mfVEPs are generated in the striate cortex (V1), and that topographic analysis of mfVEPs may be able to distinguish striate from extrastriate lesions.[17] Recently, a patient was reported with a congruous quadrantic defect and a loss of the fMRI signal in the corresponding extrastriate area, with normal signals in V1.[21]

Some investigators[1–5,39–44] have successfully recorded VEPs in cases with early compressive lesions of the optic tract, especially in the chiasmal or retrochiasmal regions. They reported that the VEP recordings were a sensitive and objective method for examining patients with visual field loss using full- or hemi-field stimulation or single or multichannel recordings. Flanagan and associates[40] reported that VEPs recorded with multichannel electrodes were useful and even more sensitive for detecting compressive lesions than subjective perimetry. In contrast, Maitland and associates[1] concluded that it was possible to lateralize the brain lesion, but not to predict the site of the lesion within the hemisphere. They also stated that VEP analysis is of only limited value in assessing patients with homonymous or bitemporal hemianopias.

On the basis of these findings, advanced mfVEP techniques with the dartboard pattern stimuli and recordings using bipolar occipital cross electrodes, as used in this study, enhanced the sensitivity of the VEPs in detecting postchiasmal optic nerve damage. Our findings clearly illustrate that the responses that were summed within each of the four quadrants were well-correlated with subjective perimetry in some patients. Moreover, this method was sensitive enough to detect visual field impairments even with macular sparing (Cases 7 and 10). Bradnam and associates[5] reported that a 90-minute check size used in their study, which is optimal for peripheral areas of the visual field, was minimally affected by central sparing of their VEP recordings. Although we are not certain whether full-field VEPs and mfVEPs are measuring the same thing, the dartboard pattern stimulus used in our study should stimulate the receptive fields optimally at each retinal eccentricity.[23] Therefore, they could improve the detection of incomplete hemi-field and quadrantic visual field defects and would be minimally affected by macular sparing. Further application of this technique on the large number of patients with visual field defects in the central area will be helpful in establishing whether this objective visual field testing can be used in patients with or without macular sparing.

In conclusion, there are some cases with lesions in the visual centers whose mfVEPs are not concordant with the subjective visual field. Two patients with this discordance had their hemianopsia recovered completely and three did not. We suggest that the mfVEPs originated from the primary area (area 17/V1). If the visual function arose from damage by a lesion in the higher visual centers, it is possible for the subjectively determined visual field to be dissociated from the mfVEPs. This means that damage of the visual pathway from the retina to the primary visual cortex (area 17/V1) would decrease the mfVEPs, but the damage of the higher visual cortex could lead to a discordance between the subjective visual field test and the mfVEPs. We suggest that the primary visual cortex was not affected severely in cases 7 and 10, and the transient tissue edema or circulation disturbance after neurosurgery was responsible for the subjectively determined visual field defects. In cases 6, 8, and 9, the brain tumor was not located in the primary visual cortex, and the mfVEPs could be recorded (V1). The mfVEPs even with conventional visual field defect can be interpreted to show that the primary visual cortex is not affected. In these cases, the visual field defect that is detected by conventional subjective visual field testing may recover. However, the recovery of visual field in the two cases may be associated with the cause of the visual field defect such as edema. Further investigation will be necessary to elucidate the mechanism of the recovery.[31]

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INFLUENCE OF VERTEPORFIN PHOTODYNAMIC THERAPY ON INFLAMMATION IN HUMAN CHOROIDAL NEOVASCULAR MEMBRANES SECONDARY TO AGE-RELATED MACULAR DEGENERATION

AQ: 1

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Purpose: To examine the short- and long-term consequences of verteporfin photodynamic therapy (PDT) on inflammation with regard to infiltration of macrophages and leukocytes and expression of thy-1 in human choroidal neovascularization membranes (CNV) secondary to age-related macular degeneration (AMD).

Methods: Retrospective review of an interventional case series of 43 patients who underwent removal of CNV. Twenty patients were treated with PDT 3 to 246 days preoperatively. Twenty-three CNV without previous treatment were used as control. CNV were stained for CD34, CD105, cytokeratin18, Ki-67, thy-1, an endothelial cell glycoprotein known to be upregulated only by inflammatory cytokines, CD68 (macrophages), and CD45 (common leukocyte antigen).

Results: Specimens treated by PDT 3 days previously showed significantly reduced endothelial thy-1 expression ($P = 0.008$), leukocyte ($P = 0.04$) and macrophage ($P = 0.0063$) infiltration, and proliferative activity ($P = 0.02$) compared to control CNV. Specimens at longer intervals after PDT, in contrast, disclosed a significantly increased expression of thy-1 ($P = 0.004$), infiltration with leukocytes ($P = 0.044$) and macrophages ($P = 0.01$), and proliferative activity ($P = 0.03$) compared to CNV excised 3 days after PDT.

Conclusions: The rebound effect after PDT seems to be based on an inflammatory response that contributes to enhanced proliferation. These data support the need for an anti-inflammatory therapy as adjuvant to PDT.

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Age-related macular degeneration (AMD) is the leading cause of legal blindness in patients older than 60 years in the Western world.[1] Neovascular AMD with the development of a choroidal neovascularization (CNV) in the macular area accounts for 80% of the severe loss of visual acuity due to AMD.[2,3]

Numerous treatment modes have been attempted to destroy the pathologic blood vessels. PDT with verteporfin (Visudyne, Novartis AG, Buelach, Switzerland) is an effective treatment for choroidal neovascularization secondary to AMD in certain circumstances.[4–10] A recurrence rate of approximately 90% within 3 months and a retreatment rate of 5.6 in 2 years was reported to limit its effectiveness.[4,5]

The Submacular Surgery Trials (SST) demonstrated that patients do not benefit from surgical extraction.[11] Surgery, especially if combined with macular translocation, might be, however, a treatment option for those who do not sufficiently benefit from prior PDT (Johnson MW; ARVO meeting, 2005; abstract; Suesskind D; ARVO meeting, 2006; abstract).[12,13]

The evolution of antiangiogenic drugs, however, is changing the treatment philosophy to a more modifying rather than destructive approach.[14–19] Additionally, antiangiogenic or angiostatic treatment modalities may be used also as adjuvants to PDT to overcome its limitations and to increase its efficacy.[14,15,20,21] In experimental CNV, a greater reduction in angiographic leakage was achieved when PDT was combined with intravitreal injection of ranibizumab.[22] Clinical data with different adjuvants and variable treatment regimens suggest a clinical improvement.[14,15,20,21]

Herein, we present the clinicopathologic evaluation of cases treated with verteporfin PDT before the surgery. This analysis focuses on the inflammatory and proliferative activity.

The level of vascularization was determined by immunodetection of CD34 and CD105. CD34 was used as a panendothelial marker.[23] CD105 has a pronounced expression in activated endothelial cells that are the constituents of vessels in tissues undergoing angiogenesis.[24] Thy-1 is a cell surface marker expressed on vascular endothelial cells which is upregulated by inflammatory cytokines interleukin (IL)1β and tumor necrosis factor (TNF)-α but remains unaffected by growth factors such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor, and platelet derived growth factor.[25] Ki-67 is a cell-cycle associated antigen that is only present in proliferating cells, so it is used as a nuclear marker of cell proliferation.[26] Macrophages and leukocytes were detected by immunostaining for CD68 and CD45, respectively.[27–29] Cytokeratin18 was used to detect retinal pigment epithelium (RPE) cells.[27]

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Methods

Subjects and Treatments

We retrospectively reviewed 43 eyes of 43 consecutive patients with AMD who were treated by surgery for CNV extraction at four distinct surgical sites between 1997 and 2005. In 20 of these patients, surgery was performed after verteporfin PDT. Clinical characteristics of the patients treated with PDT preoperatively are summarized in Table 1. Fourteen female and nine male patients (mean age ± SD: 76.17 ± 5.1, range: 64–88) without any preoperative treatment composed the control group. In addition to the complete ophthalmologic examination, for patients receiving verteporfin PDT, stereoscopic fluorescein angiography was performed before the treatment and thereafter perioperatively. CNV were classified according to the guidelines of the Treatment of Age-related Macular Degeneration with Photodynamic Therapy (TAP) and Verteporfin In Photodynamic Therapy (VIP) studies.[4–9] Therapy options, including observation, conventional thermal laser photocoagulation, PDT retreatment, intravitreal triamcinolone injection either alone or with PDT, macular translocation with 360° retinotomy, and CNV membrane extraction, were discussed with the patients. Surgical intervention and removal of the CNV were offered when 1) visual acuity was below 20/200 being the minimum visual acuity to recommend the first PDT according to the TAP Studies,[4,5] 2) visual deterioration progressed after initial PDT, 3) the patient refused retreatment with PDT due to continuous visual deterioration in the fellow eye in spite of PDT, and 4) retreatment with PDT was impossible due to recurrent or massive submacular hemorrhage. Four eyes underwent CNV extraction 3 days after PDT. Three of these four eyes had subfoveal classic CNV. The visual acuity of these three eyes was between 4/200 and 10/200, less than the 20/200 that was the lowest permissible visual acuity for PDT in the TAP Investigation.[4,5] The fourth patient with predominantly classic CNV had experienced decrease in visual acuity from 60/200 to

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Table 1. Clinical Characteristics of Patients Treated With PDT Before Surgical Removal of the CNV

Case	Eye	Age, yr/Sex	CNV type	No. of PDT	Time to Surgery From the First PDT/Last PDT, d
1	L	76/M	Classic	1	3
2	R	78/F	Classic	1	3
3	L	54/M	Pred. classic	2	113/3
4	L	84/M	Classic	1	3
5	R	74/F	Occult	1	21
6	L	83/M	Classic	1	34
7	L	85/F	Classic	1	37
8	R	73/F	Occult	3	208/138/40
9	L	79/M	Classic	1	55
10	R	80/F	Classic	2	172/69
11	L	77/M	Min. classic	1	84
12	R	93/M	Classic	2	95
13	L	87/M	Pred. classic	1	108
14	R	71/M	Classic	1	112
15	R	70/F	Classic	2	151/132
16	L	77/M	Classic	3	329/245/147
17	L	72/M	Pred. classic	2	232/156
18	R	74/F	Hemorrhagic	1	246
19	L	73/F	Classic	4	677/558/467/383
20	L	77/F	Pred. classic	7	Unknown*/772/655

* Time of first to fifth PDT session unknown.
PDT = photodynamic therapy; CNV = choroidal neovascular membrane; min = minimally; pred = predominantly.

10/160 accompanied by leakage in fluorescein angiography 3 months after the first PDT. He opted to proceed with macular surgery rather than PDT retreatment. PDT 3 days before surgery was intended to reduce the risk of bleeding at the time of surgical extraction in all cases.

Each patient gave written informed consent after the experimental nature of the procedure and the risk and benefits of all alternative therapy options had been fully explained. The study followed the guidelines of the Declaration of Helsinki as revised in Tokyo and Venice. The study and the histologic analysis of the specimens were approved by the local Institutional Review Board.

Tissue Preparation and Immunohistology

Within minutes after surgery, excised CNV membranes were fixed in 3.7% formalin and embedded in paraffin. Each specimen was serially mounted on poly-L-lysine coated glass slides (Dako, Glostrup, Denmark) for immunohistochemical staining.

After serial sections were deparaffinized and rehydrated, antigen retrieval was accomplished by proteolytic digestion with 0.5% protease XXIV (Sigma, St. Louis, MO) for thy-1 and cytokeratin 18. For Ki-67, CD34, CD105, CD45, and CD68, the method of antigen retrieval was heat treatment in citrate buffer (0.01 moles/L, pH: 6.0) in a pressure cooker.

Immunohistochemical staining with the primary antibodies specific for human CD105 (mouse, MAb, Clone SN6 h, Dako), CD34 (mouse, MAb, Immunotech, Hamburg, Germany), Ki-67 (mouse, MAb, Clone Ki-S5, Dako), cytokeratin 18 (mouse, MAb, Progen, Heidelberg, Germany), and CD45 (mouse, monoclonal, Dako) was performed using the horseradish peroxidase as previously described.[30]

Immunohistochemical staining with the primary antibodies specific for human thy-1 (a purified mouse anti-CD90 antibody, BD Pharmingen, BD Biosciences, San Jose, CA) and CD68 (a mouse monoclonal antihuman, Dako) was performed by the alkaline-phosphatase method as we previously described.[30]

Hematoxylin III according to Gill (Merck, Darmstadt, Germany) was used as a counterstain. For negative controls, the primary antibodies were substituted by appropriate normal sera or omitted.

Analysis

Serial sections from a specimen were analyzed independently by two masked observers (O.T., S.G.) by light microscopy.

Vascularization was evaluated by counting the number of CD34 and CD105 positive vascular-like patterns. Thy-1 expression was evaluated as the percentage of thy-1 expressing vessels in the overall vascularization of each membrane.

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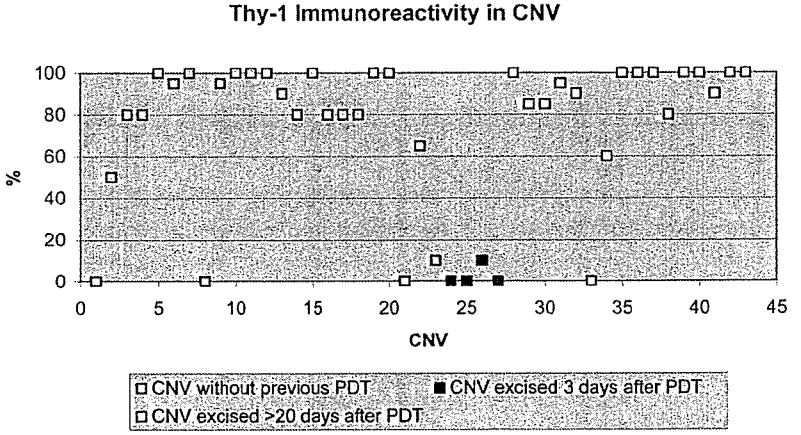

Fig. 1. Graph showing the percentage of thy-1 immunoreactive vessels in choroidal neovascularization (CNV) membranes without previous photodynamic therapy (PDT), extracted 3 days or longer than 20 days after PDT.

Each specimen was documented under ×50 magnification with a Zeiss microscope (Axioskop, Zeiss, Oberkochen, FRG) connected to a digital camera (HC-300Z, Fujix, Japan). Area of each specimen was measured using the appropriate hardware and software (AxioVision, Version 3.1, Carl Zeiss, Göttingen, Germany).

All Ki-67 positive nuclei, CD45 immunoreactive leukocytes, and CD68 immunopositive macrophages were counted in each specimen. Rate of infiltration of CNV by leukocytes and macrophages was determined separately by the ratio of total number of leukocytes and macrophages to the total area of the membrane (mm^2), respectively. Proliferative activity in each specimen was determined quantitatively by calculating the number of Ki-67 positive nuclei in 1 mm^2 area of CNV.

Intensity of infiltration of leukocytes and macrophages, proliferative activity, frequency of thy-1 expression, and the surface area of the CNV treated with PDT preoperatively were compared with CNV without any kind of previous treatment before surgery using the Mann–Whitney U test. $P \leq 0.05$ was considered significant.

Results

Immunohistopathologic findings are summarized in F1-3 Figures 1–3.

Immunohistopathologic Findings of Cd34, Cd105, and Thy-1 Expression

CNV without any previous treatment (n = 23) disclosed healthy EC expressing CD34 and CD105 F4 (Figure 4, top left). In 34.8% (8 of 23) of the samples thy-1 was expressed in all vessels. In the other specimens the percentage varied from 50%–95% (median: 80%, range: 0–100) (Figures 1 and 4, top right) whereas three CNV (13%) did not display thy-1 at all.

In CNV extracted 3 days after PDT (n = 4), immunohistology with CD34 and CD105 disclosed mostly collapsed vessels. Morphologically, the EC lining the vessels appeared severely damaged (Figure 4, middle left). Thy-1 expression was detected in only one of the specimens in 10% of the vessels (median: 0, range: 0–10) (Figures 1 and 4, middle right). Percentage of thy-1 expressing vessels was significantly less in CNV excised 3 days after PDT when compared to CNV without any pretreatment ($P = 0.0079$).

In CNV extracted at longer post-PDT intervals (n = 16), CD34 and CD105 immunoreactive vessels were detected in all but one membrane. The vessels were all patent and lined with endothelial cells displaying prominent nuclei (Figure 4, bottom left). Thy-1 was expressed in all vascularized specimens, but again in a varying percentage of the vessels (range: 0%–100%, median: 97.5). All vessels expressed thy-1 in eight of the specimens and in only one specimen the frequency of thy-1 immunoreactive vessels was as low as 60% (Figure 1). The frequency of thy-1 expressing vessels was considerably higher in CNV extracted at longer post-PDT intervals in comparison to CNV extracted 3 days after PDT ($P = 0.0038$, Figure 4, bottom right) but comparable to its frequency in CNV without previous therapy ($P = 0.16$).

Histopathologic Findings of CD68 Expression and CD45 Expression

In control group without any pretreatment (n = 23), specimens were mostly inflammatory active. CD68 immunoreactive macrophages were detected in all CNV (median: 918.72, range: 183.94–7955.8 macrophages/

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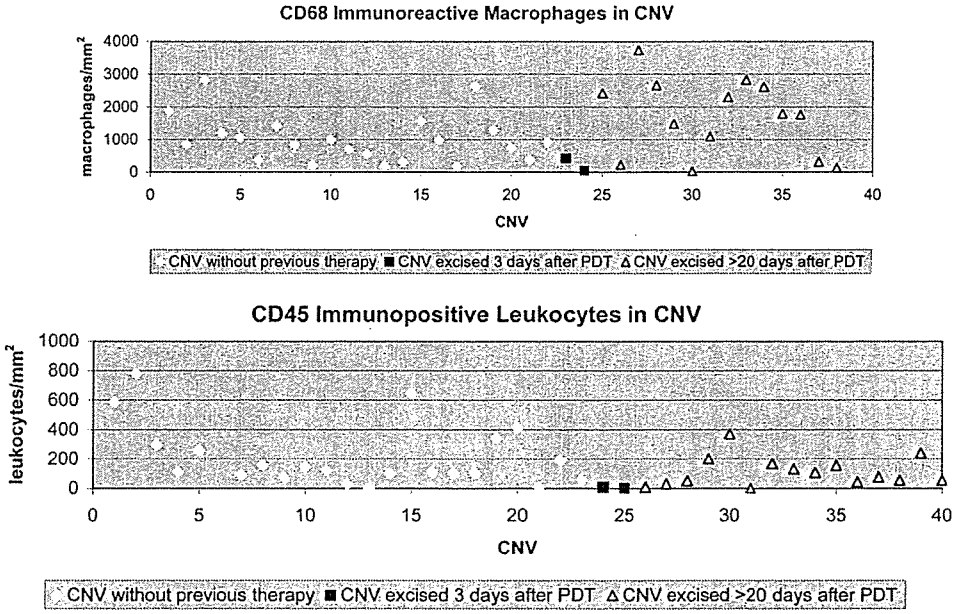

Fig. 2. Graphs showing number of macrophages (top) and leukocytes (bottom) in 1 mm^2 area of a choroidal neovascularization (CNV) membrane without previous photodynamic therapy (PDT), extracted 3 days or longer than 20 days after PDT. Infiltration of CNV by macrophages and leukocytes was determined quantitatively by the ratio of total number of macrophages or leukocytes to the area of the membrane (mm^2), respectively. A specimen with 6,296,082 macrophages/mm^2 in the subgroup of CNV without previous therapy and two samples with 21477,416 macrophages/mm^2 and 6,296,082 macrophages/mm^2 in the subgroup of CNV excised longer than 20 days after PDT were not plotted on the graph to show the rest of the values with a bigger scale (Top). Likely, a CNV without previous PDT which has 2,746.3 leukocytes/mm^2 was not plotted on the graph (Bottom).

F5 mm^2) (Figure 2, top, Figure 5, top left). Macrophages were present within the cytokeratin-18 expressing RPE layer, perivascular area, and stroma in 20 (86.9%), 5 (21.7%), and 21 (91.3%) of the specimens, respectively. CD45 immunoreactive leukocytes were found to be present in all but one CNV (95.6%). Number of infiltrating leukocytes varied between the membranes (range: 0 –2746 leukocytes/mm^2, median: 122.087) (Figure 2, bottom, Figure 5, top right).

Infiltration of leukocytes and macrophages could be evaluated in only two of the specimens excised 3 days after PDT due to lack of serial sections from the other two membranes. Both membranes were hypocellular and inflammatory inactive (Figure 5, middle left and right). A few macrophages were detected in stroma and RPE layer of both of the specimens. The density of macrophages (median: 235.94, range: 50.31–421.58 macrophages/mm^2, Figure 2, top) and leukocytes (range: 0–7.46, median: 3.73) (Figure 2, bottom, Figure 5, middle right) was found to be significantly lower than in the CNV without any previous therapy ($P = 0.0063$, $P = 0.04$, respectively).

At longer post PDT intervals, macrophage infiltration was detected within the RPE cell layer, perivas-

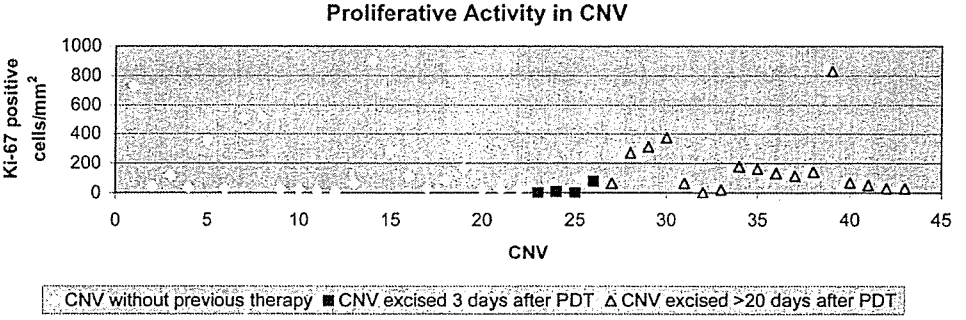

Fig. 3. Graph showing number of Ki-67 positive proliferating cells in 1 mm^2 area of a choroidal neovascularization (CNV) membrane without previous photodynamic therapy (PDT), extracted 3 days and longer than 20 days after PDT. Proliferative activity was determined quantitatively by the ratio of total number of proliferating cells to the area of the membrane (mm^2), respectively. Only a CNV without previous PDT which has 2,265 proliferating cells/mm^2 was not plotted on the graph to show the rest of the values with a bigger scale.

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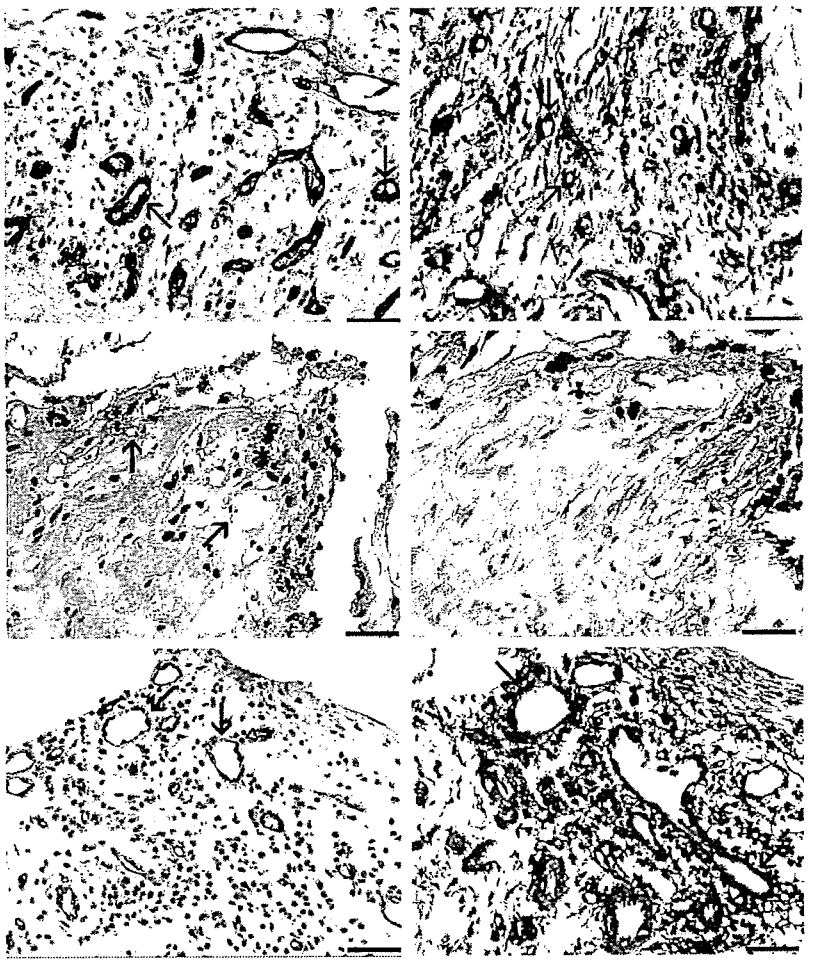

Fig. 4. Photomicrographs of surgically excised choroidal neovascularization (CNV) probed with antibodies against endothelial cell markers CD34 (top and bottom left), CD105 (middle left), and thy-1 (top, middle, and bottom right). CD34 and CD105 immunoreactivities were detected with 3-diaminobenzidine resulting in a brown chromogen whereas thy-1 was labeled with red chromogen. Hematoxylin was used as counterstain. Brown chromogen (arrows) can be distinguished from the melanin granula (asterisk) contained in pigmented cells (middle left). CNV without previous PDT disclosed vessels lined with healthy endothelial cells (top left, arrows) which are shown to be thy-1 immunopositive (top right, arrows) in the serial section. Severely damaged endothelial cells (middle left) in CNV excised 3 days after PDT (Case 1 in Table 1) are not immunoreactive for thy-1 (middle right) in the serial section of the same membrane. Serial sections of the CNV excised 55 days after PDT (Case 9 in Table 1) display vessels lined with healthy endothelial cells (bottom left, arrows) which are thy-1 immunoreactive (bottom right, arrows). Scale bars: 50 μm.

cular layer, and stroma of 75% (12 of 16), 6.25 (1 of 16), and 87.5% (14 of 16) of the specimens, respectively. The density of infiltrating macrophages (median: 2044.035, range: 26.01–21477.416 macrophages/mm^2) was significantly higher than in the CNV excised 3 days after PDT ($P = 0.01$). The level of macrophage infiltration in these CNV was considerably higher than that in CNV without previous treatment even though the result did not reach significance ($P = 0.07$) (Figure 2, top, Figure 5, bottom left). Infiltration of leukocytes was evaluated in 15 of the specimens. CD45 expressing leukocytes were absent only in one membrane (6.67%). The number of infiltrating leukocytes increased also significantly (range: 0–371.79 nuclei/mm^2, median: 76.78) in comparison to the CNV excised 3 days after PDT ($P = 0.04$). The leukocytic infiltration in these CNV was comparable to the CNV without previous treatment ($P = 0.1$) (Figure 2, bottom, Figure 5, bottom right).

Histopathologic Findings of Ki-67 Expression and Evaluation of Proliferative Activity

In CNV membranes of the control group, Ki-67 positive proliferating cells were detected in all specimens. Most of the Ki-67 positive cells appeared to belong to cells within the stroma. Proliferative activity ranged between 6.68 nuclei/mm^2 and 2265 nuclei/mm^2 (median: 62.332) (Figures 3 and 6, top). F6

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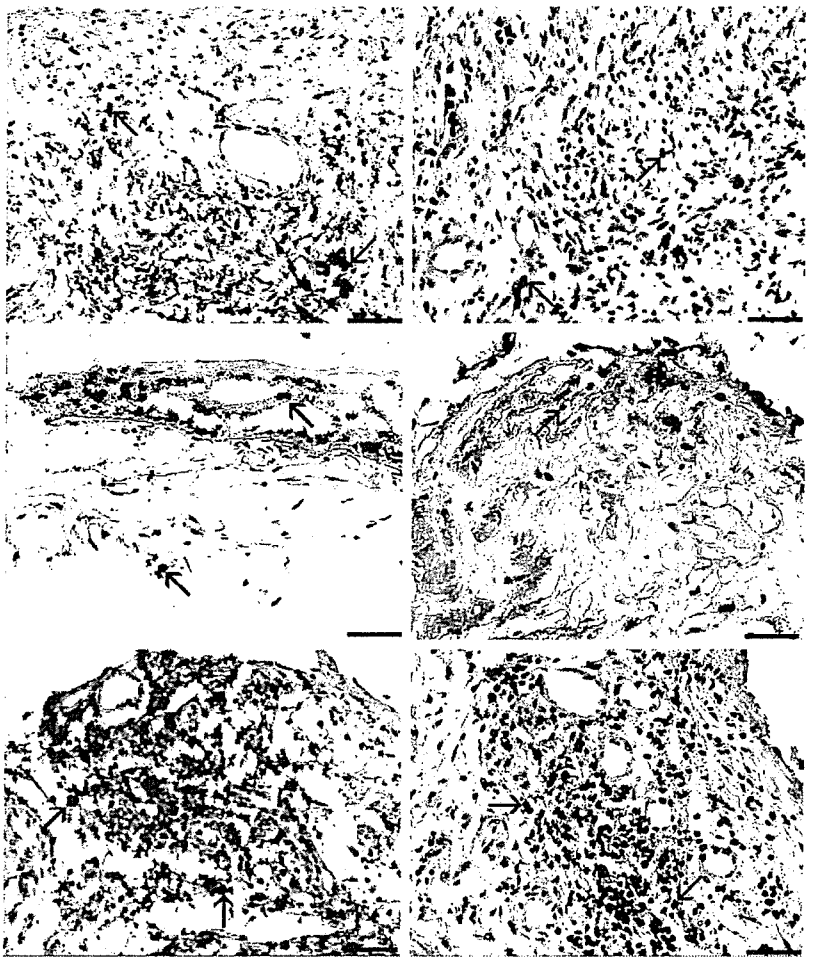

Fig. 5. Photomicrographs of choroidal neovascularization (CNV) membranes probed with CD68 (top, middle, and bottom left) and CD45 (top, middle, and bottom right) labeling macrophages (red chromogen) and leukocytes (3-diaminobenzidine brown chromogen), respectively. CNV without previous PDT (serial section from the same control specimen shown in Figure 2) displayed many macrophages (top left, arrows) and leukocytes (top right, arrows). CNV excised 3 days after PDT was hypocellular with a few macrophages (Case 4 in Table 1, middle left, arrows) and leukocytes (Case 1 in Table 1, middle right, arrows). CNV excised 55 days after PDT (Case 9 in Table 1) discloses many macrophages (bottom left, arrows) and leukocytes (bottom right, arrows). Scale bars: 50 μm.

In membranes extracted 3 days after PDT (n = 4), Ki-67 expressing cells were completely negative in two cases and relatively high only in one specimen. The surface area of these membranes (median: 0.18, range: 0.038 mm^2–0.457 mm^2) did not differ significantly from the control CNV without previous PDT (median: 0.27, range: 0.035 mm^2–2.246 mm^2, P = 0.245). However, proliferative activity in these specimens excised 3 days after PDT (median: 4.85 nuclei/mm^2, range: 0–9.71) was significantly smaller than that in the CNV membranes without previous PDT (P = 0.0243) (Figures 3 and 6, middle). At longer intervals following PDT, although the surface area of the membranes (median: 0.369, range: 0.0193 mm^2–3.259 mm^2) did not show any significance (P = 0.18), proliferative activity increased significantly (median: 122.39 nuclei/mm^2, range: 0–829.29, P = 0.0295) in comparison to CNV excised 3 days after PDT. Neither surface area nor proliferative activity of these specimens excised long after PDT showed a significance in comparison to the control group (P = 0.42, P = 0.54, respectively) (Figures 3 and 6, bottom).

Discussion

Recently, inflammatory mechanisms and cells have been confirmed to play a pivotal role in the multifactorial pathogenesis of CNV.[28,31–36] These inflammatory factors might, therefore, be potential targets to develop new treatment strategies to be used either alone or as adjuvant to PDT. Additionally, they also influence the potential effectiveness and impacts of the other treatment modalities. Although the effect of PDT with different photosen-

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Fig. 6. Photomicrographs of choroidal neovascularization (CNV) membranes probed with Ki-67 antibody which labels proliferating cells. CNV without previous photodynamic therapy (PDT) (serial section from the same control membrane in Figures 2 and 3) disclosed some proliferating cells stained brown with the 3-diaminobenzidine chromogen (top, arrows). Serial section of the specimen excised 3 days after PDT (Case 1 in Table 1) displayed only one proliferating cell (middle, arrow). The membrane extracted 40 days after the third PDT (Case 8 in Table 1) was hypercellular and many proliferating cells were detected (arrows, bottom). Scale bars: 50 μm.

sitizers on inflammation and inflammatory cell cytokines was evaluated in different types of healthy tissues and tumors in several studies,[37–42] knowledge of impact of verteporfin PDT on inflammation in human CNV is still limited.[43–46] PDT can modulate immunologic effects either by enhancing[39–42] or inhibiting[37,38] inflammatory cells and cytokines. Tissue type, the primary pathology, and the type of the photosensitizer and contribution of the host contribute to the specific response of each tissue. Since it is crucial to establish the potential beneficial combination therapy and the optimal protocol, we evaluated the early and late consequences of PDT on inflammation in human CNV.

In our series of control CNV without any preoperative treatment, macrophages and leukocytes were found to be present in nearly 90% and 95% of the specimens, respectively. Macrophages and leukocytes were previously reported to be present in surgically excised CNV up to a frequency as high as 100%.[27–29,47–52] Grossniklaus and associates have shown that CNV is a wound healing tissue similar to granulation.[27,29] Wound healing is a dynamic process characterized by inflammation and angiogenesis in the active stages. Grossniklaus et al described inflammatory active stage of CNV by macrophage influx into the CNV.[27] It is not surprising that most of the specimens either in ours or in the other studies were inflammatory active with high frequency of macrophages and leukocytes since submacular surgery was offered to patients with clinically active CNV. Macrophages synthesize the cytokines interleukin (IL)-1 and tumor necrosis factor (TNF)-α actively.[34,52–54] Leibovich et al showed that TNF-α is the mediator of macrophage-induced angiogenesis.[54] Cousins et al reported that patients with activated monocytes with the highest TNF-α expression develop more CNV.[55] BenEzra and associates have demonstrated that IL-1 can induce ocular neovascularization in vivo.[56] IL-1 and TNF-α are both expressed in experimental and human CNV membranes in macrophages, RPE cells, and vessels.[28,35] Macrophages were shown to affect and stimulate human RPE cells to secrete cytokines through IL-1 and TNF-α by Jaffe and collagues.[57] Oh et al suggested that macrophages amplify the local VEGF response by inducing VEGF production in RPE through IL-1 and TNF-α in human CNV.[28] Thy-1 is a cell surface marker expressed on vascular EC of adult angiogenesis. Lee et al demonstrated that Thy-1 is upregulated by inflammatory cytokines IL-1 and TNF-α, but remains unaffected by growth factors such as VEGF.[25] Presence of thy-1 expression in 87% of the membranes in our series seems to correlate well with the 90% frequency of macrophage and 95% frequency of leukocytes presence in our specimens.

Contrary to control CNV, in membranes extracted 3 days after PDT, density of leukocytes and macrophages was significantly smaller than in the control CNV. Consequently, expression of thy-1 was also considerably less, which reflects decreased inflammatory cytokines such as IL-1β and TNF-α in these specimens.

One week after verteporfin PDT, macrophages were found in the periphery of affected vessels of healthy human choroid. Schmidt-Erfurth et al suggested that release of cytokines such as IL-1, IL-2, and TNF-α from attracted macrophages might facilitate vessel closure.[46] This was not supported by our results. In our series, 3 days after PDT, choroidal nonperfusion was detected in the targeted area (data not shown) by

fluorescein angiography. Histologic examination, however, disclosed significantly lower inflammatory infiltration and activity. Macrophages and leukocytes were found to be critical for the development of experimental CNV. In macrophage depleted mice or MCP-1 receptor CCR2 knockout mice, reduction in the number of infiltrating macrophages and neutrophils correlated with reduction in VEGF protein levels in smaller, less cellular, and less vascular CNV.[31–34,36] Therefore, significant reduction in the inflammatory infiltration and cytokines could have been a favorable effect of PDT if it were permanent. However, in longer intervals following PDT, number of infiltrating leukocytes and macrophages was significantly increased. The contribution of inflammatory cytokines such as IL-1 and TNF-α also seemed to increase in longer intervals as evidenced by significantly increased percentage of thy-1 expressing vessels.

Many factors might contribute to enhanced infiltration of macrophages and leukocytes as well as increased activity of inflammatory cytokines in longer intervals after PDT. Verteporfin PDT was suggested to exert its effect through endothelial cell damage,[30,44,45] which we have recently shown to be through apoptosis.[58] Macrophages recognize and engulf apoptotic cells.[55] Phagocytosis of injured dead tumor cells was found to be the mechanism of their eradication in an experimental tumor study following PDT.[59] Therefore inflammatory cells may function to remove the apoptotic EC after PDT. Ghazi et al[44] showed macrophages wrapping an occluded vessel lined with degenerated EC and phagocytosing those EC cells in a CNV excised 27 days after PDT. Herein we have also seen that enhanced inflammation is a late term effect of PDT. Secondly, we have previously reported enhanced expression of VEGF in RPE cells early after PDT and in EC and stromal cells in longer intervals after PDT.[30] VEGF activates monocytes and macrophages induce chemotaxis and migration of monocytes across EC monolayers[61] and therefore promotes their recruitment.[62] Since macrophages also synthesize VEGF themselves[31–34,53,63] and induce RPE[28,57] and fibroblasts to synthesize VEGF, there seems to be a closed but amplifying circle between enhanced VEGF expression and macrophage infiltration with enhanced inflammatory cytokines.

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Proliferative activity, which decreased significantly in comparison to the control CNV 3 days after PDT, increased considerably in longer post-PDT intervals. We have previously reported that proliferative activity was significantly higher in inflammatory active CNV.[30,64] In our series, proliferating cells were mostly stromal cells rather than EC or RPE. Therefore, increased proliferative activity in longer post-PDT intervals might be due to enhanced inflammatory cell infiltration. Arras et al showed that macrophage accumulation correlated with maximal proliferation and the number of newly formed vessels following vascular occlusion.[65] In CNV following PDT, inflammatory activity seems to accompany the enhanced proliferation.

The authors are unaware of previous reports of immunohistopathologic evaluation demonstrating impact of PDT on inflammatory cell infiltration and thy-1 expression in human CNV membranes treated by PDT and could find no reference to it in a computerized search utilizing PubMed. Schmidt-Erfurth et al suggested that induction of a local inflammatory reaction with release of cytokines and angiogenic factors reinitiates the wound healing process and growth of recurrent CNV following PDT.[45] Our findings confirm this suggestion. The proper interpretation of this study, however, is limited by the fact that our cases may represent a negative selection and the histopathologic findings in patients who profit from verteporfin-PDT might differ. Still, it is conceivable that PDT causes a trauma followed by enhanced inflammation contributing to induced proliferation. The upcoming antiangiogenic and anti-inflammatory adjuvant therapies were postulated to inhibit the PDT induced angiogenic response, therefore decreasing CNV recurrence rate and PDT retreatment rate. Our findings also reveal the necessity of an adjuvant therapy to inhibit the ongoing cascade. Still, the impact of each adjuvant therapy on inflammation in human CNV needs to be evaluated specifically to establish the most beneficial and rational protocol for combination therapy.

Key words: age-related macular degeneration, choroidal neovascular membranes, verteporfin photodynamic therapy, CD68, Thy-1, CD45, Ki-67.

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Visual Sensations during Vitrectomy

Dear Editor:

The visual sensations experienced by patients during cataract surgery have been well documented.[1–3] Here we report the visual sensations described by patients during vitrectomy under retrobulbar anesthesia. Twenty-eight men and 28 women with a mean age of 61.4±12.5 years and a variety of vitreoretinal pathologies (Table 1 [available at http://aaojournal.org]) were studied. The patients were questioned about their visual sensations during and within 3 hours after vitrectomy (Table 2 [available at http://aaojournal.org]). Fifty-four (96.4%) of the patients reported seeing lights, 46 (82.1%) reported seeing ≥1 colors, and 37 (66.1%) reported seeing movements or moving objects. Of the latter 37, 34 saw instruments, and 6 (13.0%) saw the surgeon's fingers or hands. In the 51 cases of triamcinolone-assisted vitrectomy, 24 (47.1%) reported seeing many diffuse whirling black spots. Four patients (7.1%) found the

visual experiences frightening. There was no obvious difference in the incidence of the type of sensation for the different vitreoretinal pathologies (Table 3 [available at http://aaojournal.org]). The amount of anesthesia, gender, age, and pain did not seem to affect the number of patients reporting the various visual sensations (Table 4 [available at http://aaojournal.org]). The patients who had lower amounts of anesthesia tended to experience light and color sensations more frequently, but the rates were not statistically significant. A representative patient saw colorless swirling fluid during the early phase of vitrectomy, which probably corresponded to the intraocular irrigation used during core vitrectomy. Later, he reported seeing numerous swirling black and gray spots resembling snowflakes when the white triamcinolone acetonide crystals were injected into the vitreous. Twenty-two patients described a sharply tapered shadow that moved into the center of the field just before the membranelike material was peeled off. The accuracy and precision of the descriptions was unexpected, although we have reported on a patient who not only described but also drew what he saw during vitrectomy with great accuracy.[4] These drawings illustrated how well the visual perception of the patient corresponded with the surgical procedure being performed, even when they were not focused on the retina through the optical system of the eye. The basis for the visual sensations was considered to be similar to that of other entoptic phenomena,[5] as in vitreal floaters in patients with a posterior vitreous detachment. The closer the object to the retina, the more exact will its shadow correspond to the actual shape and size of the object. This explains why some of our patients reported seeing a decrease in the thickness of the object in the center of the visual field (VF) when a rod-shaped instrument was inserted into the vitreous. In addition, patients reported only one instrument on the left side of the VF, and the shaft of the light pipe was not seen. This can be interpreted as the shadow of the instrument but not of the endoillumination probe. It is of some interest that some patients perceived blood as being red; this has been reported previously.[6] Additional investigations are needed to determine the mechanism for this phenomenon. Visual sensations are experienced by the majority of patients despite full pain control, and surgeons should warn patients of these possibilities, as they can be frightening. This should minimize the patients' anxiety and stress during surgery.

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