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Monocyte Chemotactic Protein-1 Levels in the Vitreous of Patients with Proliferative Vitreoretinopathy

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Purpose: To assess the potential role of monocyte chemotactic protein-1 (MCP-1) in the pathogenesis of proliferative vitreoretinopathy (PVR) and to investigate its possible interaction with the macrophage migration inhibitory factor (MIF).

Methods: We assayed MCP-1 and MIF levels in the vitreous samples of 85 consecutive patients with PVR (29 eyes), rhegmatogenous retinal detachment (RRD; 22 eyes), and macular hole or idiopathic epimacular membrane (controls; 34 eyes), by enzyme-linked immunosorbent assay.

Results: Vitreous levels of MCP-1 were 1760.7 ± 471.3 pg/mL (mean ± SD) in PVR patients, 1200.4 ± 579.8 pg/mL in RRD patients, and 436.3 ± 286.1 pg/mL in the controls. Vitreous MCP-1 levels in PVR patients were significantly higher than those in RRD patients and in the controls ($P < .0001$, respectively). MCP-1 levels in grade C of PVR (1883.7 ± 479.5 pg/mL) were significantly greater than those in grade D (1437.8 ± 258.8 pg/mL) ($P = .0112$). Vitreous concentrations of MCP-1 had no correlation with those of MIF.

Conclusions: The results indicate the possibility that MCP-1 may have a role mainly in the early stage of PVR and that the role of MCP-1 in PVR may differ from that of MIF. **Jpn J Ophthalmol 2002;46:218–221**

Key Words: Macrophage migration inhibitory factor, monocyte chemotactic protein-1, proliferative vitreoretinopathy, vitreous.

Introduction

Although the pathogenesis of proliferative vitreoretinopathy (PVR) is not completely understood, it is considered that inflammatory reactions have an important role in its pathogenesis. It was reported that fibrovascular proliferation and retinal detachment were observed after intravitreal injection of activated macrophages in the rabbit eye.[1] Monocyte chemotactic protein-1 (MCP-1) is the chemokine that induces monocyte and macrophage infiltration into tissues. It has been reported that the vitreous MCP-1 level was significantly increased in PVR.[2–4] On the other hand, the macrophage migration inhibitory factor (MIF) is the lymphokine that prevents random migration of macrophages and recruits macrophages at inflammatory loci. We have reported that vitreous MIF levels increased in PVR and that there was a significant association between MIF levels and PVR grades.[5] In this study, we investigated the relationship between vitreous MCP-1 levels and

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clinical grades of PVR as well as the correlation between MCP-1 and MIF levels.

Materials and Methods

This study included 85 consecutive patients, 47 women and 38 men ranging in age from 28 to 80 (mean = 56.3 years). There were 29 eyes with PVR, 22 eyes with rhegmatogenous retinal detachment (RRD) without PVR; and 25 eyes with macular hole and 9 eyes with idiopathic epimacular membrane, which served as controls. Patients had undergone pars plana vitrectomy in Toho University Sakura Hospital between 1998 and 1999. All PVR cases were grade C2 or worse according to the old classification recommended by the Retina Society Terminology Committee (grade CP-4 or worse according to the new classification by this Committee). The indications for vitrectomy in eyes with RRD were bullous retinal detachment with giant breaks (3 eyes), macular holes (3 eyes), and complex arrangement of breaks (16 eyes). We excluded the cases with perforating trauma, uveitis, or vitreous hemorrhage. Informed consent was obtained prior to the sampling in each patient for collection of the vitreous samples and investigation of cytokine levels.

The vitreous samples were obtained during the pars plana vitrectomy before intraocular infusion. The enzyme-linked immunosorbent assay was used to determine the MCP-1 levels with a commercially available immunoassay kit (BioSource, Camarillo, CA, USA) and to determine the MIF levels as described previously.[6] The measurement of MCP-1 was performed according to the manufacturer's standard protocol. Briefly, 50 μL of samples and 50 μL of standard diluent buffer were placed in duplicate in MCP-1 antibody-coated wells. After that, 50 μL of biotinylated anti-MCP-1 antibody was added to the wells and incubated together with the buffer and samples for 2 hours at 25°C. After the samples were washed, 100 μL of streptavidin-horseradish peroxidase working solution was added to each well. Following incubation for 30 minutes, the substrate was added to individual wells, followed by incubation for 30 minutes in the dark. The reaction was terminated with 100 μL of 1 N sulphuric acid. The absorbances at 450 nm and 630 nm were measured using a Sjeia Auto Reader (Model ER-8000, Sanko, Tokyo). Duplicate readings of each sample were averaged. The assay detection limit was defined as 20 pg/mL for MCP-1 and 1 ng/mL for MIF. For statistical calculations, samples with undetectable levels were entered at the assay detection limit.

Data were statistically analyzed with the Mann-Whitney *U*-tests for comparison of the two groups and with the Kruskal-Wallis test for multiple groups in the vitreous concentrations of MCP-1. Correlations between vitreous levels of MCP-1 and MIF were examined by Pearson's correlation test. Levels of statistical significance were set at $P < .05$.

Results

MCP-1 concentrations were detectable in the eyes of all subjects (85 eyes). The vitreous levels of MCP-1

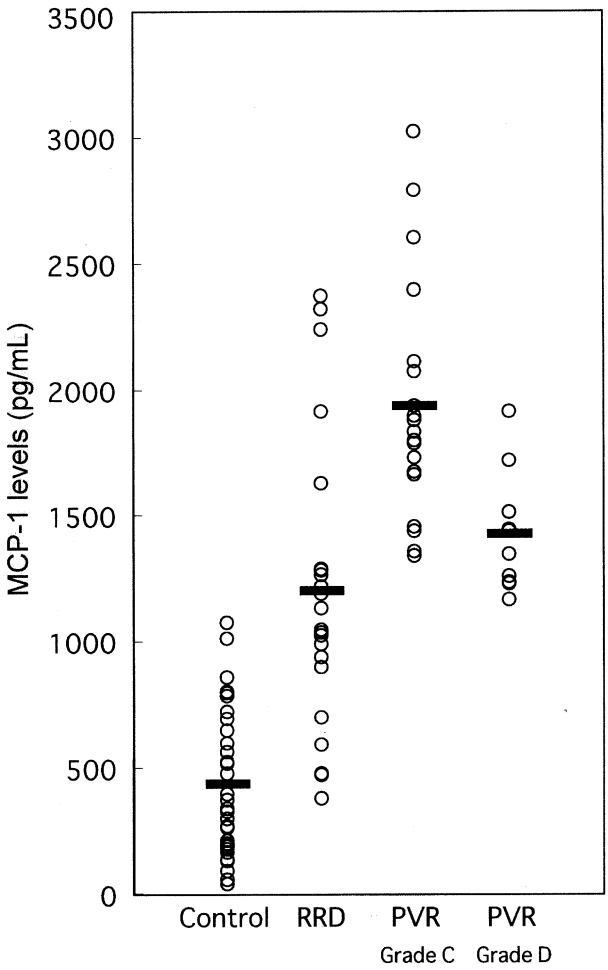

Figure 1. Monocyte chemotactic protein-1 (MCP-1) levels in the vitreous samples from eyes with macular hole or idiopathic epimacular membrane (control subjects, n = 34), rhegmatogenous retinal detachment (RRD, n = 22), and proliferative vitreoretinopathy (PVR, grade C; n = 19, grade D; n = 10) The horizontal lines indicate the mean concentration in each group. The vitreous levels in PVR patients were significantly greater than those in RRD patients and controls, respectively ($P < .0001$, Kruskal-Wallis test and Fisher's protected least statistical difference. The MCP-1 levels were significantly greater in grade C of PVR than in grade D ($P = .0112$, Mann-Whitney *U*-tests).

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were 1760.7 ± 471.3 pg/mL (mean ± SD) in PVR patients, 1200.4 ± 579.8 pg/mL in RRD patients, and 436.3 ± 286.1 pg/mL in the controls (Figure 1). Significant differences were found among these 3 groups ($P < .0001$, Kruskal-Wallis test). The vitreous levels in PVR patients were significantly higher than those in the RRD patients and control patients (both $P < .0001$, Fisher's protected least significant difference test). With respect to control subjects, significant difference was not found between MCP-1 levels in the macular hole group (429.6 ± 290.6 pg/mL) and those in the idiopathic epimacular membrane group (455.0 ± 289.7 pg/mL) ($P = .8606$, Mann-Whitney *U*-tests). There was no significant correlation between vitreous MCP-1 levels and age (Pearson's correlation tests) or sex (Mann-Whitney *U*-tests).

In the eyes with PVR, the MCP-1 concentrations were 1936.0 ± 472.7 pg/mL in grade C of PVR (19 eyes) and 1427.7 ± 237.0 pg/mL in grade D (10 eyes) according to the old classification (Figure 1). The MCP-1 levels were significantly higher in grade C than in grade D ($P = .0025$, Mann-Whitney *U*-tests). According to the new classification, vitreous MCP-1 levels were 2081.4 ± 520.9 pg/mL in grades CP4–7 (12 eyes), 1656.7 ± 188.5 pg/mL in grades CP8–12 (7 eyes), and 1448.8 ± 286.6 pg/mL in grades CA1–12 (10 eyes). None of the cases in grades CP4–7 or CP8–12 were accompanied by anterior PVR (CA), and all cases in grades CA1–12 were accompanied by posterior proliferation (CP). Significant differences were found among these three groups ($P = .0044$, Kruskal-Wallis test). Vitreous MCP-1 levels in PVR were 1479.8 ± 261.3 pg/mL in patients with unsuccessful preoperative vitreoretinal surgery (16 eyes) and 2106.5 ± 445.2 pg/mL in patients without preoperative surgery (13 eyes). MCP-1 concentrations were unrelated to the period from the preoperative surgery to the sampling in patients with unsuccessful preoperative surgery ($r = 0.095$; $P = .727$, Pearson's correlation tests).

MIF concentrations were undetectable in only 8 samples of vitreous from control patients. There was no significant correlation between vitreous concentrations of MCP-1 and those of MIF in any of the subjects' eyes ($r = 0.193$; $P = .0775$) or the eyes with RRD or PVR ($r = 0.251$; $P = .762$) (Pearson's correlation test) (Figure 2).

Discussion

MCP-1 primarily is chemotactic for monocytes and lymphocytes, causing monocyte and macrophage infiltration into tissues. MCP-1 is also a strong activator of monocytes and macrophages. Activated macrophages can produce multiple growth factor, including transforming growth factor-β, platelet-derived growth factor, and fibroblast growth factor. In this study, the vitreous MCP-1 levels in PVR were significantly higher than those in RRD and controls, which is consistent with the previous reports. Capeans et al[4] described that MCP-1 may be involved in recruiting macrophages and monocytes into the vitreous and in the pathogenesis of PVR. This study also indicated that vitreous MCP-1 levels were significantly greater in grade C of PVR than in grade D. Elner et al[7] reported that no correlation of vitreous MCP-1 levels with PVR grade was found. By calculating from data presented in their article, however, MCP-1 levels were higher in grade C (8.2 ng/mL [n=10]) than in grade D (5.9 ng/mL [n = 3]). We speculate that MCP-1 may induce monocyte and macrophage infiltration into the vitreous mainly in the early stage of PVR.

Vitreous MCP-1 levels in PVR were higher in patients without preoperative surgery than in those with preoperative surgery. This result suggests that the effect of inflammation due to unsuccessful preoperative surgery is considered minimal.

Because the serum normally does not contain MCP-1, breakdown of the blood—retina barrier might play a

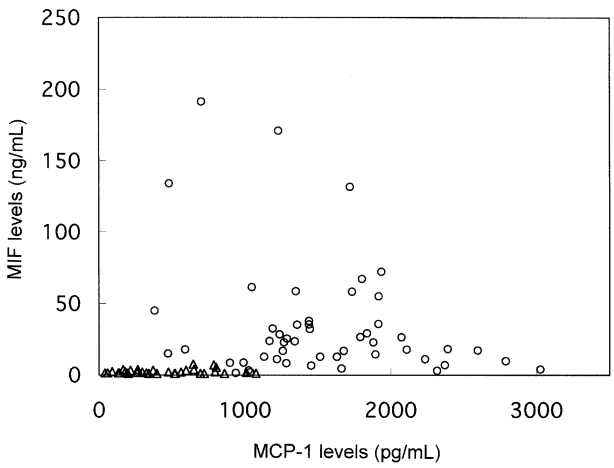

Figure 2. The relationship between the vitreous levels of monocyte chemotactic protein-1 (MCP-1) and macrophage migration inhibitory factor (MIF). Open triangles indicate macular hole or idiopathic epimacular membrane (control subjects) and open circles indicate rhegmatogenous retinal detachment or proliferative vitreoretinopathy. There was no significant correlation between vitreous concentrations of MCP-1 and those of MIF in all subjects' eyes ($r = 0.193$; $P = .0775$) or the eyes with RRD or PVR ($r = 0.251$; $P = .762$) (Pearson's correlation test).

minor role in the increased levels of vitreous MCP-1 in PVR patients. It is suggested that increased vitreous concentrations of MCP-1 reflect intraocular production of MCP-1. Retinal pigment epithelial cells are among the possible sources for MCP-1 in the vitreous of PVR patients. It was reported that MCP-1 can be secreted by retinal pigment epithelial cells and that the secretion is significantly up-regulated by stimulation by interleukin-1 and the tumor necrosis factor, which are known to be present in the eyes of PVR patients.[7]

It has also been reported that vitreous MIF levels were significantly increased in PVR patients.[5] In this study, vitreous MCP-1 concentrations were unrelated to MIF concentrations. We have already reported that MIF levels were significantly greater in grade D PVR patients than in grade C patients,[5] whereas MCP-1 levels of grade C were greater than those of grade D in this study. These findings suggest that the role of MCP-1 in PVR may differ from that of MIF, which may have a role only in the later stage of PVR.

In conclusion, the findings of this study indicate the possibility that MCP-1 may have a role mainly in the early stage of PVR and that the roles of MCP-1 in PVR may differ from that of MIF. However, we could not clarify whether the increased MCP-1 levels are the cause or the effect of PVR in this study. Therefore, further investigations that will prospectively investigate vitreous MCP-1 levels in RRD patients with and without postoperative PVR are under way.

References

1. Hui YN, Goodnight R, Sorgente N, Ryan SY. Fibrovascular proliferation and retinal detachment after intravitreal injection of activated macrophages in the rabbit eye. Am J Ophthalmol 1989;108:1762–84.
2. Elner SG, Elner VM, Jaffe GJ, Stuart A, Kunkel SL, Strieter RM. Cytokines in proliferative diabetic retinopathy and proliferative vitreoretinopathy. Curr Eye Res 1995;14:1045–53.
3. Abu El-Aslar AM, Van Damme J, Put V, et al. Monocyte chemotactic protein-1 in proliferative vitreoretinal disorders. Am J Ophthalmol 1997;123:599–606.
4. Capeans C, Victoria de rojas M, Lojo S, Salorio MS. C-C chemokines in the vitreous of patients with proliferative vitreoretinopathy and proliferative diabetic retinopathy. Retina 1998;18:546–50.
5. Mitamura Y, Takeuchi S, Matsuda A, Tagawa Y, Mizue Y, Nishihira J. Macrophage migration inhibitory factor levels in the vitreous of patients with proliferative vitreoretinopathy. Am J Ophthalmol 1999;128:763–5.
6. Mitamura Y, Takeuchi S, Matsuda A, Tagawa Y, Mizue Y, Nishihira J. Macrophage migration inhibitory factor levels in the vitreous of patients with proliferative diabetic retinopathy. Br J Ophthalmol 2000;84:636–9.
7. Elner SG, Strieter RM, Elner VM, Rollins BJ, Del Monte MA, Kunkel SL. Monocyte chemotactic protein gene expression by cytokine-treated human retinal pigment epithelial cells. Lab Invest 1991;64:819–25.