experimental work has shown that in healthy attached retina, PDEG is localized throughout the cone outer segments and the cytoplasm of the cone inner segments, somata, and axons.\textsuperscript{21} In rods, PDEG is localized to the outer segments only.\textsuperscript{21} Rhodopsin is localized to the outer segments of rods.\textsuperscript{22} Thus, in the opercula described here, positive PDEG labeling confirms the presence of photoreceptors, and negative rhodopsin labeling confirms the photoreceptors to be cones rather than rods, which is consistent with central foveal tissue.

Although we cannot exclude the possibility that opercula with very few or no cones represent opercula in which photoreceptors have degenerated with time, both the current study and our previous data\textsuperscript{18} showed no correlation between the presence of photoreceptors and the duration of symptoms. This suggests that the distinction between photoreceptor-rich opercula and opercula with few or no photoreceptors is a real one. On the other hand, it is likely that the number of cones demonstrated in any operculum by immunocytochemical labeling represents only a proportion of those originally avulsed, with a significant number having undergone degeneration in the operculum. The numerous cells in opercula that show positive DAPI nuclear labelling but negative reactivity for both anti-PDEG and antirhodopsin represent glial cells, which constitute the majority cell population within opercula.

The paucity of photoreceptor inner and outer segments in cones found in opercula during this and the previous study\textsuperscript{18} is not unexpected since separation of the retina from the RPE in a chronic retinal detachment leads to marked degeneration of outer segments within 2 to 4 weeks,\textsuperscript{23,24} with slower inner segment degeneration after months. In the vast majority of eyes with stage 3 FTMH at the time of vitrectomy, any foveal cones present in the operculum would have been separated from the RPE, first during the initial foveal detachment phase (stage 1) and then during the time between progression from stage 2 to stage 3 FTMH. This occurs during a period of weeks or months. In photoreceptor-rich opercula, the lack of healthy retinal architecture, rounding of the edges of the operculum, the paucity of inner and outer segments, and the marked glial response are consistent with gliotic, chronically detached retinal tissue rather than retinal tissue avulsed from the fovea at the time of surgery.

The findings described in this study provide further evidence that a significant proportion of macular holes arise from avulsion of foveal neural tissue rather than from a pure foveal dehiscence without tissue loss or avulsion of only superficial inner-retinal glial tissue. The variation in the quantity of photoreceptors reflects a variable degree of foveal tissue loss during hole formation. The presence of numerous cones and the lack of rods in opercula is consistent with tissue from the central fovea, with the abundance of cone nuclei in some cases suggesting that these operculum contains tissue from deeper within the fovea.

The current study also supports some of Gass’s concepts regarding healthy foveal architecture and the mechanism of FTMH formation. In his most recent hypothesis\textsuperscript{25} based on the interpretation of previous studies of Hogan and Alvarado\textsuperscript{26} and Yamada,\textsuperscript{27} he emphasizes the presence of an inverted “cone” of Müller cells at the central foveola. These cells may provide important anatomical support for the healthy fovea, without which the underlying retinal tissue would be susceptible to disruption. They may also act as the primary site of xanthophyll storage within the healthy fovea and play an primary role in FTMH formation. In this process, migration and proliferation of the Müller cells (an aging process) into the prefoveolar vitreous cortex causes contraction of the vitreous cortex and further disruption of the Müller cell “cone.” This in turn results in a full-thickness foveal defect, centrifugal retraction of photoreceptors, and formation of a prehole opacity, which represents an avulsed segment of the Müller cell cone.

These hypotheses are consistent with the findings of our current and previous studies.\textsuperscript{17,18} The spectrum of tissue found in opercula reflect the degree of tissue avulsion from the Muller cell cone. At one end of the spectrum are the majority of opercula (accounting for ap-
proximately 80% of all lesions) formed by avulsion of tissue from around the superficial base of the Muller cell cone. This base consists of glial elements (found in 100% of opercula\textsuperscript{17,18}) and internal limiting membrane (found in 61%-100% of opercula\textsuperscript{17,18}) with or without occasional cone photoreceptors from the more superficial layers of the neuroretina at the fovea. In these specimens, it is impossible to distinguish histologically between glia originating from beneath (ie, from superficial inner retina) or superficial to the internal limiting membrane (ie, epiretinal). It is likely that in the majority, both inner-retinal and epiretinal glia are avulsed within the operculum. The presence of cone pedicles and second-order neurites in some opercula would be consistent with tissue avulsed from more superficial peripheral inner neurites in some opercula. Even accounting for photoreceptor degeneration within the free operculum tissue from around the superficial base of the Muller cell cone, with or without occasional photoreceptor elements. They also suggest that a more useful distinction would be between "true" and "pseudo" opercula. The worse anatomical results after surgery in cases with opercula containing photoreceptor tissue could be accounted for by (1) a larger central foveal neuroretinal defect before surgery and/or (2) a more pronounced glial response occurring around the edges of the hole as a result of greater tissue disruption at the fovea. Both factors would make the hole edges less likely to reappose and heal after surgery. This is also supported by previous histological studies\textsuperscript{28-30} on postmortem eyes with successfully treated FTMH showing variable residual retinal defects of between 16 µm and 230 µm, which may reflect a variable amount of foveal tissue loss. Although the studies of Funata et al\textsuperscript{28} and Madreperla et al\textsuperscript{29} showed effective reapposition of the hole edges, consistent with minimal or no foveal tissue loss, the study by Rosa et al\textsuperscript{30} showed a much larger residual foveal defect of 250 µm in a healed macular hole after surgery, consistent with significant loss of central foveal tissue. The overall primary surgical closure rate of 64% in the combined series of 30 eyes is lower than those reported by other series but is comparable with the 69% closure rate reported for stage 3 and stage 4 holes by Freeman et al\textsuperscript{13} in their prospective randomized study. The eyes included in the our 2 studies may represent one end of the spectrum of stage 3 holes, in which greater foveal tissue loss and more pronounced glial proliferation have occurred, resulting in a worse prognosis following surgery. Once anatomical closure has been achieved after reoperation, the lack of correlation between the presence of cones in opercula and a worse visual outcome is likely to reflect the small number of foveal cones avulsed in even the most photoreceptor-rich operculum. Even accounting for photoreceptor degeneration within the operculum before surgery, this number would still represent only a very small portion of the foveal cone population of 2500. In terms of visual function, the loss of such a small number of cones is probably of less consequence compared with the improvement of photoreceptor function in the rest of the macula following successful reapposition of photoreceptors to the RPE with resolution of the fluid cuff. In any case, these clinicopathological correlates should be interpreted with caution in view of the sample sizes of the studies, which do not permit detailed statistical analysis. Ideally, larger studies should be conducted to allow adequate stratification and analysis of the different types of opercula and their clinical outcomes.

Although the current study provides further insight into the spectrum of tissue avulsed from the central fovea, the primary mechanism initiating vitreofoveal traction in the early stages of hole formation remains unclear. Gass\textsuperscript{16,25} has proposed that glial proliferation and migration forms in the Muller cell cone into the prefoveal vitreous cortex (demonstrated histologically in prefoveal vitreous removed during surgery for impending holes\textsuperscript{31,32}) results in prefoveal vitreous contraction, and is the primary initiating mechanism. Others\textsuperscript{33} have suggested that different factors such as mechanical forces transmitted to the fovea in the presence of a partial vitreomacular separation with residual foveal tethering (as seen on optical coherence tomography\textsuperscript{33}) may be the primary mechanisms. Thus the glial activity around the fovea, as in opercula\textsuperscript{17,18} and epiretinal membranes\textsuperscript{34} found at the edges of FTMHs might represent a secondary healing response.17 Although in vitro and in vivo data suggest that glial membranes may contract as a result of cellular migration\textsuperscript{35} rather than cellular myofibroblastic contraction,\textsuperscript{36-38} it remains uncertain whether or not such forces are sufficient per se, or can contribute significantly to FTMH. Indeed, as much as 20% of apparently healthy asymptomatic eyes may show epiretinal glial membranes at postmortem examination without any evidence of epimacular traction.\textsuperscript{39-40} The nebulous epiretinal membranes observed in intraoperatively in some cases of FTMH\textsuperscript{41} may not necessarily be associated with significant traction. Interestingly, case 7, observed to progress from a pericentral stage 2 lesion to a stage 3 lesion, was associated with the formation of a photoreceptor-rich operculum. This suggests that significant foveal tissue may be avulsed in some cases, and that in such opercula, avulsion of a segment of glial membrane overlying an "occult" hole leads to avulsion of the adherent underlying foveal cones. In fact,
previous clinical data have suggested that pericentric stage 2 holes are associated with a worse prognosis and this may reflect the larger neuroretinal tissue defect in these cases. In summary, our data suggest that although some FTMHs are associated with minimal or no foveal tissue loss (ie, begin as an umbo dehiscence as described by Gass), others are associated with more extensive central tissue avulsion including neuroretinal tissue with cone photoreceptors, from the fovea. The correlation between the spectrum of histological disease and variation in clinical outcome following surgery requires further elucidation with studies of greater numbers of operators to allow more detailed statistical analysis.

Accepted for publication November 3, 2000.

This article was supported by grant 301 from the Guide Dogs for the Blind Association, London, England; grant 311 from the Stenger Bequest to the Special Trustees of Moorfields Eye Hospital, London, England (Dr Ezra, Gregor, and Aylward); grant EYO-1311 from the National Institutes of Health, Bethesda, Md (Dr Milam); the Foundation Fighting Blindness, Hunt Valley, Md (Dr Milam); and the Paul and Evania Bell Mackall Foundation Trust, New York (Dr Milam).

The authors would like to thank Paulette Brunner from the W. M. Keck Centre for Advanced Studies of Neural Signaling, University of Washington, Seattle, and Glen MacDonald of the Virginia Merrill Hearing Research Center and the Center on Human Development and Disability, University of Washington, Seattle, for their help and advice on confocal image analysis. We also thank professors A. C. Bird, MD, FRCS, FRCOphth, and W. R. Lee, FRCPath, FRCOphth, for their contributions to the discussion. The authors also thank B. K. Fung, PhD, from the Department of Ophthalmology, Jules Stein Institute, University of California Los Angeles, who provided the anti-PDE5 antibody; R. S. Molday, PhD, from the Department of Biochemistry and Molecular Biology, University of British Columbia, Vancouver, who provided the antihodophilin (Rho 4D2); and J. C. Saari, PhD, from the Department of Ophthalmology, University of Washington, Seattle, who provided the anticlecular retinaldehyde binding protein (Immuno globulin B3).

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