Early Effects of Intravitreal Triamcinolone Acetonide on Inflammation and Proliferation in Human Choroidal Neovascularization

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eFigure 1. Photomicrographs of choroidal neovascularization membranes without previous therapy (A), excised 3 days after verteporfin photodynamic therapy (PDT) (B), excised 4 days after intravitreal triamcinolone (TA) injection (C and E), and excised 4 days after intravitreal TA and 5 days after PDT (D and F). Specimens were probed with antibodies against intercellular adhesion molecule (ICAM)-1 (A-D) or E-selectin (E and F) and stained with 3-amino-9-ethylcarbazole red chromogen. Hematoxylin was used as a counterstain. A-D, Intense ICAM-1 expression was detected in the retina pigment epithelium (RPE) (asterisk). Choroidal neovascularizations treated with TA monotherapy (C) or PDT/TA combination therapy (D) also display intense ICAM-1 expression in endothelial cells (arrow) and stromal cells (arrowhead). E and F, Some RPE cells display E-selectin (asterisk).

eFigure 2. A, B, and F, Photomicrographs of choroidal neovascularization (CNV) membrane extracted 5 days after verteporfin photodynamic therapy (PDT) and 4 days after intravitreal triamcinolone acetoneide (TA) injection. A, Late phase of fluorescein angiography on the day of surgery displays nonperfusion of the CNV and laser spot area. B, The specimen was probed with antibody against CD34 and stained with 3-diaminobenzidine, resulting in brown chromogen. Most of the vessels are occluded and were lined with damaged endothelial cells (EC) (arrows). C-F, Photomicrographs of CNV membranes probed with antibody against Thy-1 and stained with red chromogen. Hematoxylin was used as a counterstain. In control CNV membranes without previous therapy (C), some EC were immunopositive for Thy-1 (arrow), whereas some EC were immunonegative (arrowhead). D, The CNV excised 3 days after PDT displayed no Thy-1 immunoreactive EC. E, The CNV excised 3 days after intravitreal TA injection and those extracted after PDT+TA combination therapy (F) showed strong Thy-1 expression in all vessels (arrows). Scale bars, 50 µm.
**Figure 3.** Photomicrographs of choroidal neovascularization (CNV) membranes probed for macrophages with CD68 antibodies and stained with red chromogen (A-D), for leukocytes with CD45 antibody (E and F), and for proliferating cells with Ki-67 antibody (G, H) stained with the brown chromogen 3-diaminobenzidine. Hematoxylin was used as a counterstain. A, In control CNV membranes without previous therapy, some macrophages were detected in the retina pigment epithelium (RPE) cell layer (asterisk) and stroma (arrow). Contrary to CNV excised 3 days after photodynamic therapy (PDT) (B), CNV excised 3 days after triamcinolone acetonide monotherapy (TA CNV) (C) and CNV extracted 5 days after PDT and TA combination therapy (PDT + TA CNV) (D) displayed many macrophages in the RPE cell layer (asterisks) and within the stroma (arrows), but only a few leukocytes (E and F, arrows). Only a few proliferating cells were detected in TA CNV membranes (G, arrow). The PDT + TA CNV disclosed no proliferating cells (H). Scale bars, 50 µm.

**Figure 4.** Photomicrographs of choroidal neovascularization (CNV) membranes probed with antibody against vascular endothelial growth factor (VEGF) stained with red chromogen. Hematoxylin was used as a counterstain. In control CNV without previous therapy (A), the retina pigment epithelium (RPE) did not display VEGF (asterisk), but VEGF staining was detected within some cells in the stroma (arrow). In a CNV membrane excised 3 days after photodynamic therapy (PDT) (B), strong VEGF expression was especially prominent in the RPE (asterisk). In a CNV membrane excised 4 days after intravitreal triamcinolone acetonide (TA) injection (C), strong VEGF expression was present in the RPE (asterisk) and stromal cells (arrow). In a CNV membrane excised 3 days after PDT and 4 days following intravitreal TA injection (D), RPE (asterisk) and stromal cells (arrows) showed strong VEGF immunoreactivity. Scale bars, 50 µm.