Monocyte Chemoattractant Protein 1, Interacellular Adhesion Molecule 1, and Vascular Cell Adhesion Molecule 1 in Exudative Age-Related Macular Degeneration

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Objective: To examine intraocular concentrations of monocyte chemoattractant protein 1 (MCP-1), soluble intercellular adhesion molecule 1 (sICAM-1), soluble vascular cell adhesion molecule 1 (sVCAM-1), and vascular endothelial growth factor (VEGF) in eyes with exudative age-related macular degeneration (AMD).

Methods: The investigation included a study group of 28 patients (28 eyes) with exudative AMD and a control group of 25 patients (25 eyes) with cataract. The concentrations of MCP-1, sICAM-1, sVCAM-1, and VEGF in aqueous humor samples obtained during surgery were measured using a solid-phase chemiluminescence immunoassay.

Results: The study group as compared with the control group had higher aqueous concentrations of sICAM-1 (mean [SD], 844 [2073] vs 246 [206] pg/mL, respectively; \(P < .001\)), sVCAM-1 (mean [SD], 7978 [7120] vs 2999 [1426] pg/mL, respectively; \(P < .001\)), and MCP-1 (mean [SD], 587 [338] vs 435 [221] pg/mL, respectively; \(P = .07\)). The concentration of VEGF did not vary significantly between the groups (\(P = .76\)). The MCP-1 concentration was significantly associated with macular thickness (\(r = 0.40; P = .004\)). It decreased significantly with the type of subfoveal neovascular membrane (classic membrane type, occult membrane, retinal pigment epithelium detachment) (\(P = .009\)). The concentrations of sICAM-1, sVCAM-1, and VEGF were not significantly associated with membrane type and macular thickness (\(P > .18\)).

Conclusions: Concentrations of MCP-1, sICAM-1, and sVCAM-1 are significantly associated with exudative AMD, even in the presence of normal VEGF concentrations. Intraocular MCP-1 concentrations are correlated with the subfoveal neovascular membrane type and the amount of macular edema. One may infer that MCP-1, sICAM-1, and sVCAM-1 could potentially be additional target molecules in therapy for exudative AMD.


A GE-RELATED MACULAR DEGENERATION (AMD) is one of the leading causes of severe, often irreversible visual impairment in the elderly population in Western countries,\(^1\)-\(^4\) while East Asian societies have a lower prevalence of the disease.\(^5\)-\(^6\) The neovascular exudative form of AMD as compared with the nonexudative or dry form is responsible for most severe visual impairment resulting from the disease.\(^7\) After the landmark studies by Aiello et al\(^8\) on the association of retinal neovascularization and vascular endothelial growth factor (VEGF), evidence has been accumulating that VEGF is associated also with the development of subfoveal choroidal neovascular membranes in eyes with exudative AMD.\(^9\)-\(^10\) In particular, the intravitreous application of anti-VEGF drugs such as bevacizumab and ranibizumab resulted in a highly significant improvement in visual outcome compared with nontreated control groups.\(^11\)-\(^13\)

Studies have also shown, however, that although the intraocular concentrations of VEGF were markedly higher in eyes with diabetic retinopathy than in eyes with exudative AMD, the latter eyes did not always show significantly higher VEGF concentrations as compared with age-matched control eyes.\(^14\)-\(^16\) In a similar manner, the intraocular concentrations of other cytokines such as erythropoietin and basic fibroblast growth factor were significantly higher in eyes with diabetic retinopathy than in eyes with exudative AMD, in which the levels were not significantly different from the concentrations in normal eyes.\(^15\)-\(^17\)

In view of these findings that eyes with exudative AMD and age-matched control eyes did not differ markedly in the intraocular concentrations of most of the examined cytokines including VEGF, it was hypothesized that other cytokines and adhesive molecules besides VEGF may be involved in the pathogenic process of exu-
The clinical interventional comparative study included a study group consisting of patients with exudative AMD who were treated by an intravitreous injection of bevacizumab and/or triamcinolone acetonide and a control group of patients with age-related cataract who underwent routine cataract surgery. All patients were treated at the same institution. An inclusion criterion was the absence of any retinal or optic nerve disease except exudative AMD in the study group and except hard drusen of the macular retinal pigment epithelium in the control group. Additionally, the volume of collected aqueous humor had to be at least 100 µL. Previous photodynamic therapy or laser photocoagulation therapy for the subfoveal neovascular membrane was an exclusion criterion, while previous intravitreous drug applications (bevacizumab or triamcinolone) were allowed. Intraocular pressure had to be within the reference range of 10 to 21 mm Hg. The Medical Ethics Committee II of the Medical Faculty Mannheim of the Ruprecht-Karls University, Heidelberg, Germany, approved the study protocol.

All patients underwent an ophthalmologic examination including refractionmetry, applanation tonometry, and slitlamp-assisted biomicroscopy of the anterior and posterior segments of the eye. The diagnosis of exudative AMD was substantiated by ophthalmoscopy, fluorescein angiography, and optical coherence tomography. The maximal thickness of the macula was measured by optical coherence tomography (OCT III Stratus; Carl Zeiss Meditec, Jena, Germany). All scans were visually inspected to make sure that the computerized thickness analysis was appropriate.

In the study group, the aqueous humor was collected after disinfection of the peribulbar skin and conjunctiva, sterile draping of the patient, and insertion of an eyelid speculum. A paracentesis was performed in the temporal limbal region, and aqueous humor was sampled using a blunt cannula and a tuberculin syringe before the intravitreal transconjunctival injection was performed in the temporal inferior quadrant. In the control group, the peribulbar skin was disinfected, the patient was draped, an eyelid speculum was inserted, and the aqueous humor was collected through a temporal paracentesis before routine cataract surgery was continued. For all patients, the aqueous humor samples were deeply frozen in liquid nitrogen within 10 minutes after collection. In the study group, the paracentesis and the release of aqueous humor were necessary because these patients underwent an injection of triamcinolone or a combined injection of bevacizumab and triamcinolone with a total injected volume of about 0.20 mL to 0.25 mL. For such a high volume to be injected intravitreally, the eye has to become hypotonous prior to the injection to avoid a marked injection-related increase in intraocular pressure. In the control group, the paracentesis was routinely performed to create temporal access to the anterior chamber for the binocular maneuvering of the lens nucleus and cortex during surgery. The technique has been described previously.

The concentrations of VEGF, MCP-1, sICAM-1, and sVCAM-1 were measured using the suspension bead array technology on a Luminex 100 IS system (Luminex Corp, Austin, Texas). Statistical analysis was performed using a commercially available statistical software package (SPSS for Windows, version 17.0; SPSS Inc, Chicago, Illinois). The refractive data were converted into the spherical equivalent for statistical analysis. Where appropriate, Kendall bivariate correlation test, independent non-parametric test (Mann-Whitney U test), and 1-way analysis of variance were used. Two-tailed probabilities of less than .05 were considered to indicate statistical significance.

The study group consisted of 28 patients (28 eyes) with exudative AMD, and the control group included 25 patients (25 eyes) undergoing routine cataract surgery. The study group and the control group did not vary significantly in age (P = .23), sex (P = .86), or intraocular pressure (P = .32). Owing to cataract-induced myopization, the mean refractive error was significantly more myopic in the study group than in the control group (P = .01) (Table). In the study group, 14 eyes (50%) were pseudophakic.

Within the study group, 7 eyes (25%) showed a predominantly or purely classic type of subfoveal neovascular membrane, 18 eyes (64%) had a purely occult type or a minimally classic type of subfoveal neovascular membrane, and 3 eyes (11%) showed a detachment of the retinal pigment epithelium. On optical coherence tomography, the mean (SD) maximum retinal thickness of the macula was 396 (179) µm (median, 350 µm; range, 245-1135 µm). The study group was further subdivided into eyes without any previous intraocular treatment (n = 17) and eyes that had previously received intravitreous bevacizumab (n = 11). In the eyes with a previous intravitreous bevacizumab application, the mean (SD) interval between the last injection and inclusion in this study was 3.5 (2.8) months (range, 1.0-8.0 months).
Comparing the groups with each other revealed that the exudative AMD study group as compared with the control group had higher concentrations of aqueous sICAM-1 (mean [SD], 844 [2073] vs 246 [206] pg/mL, respectively; \(P = .001\)) and sVCAM-1 (mean [SD], 7978 [7120] vs 2999 [1426] pg/mL, respectively; \(P = .001\)) (Figure 1A and B). The difference in the concentration of MCP-1 between the AMD group and the control group was marginally significant (mean [SD], 587 [338] vs 435 [221] pg/mL, respectively; \(P = .07\)) (Figure 1C). The concentration of VEGF did not vary between the AMD group and the control group (mean [SD], 46 [33] vs 44 [30] pg/mL, respectively; \(P = .76\)) (Figure 1D).

If the eyes with previous intravitreous bevacizumab therapy were excluded, similar results were obtained: the AMD group as compared with the control group had significantly higher concentrations of aqueous sICAM-1 (mean [SD], 1089 [2653] vs 246 [206] pg/mL, respectively; \(P < .001\)) and sVCAM-1 (mean [SD], 8653 [8302] vs 3000 [1426] pg/mL, respectively; \(P = .003\)).

Within the study group, the concentrations of MCP-1 were significantly correlated with the macular thickness (\(P = .004; r = .40\)) (Figure 2). The other adhesive molecules were not significantly associated with macular thickness (VEGF, \(P = .70\); sICAM-1, \(P = .21\); and sVCAM-1, \(P = .33\)).

Comparing the different types of subfoveal neovascular membranes showed that the aqueous concentration of MCP-1 was significantly associated with the type of subfoveal neovascular membrane (\(r = .49; P = .009\)) (Figure 3). The MCP-1 concentration was the highest in the subgroup with a classic or predominantly classic type of membrane (mean [SD], 851 [439] pg/mL), followed by the subgroup with an occult or minimally classic membrane type (mean [SD], 527 [260] pg/mL) and finally the subgroup with a detachment of the retinal pigment epithelium (mean [SD], 331 [129] pg/mL). Considered separately, the difference in the MCP-1 concentration was significant for the comparison of the retinal pigment epithelium detachment group vs the classic subfoveal membrane type group (\(P = .03\)); for the comparison between the classic subfoveal membrane type group vs the minimally classic or occult subfoveal membrane type group, the difference was marginally significant.

Figure 1. Aqueous concentrations of soluble intercellular adhesion molecule 1 (sICAM-1) (A), soluble vascular cell adhesion molecule 1 (sVCAM-1) (B), monocyte chemoattractant protein 1 (MCP-1) (C), and vascular endothelial growth factor (VEGF) (D) in aqueous humor samples of a study group of patients with exudative age-related macular degeneration (AMD) and a control group of subjects undergoing routine cataract surgery. The horizontal line in the middle of each box indicates the median, and the top and bottom borders of the box indicate the 75th and 25th percentiles, respectively. The points beyond the whiskers indicate outliers beyond the 90th and 10th percentiles.
whiskers indicate outliers beyond the 90th and 10th percentiles. The points beyond the median, and the top and bottom borders of the box indicate the 75th and 25th percentiles, respectively. The whiskers above and below the box indicate the 90th and 10th percentiles, respectively. The points beyond the whiskers indicate outliers beyond the 90th and 10th percentiles.

If eyes after an intravitreous injection of bevacizumab were excluded, the difference in the concentrations of sVCAM-1 (P < .001) and sICAM-1 (P = .003) between the study group and the control group remained statistically significant, while the 2 groups did not vary significantly in the intraocular concentrations of VEGF (P = .14) and MCP-1 (P = .27). If pseudophakic eyes were excluded from the statistical analysis, the difference in the concentrations of sVCAM-1 (P = .003) and sICAM-1 (P = .02) between the study group and the control group remained statistically significant, while the 2 groups did not vary significantly in the intraocular concentrations of VEGF and MCP-1.

The finding that the intraocular concentrations of MCP-1 were significantly associated with the degree of macular edema and with the clinical types of subfoveal neovascular membranes is corroborated by results of previous investigations. These studies revealed that inflammation is critically involved in the formation of subfoveal choroidal neovascular membranes and may thus contribute to the pathogenesis of AMD.28,29 The recruitment of monocytes is an early step in the initiation of the inflammatory and angiogenic processes, and MCP-1 plays an important role in regulation of the migration and infiltration of monocytes and macrophages.30 It has been found that MCP-1 is constitutively released from the retinal pigment epithelium and is enhanced by proinflammatory molecules.31,32 Recently, it was put forward by Austin et al33 that the enhanced release of MCP-1 could attract resident ocular macrophages (microglia) and could contribute to the digestion of the retinal pigment epithelium and Bruch membrane as its basal membrane. Correspondingly, histologic examinations of surgically removed subfoveal neovascular membranes from patients with exudative AMD showed macrophages located in regions with atrophy of the retinal pigment epithelium, Bruch membrane breakdown, and choroidal neovascularization.34-38 These data suggest that MCP-1 essentially contributes to the formation of subfoveal choroidal neovascular membranes and are consistent with our finding that the intraocular level of MCP-1 was significantly correlated with the clinical type of subfoveal choroidal neovascular membrane and with the amount of macular edema as measured by optical coherence tomography (Figure 2). Additionally, MCP-1 has been known to facilitate angiogenesis.39,40 Yamada et al41 demonstrated that VEGF increases MCP-1 messenger RNA levels in cultured endothelial cells and that MCP-1 participates in VEGF-induced angiogenesis and vascular leakage, which may be helpful in explaining our result that the aqueous concentration of MCP-1 is positively cor-
related with the maximal retinal thickness of the macula in patients with exudative AMD.

Immunoglobulin superfamily (IgSF) molecules, including ICAM-1 and VCAM-1, are key indicators of vascular endothelial cell activation. Adhesion molecule expression, including ICAM-1, has been found in association with inflammatory cells in extracted subretinal neovascular membrane lesions. Moreover, ICAM-1 labeling of the choriocapillaris was typically more intense in the macula than in the peripheral choroid in human donor eyes. It implies that the macula may be subject to increased leukocyte trafficking. In agreement with these previous findings, our results showed that the intracellular concentrations of sICAM-1 and sVCAM-1 were significantly higher in the patients with exudative AMD than in the patients of the control group. In addition, sICAM-1 and sVCAM-1 as compared with VEGF appeared to be more closely related with neovascular AMD because the aqueous concentrations of VEGF did not vary markedly between the study group and the control group.

Our finding that the aqueous concentration of VEGF did not vary significantly between the patients with exudative AMD and the patients with cataract is in agreement with the study by Chan et al in which the aqueous VEGF concentration did not differ significantly between a study group with untreated choroidal neovascular neovascularization (mean [SD], 66.8 [35.1] pg/mL), a study group with recurrent choroidal neovascular neovascularization (mean [SD], 55.7 [63.0] pg/mL), and a control group consisting of patients with cataract (P = .31 and P = .21, respectively).

The result of our present study also agrees with the finding of our previous study on different patients in that the aqueous concentration of VEGF was not significantly different between a group of patients with exudative AMD and a control group. Patients who had previous intracocular bevacizumab application, while these subgroups did not vary significantly in the intraocular concentrations of MCP-1, sICAM-1, and sVCAM-1. Parallel to previous investigations, the findings suggest that inflammatory factors including MCP-1, ICAM-1, and VCAM-1 play an essential role in the development of exudative AMD and that, in particular, MCP-1 may markedly contribute to the development of macular edema. As such, MCP-1, ICAM-1, and VCAM-1 may be additional target molecules in therapy for exudative AMD.

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