Fibrocytes and Fibrovascular Membrane Formation in Proliferative Diabetic Retinopathy

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Purpose. The purpose of this study was to investigate whether fibrocytes participate in formation of the fibrovascular membrane (FVM) in patients with proliferative diabetic retinopathy (PDR).

Methods. Vitreous fluid and FVM samples were obtained during vitrectomy in patients with PDR. Samples from patients with macular hole or epiretinal membrane were used as controls. Vitreous fluid and FVM samples were subjected to immunohistochemical analysis. In addition, cells isolated from the vitreous fluid of PDR and control patients were cultured in serum-free medium. Fibrocytes were identified among these cells by morphological and immunohistochemical analyses. We examined the number of fibrocytes in PDR patients and control patients. Also, the concentrations of monocyte chemoattractant protein-1 (MCP-1), pentraxin3, and serum amyloid P (SAP) in vitreous fluid samples from PDR patients and control patients were determined by enzyme-linked immunosorbent assay.

Results. Fibrocytes were observed in the vitreous and FVM samples from PDR patients. Cells cultured from the vitreous samples of PDR patients were spindle shaped and expressed fibrocyte markers. TGF-β1 induced differentiation of these cells into myofibroblasts. The number of fibrocytes was higher in samples from PDR patients than in samples from control patient. The vitreous fluid concentration of MCP-1 was significantly higher in PDR patients than in controls and showed a significant positive correlation with the number of fibrocytes from the vitreous fluid. Vitreous fluid concentrations of pentraxin3 and SAP were also higher in PDR patients than in control patients.

Conclusions. These findings indicate that fibrocytes may be involved in development of the FVM in PDR.

Keywords: fibrocyte, monocyte chemoattractant protein-1, pentraxin3, serum amyloid P

In patients with diabetes, proliferative diabetic retinopathy (PDR) is characterized by the formation of a fibrovascular membrane (FVM), which is often associated with tractional retinal detachment and vitreous hemorrhage causing loss of vision. Inflammation, angiogenesis induced by ischemia and expansion of the extracellular matrix in association with the FVM growth at the vitreoretinal interface are the pathological hallmarks of PDR. The FVM is characterized by migration and proliferation of various cells, including retinal glial cells, macrophages, monocytes, hyalocytes, laminocytes, fibroblasts, pericytes, and vascular endothelial cells.[1] Among these cells, fibroblast-like cells positive for α-smooth muscle actin (α-SMA) in the collagenous matrix have a major role in causing contraction of the FVM that leads to tractional retinal detachment.[2] However, the origin of these fibroblast-like cells remains to be determined. A circulating bone marrow-derived population of fibroblast-like cells, termed fibrocytes, has recently been suggested to have a role in the pathogenesis of fibrosis in various organs. Circulating fibrocytes were originally characterized by Bucala et al.[3] in 1994. These cells express markers of both hematopoietic cells (CD34, CD45, FcγR, lymphocyte-specific protein 1, and MHC class II) and stromal cells (collagens, fibronectin, and matrix metalloproteases).[4–6] Cultured fibrocytes actively produce collagen, and TGF-β induces these cells to express α-SMA, a specific marker of myofibroblasts, resulting in the contraction of collagen gels.[7] After injury, fibrocytes transform into myofibroblasts at the wound site and increase the production of collagen.[6] In addition to acting as myofibroblast precursors, fibrocytes promote angiogenesis by secreting vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), IL-8, and platelet-derived growth factor (PDGF). Furthermore, fibrocytes promote fibroblast proliferation, migration, and collagen production by secreting TGF-β and connective tissue growth factor (CTGF).[8,9] Fibrocytes comprise 0.1% to 0.5% of the nucleated cells in peripheral blood and originate at least partly from bone marrow-derived cells, including circulating $CD14^+$ precursor cells. Cultured monocytes can differentiate into fibrocytes, but it is still unclear if maturation of fibrocytes occurs in the bone marrow, the circulation, or after migration to the tissues. Fibrocytes contribute to the innate response to injury and tissue remodeling, and these cells also mediate fibrogenesis in a number of systemic and organ-specific fibrosing disorders, such as renal fibrosis,[10] ischemic cardiomyopathy,[11,12] pulmonary fibrosis,[13,14] asthma,[15–18] and keloid scarring.[19] Recently, Abu El-Asrar et al.[20,21] demonstrated that circulating fibrocytes contributed to the population of myofibroblasts in the epiretinal membranes of patients with PDR and

proliferative vitreoretinopathy. The authors identified fibrocytes that had undergone local differentiation into myofibroblasts, using double-immunohistochemical staining to detect coexpression of CD45 and α-SMA, and they suggested that myofibroblasts derived from fibrocytes participated in the formation of FVMs in PDR and proliferative vitreoretinopathy patients. However, the role of fibrocytes in the mechanism of FVM formation has not been investigated in detail. We hypothesized that fibrocytes dependent on chemokines and chemokine receptors might be involved in FVM formation in PDR. Recent studies have demonstrated that chemokines and chemokine receptors are required for the recruitment of fibrocytes to sites of fibrosis. Fibrocytes express various chemokine receptors, including CCR3, CCR5, CXCR4, CCR7, and CCR2.[5] CCR2 is the high-affinity receptor for its ligand, which is monocyte chemoattractant protein-1 (MCP-1)/chemokine (C-C motif) ligand 2 (CCL2). In addition, there have been many reports showing that MCP-1 is increased in the vitreous fluid of PDR patients.[22–28] Therefore, at first, we investigated the presence of fibrocytes in samples of vitreous fluid and FVMs from PDR patients. We also investigated the correlation between the number of fibrocytes and the concentration of MCP-1 in vitreous fluid.

Serum amyloid P component (SAP [aka pentraxin-2]) is a highly conserved protein, and SAP is a soluble pattern recognition receptor involved in the innate immune system that regulates monocyte activation and differentiation. SAP inhibits the differentiation of circulating monocytes into fibrocytes. There have been several reports asserting that the circulating level of SAP may have an important role in fibrosis.[29–33] On the other hand, pentraxin-3 (PTX3) is one of the long pentraxins and is an acute-phase protein that has emerged as a serological marker reflecting tissue inflammation and damage under diverse pathological conditions. In contrast to SAP, which is produced by hepatocytes, PTX3 is produced by macrophages, neutrophils, endothelial cells, epithelial cells, and fibroblasts. Recently, Pilling et al.[34] reported that PTX3 promotes differentiation of human and murine fibrocytes by using an FcγRI-dependent mechanism. They also revealed that the relative levels of SAP and PTX3 at sites of fibrosis may have a significant influence on the differentiation of monocytes into fibrocytes. Therefore, second, we also examined the concentration of PTX3 and SAP in the vitreous fluid of PDR patients.

Methods

Study Population

This study was approved by the ethics committees of Juntendo University Urayasu Hospital, and the surgical specimens were handled in accordance with the Declaration of Helsinki. All patients gave informed consent before being included in the study. Samples of vitreous fluid and FVMs were obtained from PDR patients during vitrectomy. Inclusion criteria for this study were the presence of tractional retinal detachment and an FVM. Patients were excluded if they had refractory neovascular glaucoma or vitreous hemorrhage. At the start of vitrectomy, samples of undiluted vitreous fluid (0.5–1.0 ml) were aspirated under standardized conditions before commencing the intravitreal infusion of balanced salt solution. In PDR patients undergoing pars plana vitrectomy, the FVM was dissected from the retinal surface with horizontal scissors.

Vitreous samples were collected from 27 eyes of 25 patients with PDR (52 ± 16 years of age; 14 men and 11 women) during initial pars plana vitrectomy. As a control, vitreous samples were collected from 23 eyes of 23 patients (66 ± 9 years of age; 11 men and 12 women) who underwent vitrectomy for epiretinal membrane (ERM) or macular hole (MH).

Cell Culture

Samples of 1.5 ml of undiluted vitreous were collected from patients with PDR and controls and centrifuged for 20 minutes at 2000 rpm at 4°C, and the pellet was resuspended in fibroblast complete serum-free medium (FibroLife; Lifeline Cell Technology, Walkersville, MD, USA) basal medium. The fibroblast complete serum-free medium was supplemented with 10 mM HEPES (Sigma-Aldrich, St. Louis, MO, USA), 1× nonessential amino acids (Sigma-Aldrich), 1 mM sodium pyruvate (Sigma-Aldrich), 2 mM glutamine (Invitrogen, Carlsbad, CA, USA), 100 U/ml penicillin, 100 μg/ml streptomycin (Sigma-Aldrich), and 1× ITS-3 (500 μg/ml bovine serum albumin, 10 μg/ml insulin, 5 μg/ml transferrin, 5 ng/ml sodium selenite, 5 μg/ml linoleic acid, and 5 μg/ml oleic acid; Sigma-Aldrich). Vitreous pellets were cultured in 6-well flat-bottomed tissue culture plates (BD Biosciences, Franklin Lakes, NJ, USA) in a volume of 2 ml in a humidified incubator under 5% CO_2 for 2 days at 37°C. Then, cells were counted by at least two independent observers who were blinded to the experimental design.

Differentiation of Cultured Fibrocyte by TGF-β1

Undiluted vitreous samples, 1.5 ml, were collected from patients with PDR and centrifuged for 20 minutes at 2000 rpm at 4°C, and vitreous pellets were cultured using the above-described method. Cultured cells were stimulated with 10 ng / ml TGF-β1 (R&D Systems, Minneapolis, MN, USA) for 2 days.

Immunohistochemistry

For immunofluorescence, vitreous fluid and FVMs were processed using a cytospin method (Shandon Southern, Sewickley, PA, USA) for 5 minutes at 1500 rpm. These slides and the cultured cells were fixed in 4% paraformaldehyde for 10 minutes at room temperature and were subsequently washed several times in phosphate-buffered saline. Blocking of nonspecific antibody binding was performed by incubation with 2% bovine serum albumin for 5 minutes at room temperature, after which the cells were washed in staining buffer. Then the slides were incubated overnight at 4° in staining buffer with the primary antibodies. Double- immunostaining was performed using mouse anti-human CD45 monoclonal antibody (1:200 dilution; Santa Cruz Biotechnology, Dallas, TX, USA) as a hematopoietic cell marker, rabbit anti-human type 1 collagen (COL1) polyclonal antibody (1:100 dilution; Santa Cruz Biotechnology) as a mesenchymal cell marker, rabbit anti-human chemokine (C-C motif) receptor 2 (CCR2) polyclonal antibody (1:700 dilution; Abcam, Cambridge, MA, USA) to detect the MCP-1 receptor, and rabbit anti-human α-SMA polyclonal antibody (1:200 dilution; Abcam Cambridge, MA, USA) as a marker of fibroblasts. Then the cells were washed three times in staining buffer, the secondary antibody was added, and specimens were incubated for 1 hour in the dark. The secondary antibody for CD45 was Alexa Fluor 488 goat anti-mouse immunoglobulin G (IgG; H+L; 1:1000 dilution; Thermo Fisher Scientific, Rockford, IL, USA), whereas that for COL1, CCR2, and α-SMA was Alexa Fluor 594 goat anti-rat IgG (H+L) (1:1000 dilution; Thermo Fisher Scientific). The stained slides were washed three times and mounted using 50% glycerol with 4′,6-diamidino-2-phenylindole (DAPI; 1:2000 dilution; Molecular Probes, Eugene, OR, USA).

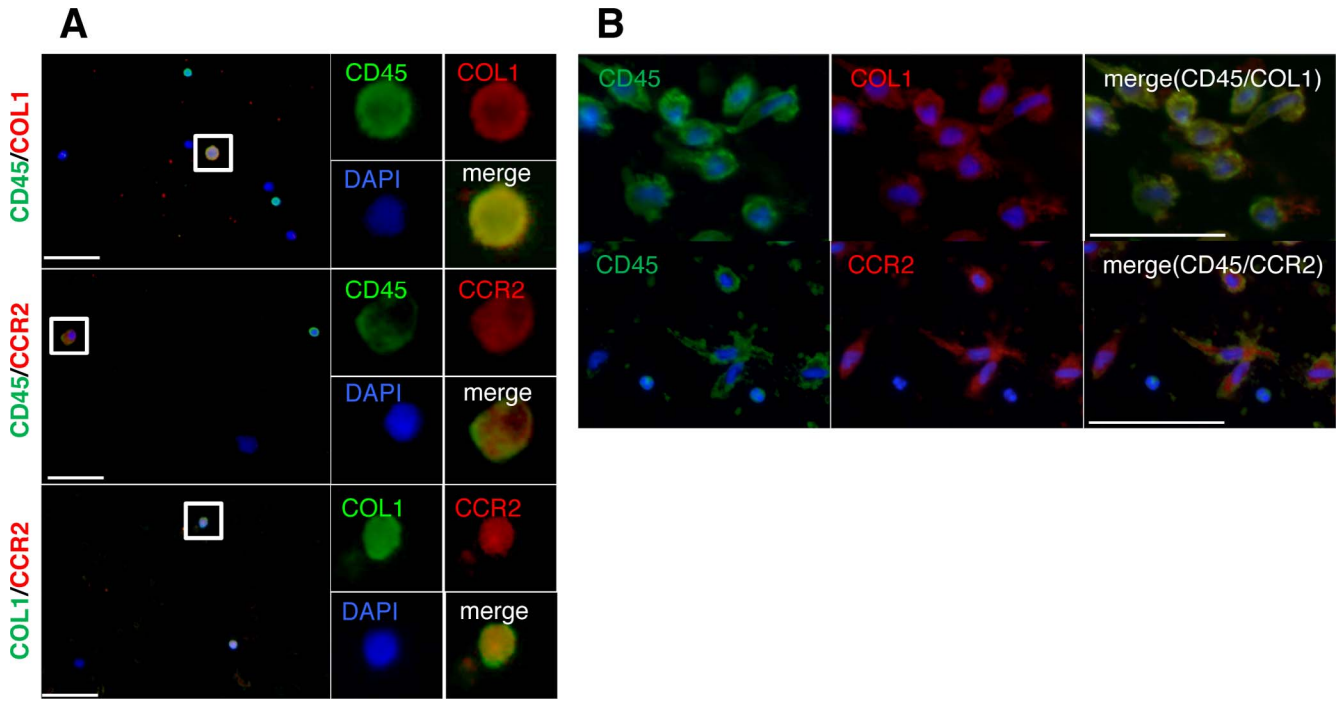

FIGURE 1. Immunostaining of cells in vitreous fluid and FVMs of patients with proliferative diabetic retinopathy. (**A**) CD45/type 1 collagen (COL1), CD45/C-C chemokine receptor type 2 (CCR2), and COL1/CCR2 dual-positive cells were observed in vitreous samples from PDR patients. Nuclei were stained with DAPI (*blue*). *Scale bar*: 50 μm. (**B**) CD45/COL1 and CD45/CCR2 dual-positive cells were detected in FVM. *Scale bar*: 50 μm.

Enzyme-Linked Immunosorbent Assay (ELISA)

The levels of MCP-1, PTX3, and SAP expression were measured in vitreous fluid by ELISA, using kits for human MCP-1, PTX3, and PTX3 (MCP-1 and PTX3 [R&D Systems]; SAP [Hycult Biotech, Uden, the Netherlands]). The vitreous samples were diluted 2.5 times before analysis; 200 μl of diluted vitreous was used for each ELISA.

Statistics

Statistical analysis was performed using Prism v4.03 software (GraphPad Software, San Diego, CA, USA). Differences between two groups were assessed by Student *t*-test. Correlation analysis was assessed by Spearman test. Significance was defined as a P value <0.05.

RESULTS

Fibrocytes in the Vitreous Fluid of PDR Patients

To detect fibrocytes, vitreous fluid samples from PDR patients were examined by immunocytochemical analysis, and CD45/COL-1 double-positive fibrocytes were observed in the samples. These cells were stained by anti-CCR2 monoclonal antibody (Fig. 1A). In contrast, no fibrocytes were detected in vitreous samples from control subjects (data not shown). Next, we used immunohistochemistry to investigate the presence of fibrocytes in FVMs obtained from PDR patients. CD45/COL1 double-positive fibrocytes were detected in FVMs, and CD45/CCR2 double-positive fibrocytes were also detected (Fig. 1B). These results indicated that fibrocytes infiltrated the FVMs of PDR patients and contributed to organization of the membranes.

Culture of Fibrocytes From Vitreous Fluid Samples of PDR Patients

A key morphological characteristic of fibrocytes is an irregular star shape or spindle shape. Cultures of vitreous fluid samples from PDR patients contained spindle-shaped cells (Fig. 2A). To determine whether these cells were fibrocytes, we examined

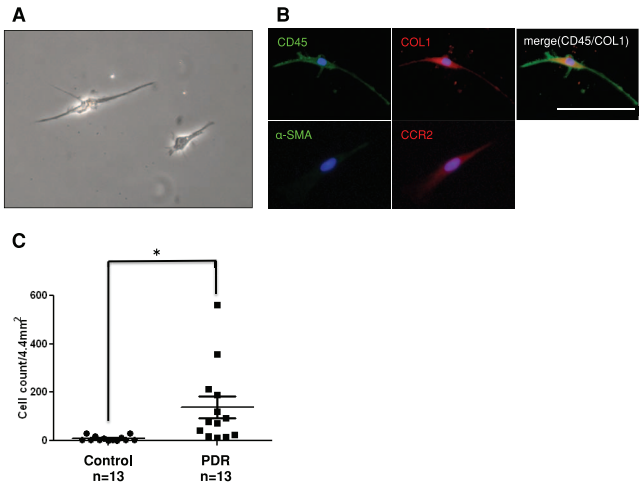

FIGURE 2. Culture of fibrocytes from vitreous fluid samples of proliferative diabetic retinopathy patients. (**A**) Spindle-shaped cells were observed in cultured cells obtained from vitreous samples from patients with PDR. (**B**) Spindle-shaped cells expressed CD45 and type 1 collagen (COL1) and CCR2 but not α-SMA. (**C**) The number of cultured fibrocytes in vitreous fluid of PDR patients (137.7 ± 44.7 cells) was significantly higher than those of controls (8.8 ± 2.9 cells); $^*P < 0.05$. *Scale bar*: 50 μm.

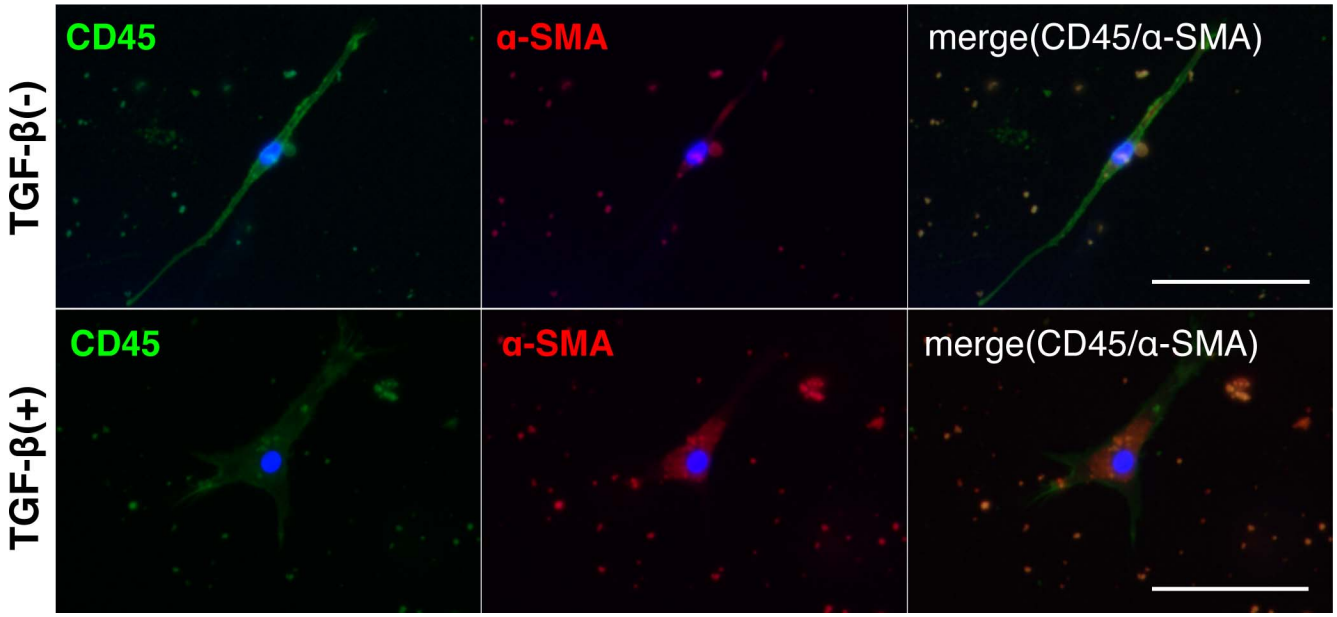

FIGURE 3. Differentiation of fibrocytes into myofibroblasts by TGF-β1. Fibrocytes without the stimulation of TGF-β1 expressed CD45 but not α-SMA. TGF-β1 increased the expression of α-SMA and decreased that of CD45. TGF-β1 also changed spindle shape to fibroblastic shape. *Scale bar*: 50 μm.

the expression of CD45/COL1/CCR2/α-SMA by immunocytochemistry. Expression of CD45/COL1 was detected, confirming that the spindle-shaped cells were indeed fibrocytes. These fibrocytes expressed CCR2 but not α-SMA, which indicated that fibrocytes cultured for 2 days did not differentiate into fibroblasts/myofibroblasts (Fig. 2B).

Next, we examined the number of fibrocytes cultured from the vitreous fluid samples of PDR patients and control subjects. Vitreous samples were collected from 13 eyes of 12 patients with PDR (55 ± 17 years of age; 5 men and 7 women). As a control, vitreous samples were collected from 13 eyes of 13 patients (67 ± 5 years of age; 5 men and 8 women) who underwent vitrectomy for ERM or MH. We defined the spindle-shaped cell as fibrocytes. Then the spindle-shaped cells were counted. We found that a significantly higher number of fibrocytes were cultured from the vitreous samples of PDR patients than from control subjects (137.7 ± 44.7 cells versus 8.8 ± 2.9 cells per vitreous sample, respectively; $P < 0.05$) (Fig. 2C).

Differentiation of Fibrocytes Into Myofibroblasts by TGF-β1

It is well known that TGF-β1 stimulates the differentiation of fibrocytes into fibroblasts/myofibroblasts. We examined whether TGF-β1 caused differentiation of the spindle-shaped fibrocytes into fibroblasts/myofibroblasts. After culture with 10 ng / ml TGF-β1 for 2 days, the spindle-shaped cells showed a morphological change to fibroblastic cells, with decreased expression of CD45 and increased expression of α-SMA. These findings demonstrated that spindle-shaped fibrocytes underwent differentiation into fibroblasts/myofibroblasts during culture with TGF-β1 (Fig. 3).

Vitreous Fluid Concentration of MCP-1/CCL2

We examined the concentration of MCP-1/CCL2 in vitreous fluid samples from PDR patients by ELISA. Vitreous samples were collected from 11 eyes of 10 patients with PDR. For control, vitreous samples were collected from 10 eyes of 10 patients. The mean ± SD MCP-1 concentration was significantly higher in vitreous samples from PDR patients than in those from control patients (2218.1 ± 1206.8 pg/ml versus 810.0 ± 413.2 pg/ml, respectively; $P < 0.05$) (Fig. 4A).

Correlation Between the Number of Cultured Fibrocytes and the MCP-1/CCL2 Concentration in Vitreous Fluid

In PDR patients and controls, the vitreous fluid concentration of MCP-1/CCL2 showed a significant positive correlation with the number of fibrocytes cultured from the vitreous samples ($r = 0.82$; $P < 0.05$) (Fig. 4D).

Vitreous Fluid Concentration of PTX3

Vitreous samples were collected from 19 eyes of 18 patients with PDR. For control, vitreous samples were collected from 13 eyes of 13 patients.

The PTX3 concentration was significantly higher in vitreous fluid samples from PDR patients ($n = 19$) than in those from control patients ($n = 13$; 901.1 ± 421.8 pg/ml versus 280.8 ± 178.4 pg/ml, respectively; $P < 0.05$) (Fig. 4B).

Vitreous Fluid Concentration of SAP

Vitreous samples were collected from 7 eyes of 7 patients with PDR. For control, vitreous samples were collected from 7 eyes of 7 patients.

The SAP concentration was significantly higher in vitreous fluid samples from PDR patients ($n = 7$) than in those from control samples ($n = 7$; 77.5 ± 53.3 ng/ml versus 1.4. ± 1.1 ng/ml, respectively; $P < 0.05$) (Fig. 4C). In PDR patients and controls, the concentration of SAP in vitreous fluid showed a significant positive correlation with the concentration of PTX3 ($r = 0.82$; $P < 0.05$) (Fig. 4E).

DISCUSSION

In the present study, we investigated whether fibrocytes had a role in FVM formation in patients with PDR. The combination

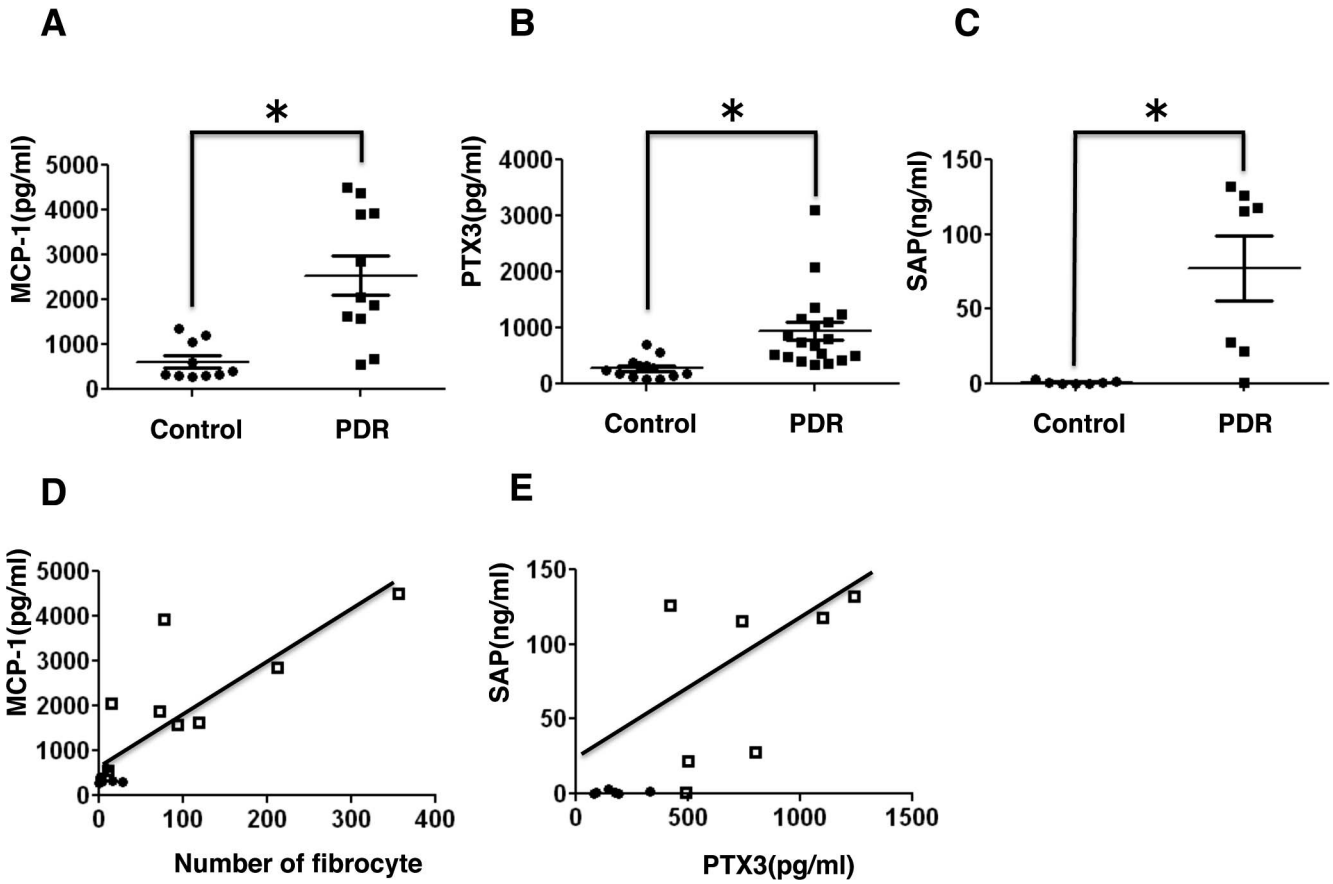

Figure 4. Concentrations of CCL2/MCP-1,PTX3 and SAP in vitreous fluid. The concentrations of MCP-1 (**A**), PTX3 (**B**), and SAP (**C**) in vitreous fluids from PDR patients were higher than those from controls. MCP-1 was 2218.1 ± 1206.8 pg/ml versus 810 ± 413.2pg/ml, respectively; PTX3 was 901.1 ± 421.8 pg/ml versus 280.8 ± 178.4 pg/ml, respectively, and SAP was 77.5 ± 53.3 ng/ml versus 1.4. ± 1.1 ng/ml, respectively (*$P < 0.05$). (**D**) Correlation of the number of cultured fibrocyte with the concentration of MCP-1 in vitreous fluids of PDR patients and control patients ($r = 0.82$, $P < 0.05$). *Square*: PDR group ($n = 8$); *black circle*: control group ($n = 7$). (**E**) Correlation of the PTX3 and SAP in vitreous fluid of PDR patients and control patients. ($r = 0.82$; $P < 0.05$). *Square:* PDR group ($n = 7$); *black circle*: control group ($n = 7$). Patients with macular hole or epiretinal membrane were used as controls.

of collagen production and hematopoietic markers (CD34, CD45) seemed to be sufficient to identify fibrocytes, and we detected CD45/COL1 double-positive fibrocytes in vitreous fluid samples and FVMs harvested from patients with PDR. In addition, the number of fibrocytes cultured from vitreous samples of PDR patients was higher than that from vitreous samples of control patients with ERM or MH. The number of cultured fibrocytes was also significantly correlated with the vitreous fluid concentration of MCP-1 in patients with PDR. Cultured fibrocytes underwent differentiation into myofibroblasts after incubation with TGF-β1. Interestingly, the percentage of fibrocytes among granular cells in the peripheral blood did not differ between PDR patients and control patients (data is not shown). These results suggest that infiltration of fibrocytes into the vitreous fluid may be involved in the pathogenesis of PDR, especially FVM formation.

Recent studies have demonstrated that chemokines and chemokine receptors are required for the recruitment of fibrocytes to sites of fibrosis. Eker et al.[35] reported that human fibrocytes express functional CCR2, after using an optimized technique for isolation of fibrocytes from human blood and demonstrating expression of functional CCR2 by a subpopulation of these cells. Signaling through ligand-CCR2 interactions induces the proliferation of human blood fibrocytes, differentiation of fibrocytes into myofibroblasts, production of type I collagen, and a chemotactic response. Sakai et al.[10] demonstrated that the number of interstitial fibrocytes and the level of urinary MCP-1 were strongly correlated in patients with chronic kidney disease, including diabetic nephropathy. On the other hand, there have been many reports showing that MCP-1 is increased in the vitreous fluid of PDR patients.[22–28] In this study, the number of fibrocytes cultured from vitreous fluid samples of PDR patients was significantly correlated with the MCP-1 concentration in the vitreous fluid of these patients. Therefore, migration of fibrocytes from the blood into the vitreous fluid of PDR patients may depend on the high vitreous concentration of MCP-1.

Fibrocytes participate not only in fibrosis but also in neovascularization. It was recently reported that fibrocytes secrete various angiogenic growth factors, including VEGF, PDGF, and FGF-2.[36] Conditioned medium from cultured fibrocytes promotes angiogenesis both in vitro and in vivo, and injection of circulating fibrocytes enhances angiogenesis during wound healing in diabetic mice.[8] Recently, Li et al.[37] cocultured fibrocytes with vascular endothelial cells and studied wound healing in mice and demonstrated that fibrocytes stabilize blood vessels during angiogenesis. These findings suggest that infiltration of fibrocytes leads to an increase of VEGF in vitreous fluid and induces neovascularization. MCP-1 has also been shown to mediate neovascularization directly. Vascular endothelial cells expressing CCR2, the

receptor for MCP-1, demonstrate chemotaxis and tube formation in response to stimulation with MCP-1 in vitro.[28] Therefore, MCP-1 may directly or indirectly participate in induction of neovascularization in PDR.

SAP is a member of the pentraxin family of proteins that includes C-reactive protein (aka pentraxin-1) and PTX3. SAP is a 27-kDa protein produced by the liver and secreted into the blood to circulate as a stable 135-kDa pentamer. In humans and most mammals, the SAP concentration remains relatively constant between 20 and 50 μg/ml.[38] SAP inhibits the differentiation of circulating monocytes into fibrocytes.[29–33] There have been several reports that the circulating level of SAP may have an important role in fibrosis. Gomer et al.[29] found that serum from patients with scleroderma or mixed connective tissue disease was less effective at inhibiting fibrocyte differentiation and contained low levels of SAP protein. In addition, it was reported that SAP injection reduced bleomycin-induced pulmonary fibrosis in animals and also reduced persistent lung inflammation with fibrosis in SAP knockout mice.[33,39] Other studies have shown that injection of human or murine SAP inhibits cardiac fibrosis[40], dermal wound healing[32] and kidney injury.[41] In a phase 1B clinical trial, recombinant human SAP improved the lung function of patients with pulmonary fibrosis.[42] These findings suggest that SAP may have a crucial role in tissue fibrosis. In the present study, the SAP concentration of vitreous fluid samples from PDR patients was higher than that in vitreous samples from control patients (77.5 ± 53.3 ng/ml versus 1.4. ± 1.1 ng/ml, respectively). The blood-ocular barrier may usually prevent SAP from entering the vitreous fluid, while breakdown of this barrier and vitreous hemorrhage may increase the vitreous fluid level of SAP in PDR patients. However, the vitreous fluid concentration of SAP in PDR patients was much lower than the average serum level (20–50 μg/ml), suggesting that it may be not high enough to prevent differentiation of fibrocytes and development of FVM. Thus, injection of recombinant human SAP into the vitreous is a potential new treatment that could inhibit the differentiation of monocytes to fibrocytes, resulting in prevention of FVM formation in PDR.

PTX3 is the prototype of the long pentraxins. PTX3 is expressed and released by hematopoietic and stromal cells in response to primary proinflammatory stimulation. In healthy humans, the plasma levels of PTX3 is low (<25 ng/ml), whereas PTX3 levels may rise to 1000 ng/ml during inflammation. Recently, Pilling et al.[34] reported that PTX3 promotes differentiation of human and murine fibrocytes by an FcεRI-dependent mechanism. However, the fibrocyte inhibitory activity of SAP is dominant over PTX3. That study also revealed that the relative levels of SAP and PTX3 at sites of fibrosis may have a significant influence on the differentiation of monocytes into fibrocytes. This study demonstrated that the PTX3 concentration of vitreous fluid samples from PDR patients was higher than in control samples. The average PTX3 concentration of vitreous fluid from PDR patients was 901.1 ± 421.8 pg/ml, while it was 280.8 ± 178.4 pg/ml in vitreous fluid from controls. Therefore, intravitreous differentiation of fibrocytes in PDR patients may depend on the imbalance between SAP and PTX3.

In conclusion, this study identified fibrocytes in vitreous fluid samples and FVMs harvested from PDR patients. There was a significant positive correlation between the number of fibrocytes cultured from vitreous fluid and the vitreous fluid concentration of MCP-1 in PDR patients. Our results suggest that the pathogenesis of FVM may be related to infiltration of fibrocytes into the vitreous fluid, which may be regulated by MCP-1, SAP, and PTX3. Accordingly, fibrocytes may be a novel therapeutic target for treatment of PDR.

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