Histopathologic and Ultrastructural Findings of Surgically Excised Choroidal Neovascularization

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Objective: To study the histologic and ultrastructural features of surgically excised choroidal neovascularization (CNV) from patients who had undergone submacular surgery.

Materials and Methods: Voluntarily submitted surgically excised CNV specimens from a subset of specimens obtained by the Submacular Surgery Trials Research Group between January 1, 1994, and December 31, 1996, were available for this study. The specimens were routinely processed for transmission electron microscopic examination. The largest horizontal and vertical dimensions, cellular and extracellular constituents, and relationship of the CNV to the neurosensory retina and retinal pigment epithelium (RPE) were recorded.

Results: Seventy-eight surgical specimens were obtained from 160 patients treated surgically in Submacular Surgery Trials centers. Sixty-one (78%) were from patients with age-related macular degeneration (ARMD) and 17 (22%) were from patients with ocular histoplasmosis syndrome or idiopathic causes (hereafter referred to as the non-ARMD group). The histologic diagnosis was fibrovascular tissue, fibrocellular tissue, or hemorrhage in all cases. Vascular endothelium and RPE were the most common constituents of the CNV. Basal laminar deposit was only present in CNV from patients with ARMD. Age-related macular degeneration specimens were larger (mean ± SD, 2042 ± 1175 [± 320] µm vs 1498 ± 792 [± 166] µm) and were more likely to have a sub-RPE (beneath the RPE) component than non-ARMD specimens.

Conclusions: All evaluated surgically excised CNV specimens in this study from patients enrolled in the Submacular Surgery Trials consisted of fibrovascular tissue, fibrocellular tissue, or hemorrhage. Surgically excised CNV associated with ARMD in this series was larger and often was located beneath the RPE compared with non-ARMD CNV, although fewer than half of all the specimens could be oriented by topographic relationship to the RPE.


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OPHTHALMIC surgeons have been performing submacular surgery to remove subfoveal choroidal neovascularization (CNV) in some eyes with age-related macular degeneration (ARMD), ocular histoplasmosis syndrome (OHS), myopia, punctate inner choroiditis, and idiopathic causes.1-3 The Submacular Surgery Trials (SST) Research Group was formed to conduct randomized clinical trials to evaluate whether submacular surgery is successful in stabilizing or improving visual acuity when compared with observation in patients with ARMD who have new subfoveal CNV (designated as group N), recurrent subfoveal CNV (group R), or large subfoveal subretinal hemorrhages (group B).4 The SST was also designed to evaluate the efficacy of surgery in patients with subfoveal CNV due to OHS or idiopathic causes (group H, hereafter referred to as the non-ARMD group). The SST investigators enrolled patients in a pilot study to evaluate the safety of this procedure for these groups. Surgically excised tissue was submitted on a voluntary basis for histopathologic examination to confirm the clinical diagnosis of CNV and to identify the tissue that was removed.

RESULTS

Of a total of 131 patients randomized to surgery during the 3-year study period, 78 specimens were submitted to and evaluated by both reviewers (H.E.G. and W.R.G.). Based on the clinical diagnosis, the specimens were divided into 2 groups—those with ARMD and those without ARMD (OHS or idiopathic). Sixty-one specimens (78%) were received from a total of 108 patients with ARMD and 17 specimens (22%) were received from a total of 23 patients without ARMD. Twenty-
MATERIALS AND METHODS

Between January 1, 1994, and December 31, 1996, 15 SST surgeons at 10 participating institutions and practices had voluntarily submitted to our laboratories surgically excised CNV tissue from patients enrolled in the SST pilot study for histopathologic examination. An informed consent form was signed by each patient prior to surgery. Patient age, sex, right or left eye, and presence or absence of ARMD were recorded by the submitting surgeon. No information was provided on whether the specimen came from group N, R, or B patients. The tissue was placed in 2.5% glutaraldehyde solution and processed for transmission electron microscopic examination. The specimens were postfixed with 0.1-mol/L cacodylate buffer and 1% osmium tetroxide solutions. Standard dehydration of the specimens was performed and the specimens were embedded in epoxy resin, sectioned, and stained with toluidine blue. Sections through the center of each specimen were evaluated for greatest linear CNV dimension and thickness, and for configuration of the CNV with regard to the neurosensory retina and retinal pigment epithelium (RPE). The diameter and thickness measurements were made with a reticule and standardized light microscope (Carl Zeiss Ltd, Oberkochen, Germany). Means of the greatest linear dimensions and thicknesses measured by the 2 independent observers (H.E.G. and W.R.G.) were recorded. The CNV configuration was listed as sub-RPE, between the neurosensory retina and RPE (subretinal), both sub-RPE and subretinal (combined), or unclassifiable.3 The orientation was accomplished by comparing the CNV with surrounding landmarks, such as photoreceptors, Bruch membrane, and basal laminar deposit (BLD). Semi-thin (0.1-µm) sections were cut and stained with uranyl acetate–lead citrate for transmission electron microscopy. A minimum of 20 micrographs per specimen was examined. Previously reported criteria6,7 were used for identification of specific cell types including RPE, vascular endothelium, fibrocytes, macrophages, myofibroblasts, glial cells, photoreceptors, lymphocytes, and extracellular components including collagen, fibrin, BLD, basal linear deposit, and fragments of Bruch membrane. The final diagnosis (CNV, fibrocellular tissue without CNV, hemorrhage, or other) was noted for each case.

seven men, 31 women, 26 right eyes, and 34 left eyes were in the ARMD group (laterality was unspecified in 1 eye) compared with 7 men, 8 women, 5 right eyes, and 8 left eyes in the non-ARMD group (laterality was unspecified in 4 eyes). The final diagnosis in the ARMD and non-ARMD groups, respectively, were as follows: CNV, 54 (89%) and 15 (88%); fibrocellular tissue without CNV, 5 (8%) and 2 (12%); and hemorrhage without CNV or fibrocellular tissue, 2 (3%) and 0. The cellular and extracellular components of the specimens are given in the Table. Vascular endothelium (Figure 1) was present in 88% of both the ARMD and non-ARMD groups. Retinal pigment epithelium (Figure 2) was present in 84%
and 94% of the ARMD and non-ARMD groups, respectively. Fibrocytes and macrophages were present in more than 50% of specimens from both groups combined. Collagen and fibrin were present in more than 50% of each group. Basal laminar deposit (Figure 3) was only present in the ARMD group and in 87% of those specimens. Basal linear deposit was present in 8% of the ARMD specimens and only present in specimens that contained basal laminar deposit (Figure 3). The mean ± SD diameter and thickness of the CNV from the ARMD group was 2042 ± 1175 µm × 320 ± 185 µm compared with 1498 ± 792 µm × 227 ± 166 µm for the CNV from the non-ARMD group. The configuration could be determined in 32 specimens in the ARMD group and in 5 in the non-ARMD group. In approximately 50% of the specimens, the configuration could not be determined owing to folding and lack of landmarks. In the ARMD group, 3 specimens were sub-RPE (Figure 4), 16 were subretinal (Figure 5), and 13 were combined (Figure 6). All 5 specimens in the non-ARMD group were subretinal.

**COMMENT**

The pathologic findings of surgically removed CNV lesions from patients with ARMD, OHS, and idiopathic causes have been previously reported. Although the previously reported cases represent specimens from selective surgical cases, they are all examples of surgically excised CNV. These previous studies have shown that the most common cellular components in the specimens are vascular endothelium and RPE, each of which have been present in approximately 85% of specimens. Other common cellular constituents that are present in at least 50% of specimens are fibrocytes and macrophages. The specimens described in our study are consistent with previous studies, as vascular endo-
thelium and RPE were present in approximately 90% of these specimens, and fibrocytes and macrophages were present in more than 50% of all specimens. Common extracellular components in these specimens included collagen and fibrin, as reported in previous studies. The presence of these constituents supports the concept that CNV is similar to granulation tissue proliferation in a wound-repair response.

This study differs from previous reports in that (1) it represents a collaborative effort among multiple surgeons and institutions, (2) basal linear deposit was present in some specimens, and (3) an attempt was made to classify the topography, greatest linear dimension, and thickness of the specimens. Two experienced ophthalmic pathologists (H.E.G. and W.R.G.) examined specimens submitted by 15 surgeons from 10 different institutions. Previous reports concentrated on surgical specimens obtained at a single institution. There are many similarities between the current study and the study of 123 surgically excised CNV specimens previously reported by Grossniklaus and coworkers. Both studies examined 78 specimens using transmission electron microscopy. Vascular endothelium was present in 88% (68 of 78 specimens) in the current study and 81% of the specimens in the previous study. The presence of vascular endothelium may have been underestimated in the previous study, since 35% of the specimens were not examined by transmission electron microscopy. There were 9 specimens in the current study that did not exhibit vascular channels, 2 specimens of which consisted of hemorrhage. The latter 2 may have been from group B patients (those with large subfoveal subretinal hemorrhages) and the surgeon may intentionally not have removed the CNV. It is possible that this avascularity represents sampling error in the pathology laboratory, although sections through the thickest portion of the specimen were examined histologically and at least 20 transmission electron micrographs covering representative portions of each specimen were studied. The vascular endothelium may have involuted, although this is unlikely since fluorescein angiography was performed and identified vascular channels prior to surgery. Alternatively, the vascular component of the lesion may not have been completely excised, which may lead to recurrent CNV in some instances.

Basal laminar deposit has been noted in previously examined surgically excised CNV specimens, and it is found almost exclusively in patients with ARMD. Although wide-spaced collagen may be found in various intraocular tissues, the presence of granular electron-dense material and wide-spaced collagen between the plasma membrane and basement membrane of the RPE forming BLD is characterized found in eyes with ARMD. Basal laminar deposit was present in 54.7% of 760 whole autopsy eye specimens with ARMD and in 56% of surgically excised CNV specimens from patients with ARMD in the other large series. We found BLD in 87% of the ARMD group and in none of the non-ARMD group. These findings confirm those of the previous series, in which all specimens with BLD but 1 were from patients with ARMD. Thus, the presence of BLD is highly suggestive of ARMD. Basal linear deposit has not previously been reported in surgically excised CNV specimens, although it has been noted in autopsy eyes with ARMD. We found basal linear deposit in 5 specimens, all of which had ARMD. Basal laminar deposit consists of electron-dense material with interspersed wide-spaced collagen located between the plasma membrane and basement membrane of the RPE, whereas basal linear deposit consists of vesicular material located external to the basement membrane of the RPE (Figure 3). Our current study and that of Green and Enger suggest that basal linear deposit is specific to ARMD. Examination of the specimens in this series and in series using autopsy eyes shows that BLD appears to be adherent to the RPE, whereas basal linear deposit often is not adherent to the RPE.

The previous large series of surgically excised CNV specimens attempted to classify the specimens as sub-RPE or subretinal. This may be theoretically important for patient selection for surgical removal of the CNV, whether the patient does or does not have ARMD, because the underlying native RPE would remain intact after removal of subretinal CNV, whereas the native RPE would be removed after removal of sub-RPE CNV. We were able to orient 37 specimens as to sub-RPE, subretinal, or combined configuration. The native RPE could be identified as such by the presence of BLDs and basal linear deposits. We were unable to orient approximately 50% of the specimens because of folding and/or the lack of landmarks. Submission of the specimen on a sponge with an accompanying diagram assisted in orientation in some cases. The ARMD group contained 3 sub-RPE, 16 subretinal, and 13 combined configurations of the specimens, whereas all 5 of the specimens in the non-ARMD group were subretinal. Thus, 16 of the 32 specimens that could be oriented in the ARMD group had a sub-RPE component (ie, they were either sub-RPE or combined). This may be owing to the intra–Bruch membrane growth pattern of CNV in eyes with ARMD, reflecting the diffuse nature of the disease. The prominent horizontal and vertical intra–Bruch membrane component associated with ARMD may also explain why the measurements of the CNV in the ARMD group (mean, 2042 × 329 μm) were larger than in the non-ARMD group (mean, 1498 × 227 μm). It is possible that there were even more specimens from the ARMD group with a sub-RPE component, owing to exclusion of only the subretinal component of 2-component (combined) CNV or incomplete specimen sampling. By SST clinical criteria, CNV from the ARMD group had to be larger than from the non-ARMD group to be eligible for removal. The fact that all 5 specimens from the non-ARMD group were subretinal indicates a propensity for non-ARMD CNV to exhibit a subretinal rather than a sub-RPE growth pattern.

Our study confirms high accuracy in the clinical diagnosis of CNV, since 69 (approximately 90%) of 78 specimens were composed of fibrovascular tissue and the remaining 9 (approximately 10%) of 78 were made up of tissue that was probably surrounding and associated with CNV. This is conceivable since the surgeons were not required to remove the CNV in group H patients (those with subfoveal CNV due to OHS or idiopathic causes) if they did not see CNV after evacuation of blood with tissue plas-
minogen activator. Basal laminar deposit was found in almost 90% (53 of 61) of specimens from patients with ARMD. The surgically excised CNV associated with ARMD is generally thicker and has a greater diameter than CNV associated with OHS or idiopathic causes. Photoreceptors were present in 28 (approximately 50%) of the 61 specimens from the ARMD group and in 3 (20%) of the 17 specimens in the non-ARMD group. This may be related to overall topographical orientation of the CNV, which tended to be subretinal in the non-ARMD group and sub-RPE in the ARMD group. Subretinal CNV theoretically is easier to remove than sub-RPE CNV without removal of adjacent structures such as photoreceptors.19 There is some evidence that topographical orientation may be predicted by preoperative fundus examination, since the histological orientation matched the clinically predicted orientation in 9 of 10 eyes in one study.5 If the topography correlates with long-term visual outcome, and surgery is preferable to no surgery, then patients may be selectively advised about postoperative visual potential after removal of submacular CNV.

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