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

Jost B. Jonas, MD; Yong Tao, MD; Michael Neumaier, MD; Peter Findeisen, MD

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 (\( P \geq .18 \)).

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, while East Asian societies have a lower prevalence of the disease. The neovascular exudative form of AMD as compared with the nonexudative or dry form is responsible for most severe visual impairment and visual loss resulting from the disease. The landmark studies by Aiello et al 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. 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. 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. 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.

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 adhesives 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 macular degeneration 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 photoocoagulation therapy for the subfoveal neovascular membrane was an exclusion criterion, while previous intravitreous drug applications (bevacizumab or triamcinolone) were described to be associated with angiogenesis in general and because the potential role of these adhesives in the pathogenesis of neovascular AMD has not yet been explored to our knowledge, we conducted this study to assess whether intraocular concentrations of MCP-1, soluble ICAM-1 (sICAM-1), soluble VCAM-1 (sVCAM-1), and VEGF are associated with ocular characteristics of patients with neovascular exudative AMD.

### METHODS

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, 245-1155 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).

### RESULTS

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 periorbital 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 intravitreous transconjunctival injection was performed in the temporal inferior quadrant. In the control group as in the study group, the periorbital 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 hypotonic 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 bimanual 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 nonparametric 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.
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; \(P = .003\)). Again, the AMD group as compared with the control group had higher, but not significantly higher, concentrations of VEGF (mean [SD], 55.8 [33.9] vs 43.6 [30.5] pg/mL, respectively; \(P = .14\)).

Within the study group, the concentrations of MCP-1 were significantly correlated with the macular thickness (\(P = .004; r = 0.40\)) (Figure 2A). 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 (\(r = 0.49; P = .009\)) (Figure 3A). 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.
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. 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. It has been found that MCP-1 is constitutively released from the retinal pigment epithelium and is enhanced by proinflammatory molecules. Recently, it was put forward by Austin et al 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. These data suggest that MCP-1 essentially contributes to the formation of subfoveal choroidal neovascular membranes and may thus contribute to the pathogenesis of AMD. 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. It has been found that MCP-1 is constitutively released from the retinal pigment epithelium and is enhanced by proinflammatory molecules. Recently, it was put forward by Austin et al 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. These data suggest that MCP-1 essentially contributes to the formation of subfoveal choroidal neovascular membranes and may thus contribute to the pathogenesis of AMD. 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. It has been found that MCP-1 is constitutively released from the retinal pigment epithelium and is enhanced by proinflammatory molecules. Recently, it was put forward by Austin et al 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.

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. 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. It has been found that MCP-1 is constitutively released from the retinal pigment epithelium and is enhanced by proinflammatory molecules. Recently, it was put forward by Austin et al 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. These data suggest that MCP-1 essentially contributes to the formation of subfoveal choroidal neovascular membranes and may thus contribute to the pathogenesis of AMD. 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. It has been found that MCP-1 is constitutively released from the retinal pigment epithelium and is enhanced by proinflammatory molecules. Recently, it was put forward by Austin et al 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.
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 intraocular 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 choriocapillaris neovascularization (mean [SD], 66.8 [35.1] pg/mL), a study group with recurrent choriocapillaris neovascularization (mean [SD], 55.7 [63.0] pg/mL), and a control group consisting of patients with cataract (mean [SD], 72 [95] pg/mL), and a control group (mean [SD], 108 [73] vs 72 [95] pg/mL), and a control group consisting of patients with exudative AMD and macular edema. Obtaining vitreous samples in the patients is, however, not possible because it would unnecessarily expand the surgical intervention.

In conclusion, the aqueous level of MCP-1 was significantly correlated with macular thickness in patients with exudative AMD, and correspondingly the aqueous MCP-1 concentration varied between the different types of choriocapillaris neovascular membranes. The aqueous levels of sICAM-1 and sVCAM-1 were significantly higher in patients with neovascular exudative AMD than in patients of a cataract control group. Patients who had previous intraocular anti-VEGF therapy showed significantly lower intraocular levels of VEGF than patients without a previous intravitreous 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.

Submitted for Publication: December 7, 2009; final revision received January 28, 2010; accepted February 8, 2010.

Correspondence: Jost B. Jonas, MD, Universitäts-Augenklinik, Theodor-Kutzer-Ufer 1-3, 68167 Mannheim, Germany (jost.jonas@augen.ma.uni-heidelberg.de).

Financial Disclosure: None reported.

Funding/Support: Dr Tao was supported by the K. C. Wong Fellowship from the German Academic Exchange Service.

REFERENCES

10. Krarup A, Nygvere PV, Berglin L, Seregard S. Subfoveal fibrovascular mem-

©2010 American Medical Association. All rights reserved.


Call for Papers

Archives of Ophthalmology, along with JAMA and other Archives specialty journals, will participate in a consortium theme issue on infectious diseases/immunology in April 2011. Manuscripts on uveitis, ocular infections, and ocular involvement in systemic infectious and immunological diseases received by October 1, 2010, will have the best chance for consideration for this theme issue.