Mesenchymal Stem Cells Modulate Light-induced Activation of Retinal Microglia Through CX3CL1/CX3CR1 Signaling

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ABSTRACT

Purpose: To evaluate the effect of CX3CL1/CX3CR1 signaling on the interaction between mesenchymal stem cells (MSCs) and retinal microglia.

Methods: Supernatants of homogenized retina were harvested from light-damaged SD rats (ISHR) to stimulate retinal microglia. Stimulated microglia were cocultured with MSCs, CX3CL1 over-expressing MSCs (CX3CL1-MSCs) or CX3CL1-blocked MSCs (anti-CX3CL1-MSCs) for 24 hours, and their molecular and functional changes were examined. Moreover, soluble CX3CL1 was directly added to microglia cultures.

Results: ISHR stimulation activated retinal microglia. MSCs coculture inhibited the protein expression of pro-inflammatory factors by activated microglia, increased the protein expression of neurotrophic factors, and was accompanied with upregulation of CX3CR1. Meanwhile, MSCs suppressed proliferative and migratory function of activated microglia, but promoted the phagocytic capability. These effects were strengthened by CX3CL1-MSCs, and reversed by anti-CX3CL1-MSCs. Soluble CX3CL1 could enhanced microglial migration.

Conclusions: MSCs might restore homeostatic functions of retinal microglia responded to light damage mainly through CX3CL1/CX3CR1 signaling.

Keywords: Mesenchymal stem cells, microglia, retinal degeneration, chemokines CX3CL1, inflammation

INTRODUCTION

Mesenchymal stem cells (MSCs) could integrate into the retina after intraocular transplantation. It has been demonstrated that MSCs may rescue retinal cells not only by direct release of various neurotrophic factors but also by promoting resident cells to secrete cytokines leading to revert functional lesion. MSCs are considered as promising candidates for retinal degeneration (RD) treatment. Nevertheless, the signal cross-talk between MSCs and retinal resident cells remains unclear.

It has been shown that the pathogenesis of RD is supposed to be dramatically associated with dysregulated inflammation in retina. As resident immunocompetent and phagocytic cells, microglia contribute to the inflammatory state of the retina. Microglia could be rapidly triggered by a variety of injurious signal inputs. It is generally thought that microglial activation has a dual effect, controlled activation of microglia is beneficial, but it can become detrimental if dysregulated. Microglia-derived proinflammatory cytokines, nitric oxide, glutamate, or caspases may result in photoreceptor death.

Chemokine (C-X3-C motif) ligand 1 (CX3CL1), also known as fractalkine, is the only member of the CX3C chemokine family. CX3CL1 acts exclusively via its receptor, CX3CR1, on microglia to regulate neuroinflammation in the central nervous system (CNS). In the eye, CX3CL1 is constitutively expressed in retinal neurons, RPE, and vascular endothelial cells. However, the role of CX3CL1/CX3CR1 signaling pathway is still controversial. In several neurological disorder models, CX3CR1 deficiency dysregulates microglial responses, leading to neurotoxicity. Conversely, other researchers have...
shown that CX3CR1-deficient mice could reduce activation of microglia, and protect neurological function.\textsuperscript{14,15}

MSCs-conditioned medium was previously reported to upregulate the expression of CX3CR1 in microglia, separated from amyotrophic lateral sclerosis (ALS) mouse model.\textsuperscript{16} Although we have also shown previously that subretinal transplantation of CX3CL1-expressing MSCs could inhibit retinal microglial activation in vivo,\textsuperscript{9} the real contribution of CX3CL1/CX3CR1 signaling in the control of retinal inflammation has not been elucidated, to the best of our knowledge.

Therefore, in this study we employed an in vitro coculture system to evaluate the effect of MSCs on regulating microglial function following light-induced microglial activation. Moreover, we tested the hypothesis that MSCs might protect against retinal inflammation triggered by microglia via CX3CL1/CX3CR1 signaling.

**MATERIALS AND METHODS**

**Animals**

All animal procedures conformed to the Association for Research in Vision and Ophthalmology (ARVO) Statement on the Use of Animals in Ophthalmic and Vision Research. Adult male Sprague–Dawley (SD) rats (Slaccas, Shanghai, China), each weighing 100–130 g, were maintained in 12-h cyclic light:dark conditions (60–80 lux). For light-induced retinal injury, rats were adapted in the dark for another 24 h and then were returned to the normal cyclic light condition.

Supernatants of homogenized retina (SHR) were harvested from 10 light-damaged rats or normal rats, as previously described.\textsuperscript{17} Briefly, retinas were collected and homogenized in DMEM/F12 (Gibco, Carlsbad, CA, USA) under sterile conditions. To remove DNA and RNA of the retina, 100 U of benzonase (Sigma, USA) was incubated with 1 mL of the suspension for 30 min at 37°C. Each suspension was centrifuged at 12 000 g and 4°C for 20 min. The supernatant was collected and diluted to a final protein concentration of 200 μg/mL with DMEM/F12. Resultant SHR solutions were stored at −20°C until use.

**Primary Retinal Microglia Culture**

The primary microglial culture was performed based on Dong et al.’s published protocol, with minor modifications.\textsuperscript{18} In brief, retinas were collected from 12 newborn SD rats and then digested with 0.125% trypsin for 30 min at 37°C to generate single cell suspension. The trypsin was subsequently inactivated by fetal bovine serum (FBS) (Gibco, Carlsbad, CA, USA). Then, the cells were resuspended in DMEM/F-12 culture medium containing 10% FBS, 1% microglia growth supplement (ScienCell, USA), 100 U/mL penicillin, and 100 μg/mL streptomycin, plated onto 75 cm\textsuperscript{2} culture flasks and incubated at 37°C, humidity 95% and CO\textsubscript{2} 5%. The culture medium was changed at 24 h. After being cultured for 2 weeks, mixed glial cells were shaken at 200 rpm for 1 h. The supernatant containing microglia were harvested to reseed, and used for the following experiments. Purity of microglia was performed using a FACSCalibur flow cytometer for each batch produced (n = 6).

**Bone Marrow-derived MSCs Culture and Expression of CX3CL1 on MSCs**

Bone marrow-derived MSCs were derived from 6–8-week-old SD rats as described previously.\textsuperscript{3} In brief, six rats were sacrificed with an overdose of pentobarbital, and their tibias and femurs were dissected. Bone marrow was flushed out with 10 mL low-glucose DMEM, supplemented with 10% FBS, 100 U/mL penicillin, 100 μg/mL streptomycin, and plated at 1.0 × 10\textsuperscript{6} cell/cm\textsuperscript{2} in 25 cm\textsuperscript{2} culture dishes. The cells at passages 3–6 were used for the following experiments. MSCs were characterized by positive expression for CD90 and CD29-FITC, and negative expression for CD45 and CD34 (Pharmacia Corporation, USA). The MSCs purity was analyzed using flow cytometry for each batch produced (n = 3).

CX3CL1 over-expressing MSCs (CX3CL1-MSCs) were obtained by lentiviral vector, as described previously.\textsuperscript{3} MSCs were seeded in 6-well plates (1 × 10\textsuperscript{5} cells/well) and allowed to adhere for 12 h. Lentivirus encoding CX3CL1 (LV-CX3CL1) or green fluorescent protein (LV-GFP) were added to MSCs at a multiplicity of infection (MOI) of 10 for each construct. The cultured medium was removed after 24 h of exposure, and the cells were washed once with DMEM/F12 and then replenished with normal medium.

**Blocking of CX3CL1 Produced by MSCs**

Cultured MSCs were treated with a CX3CL1 neutralizing antibody (1.0 μg/mL, R&D Systems, Minneapolis, MN, USA) for 2 h, an isotype goat IgG antibody incubation was set as a control. The cells were washed three times with phosphate-buffered saline (PBS) and then transferred to the co-culture. CX3CL1 release was assessed using commercial rat CX3CL1 ELISA kits (Ray Biotech, GA, USA) according to the manufacturer’s instructions.
Co-culture of Retinal Microglial Cells with MSCs

Primary microglia were reseeded on 6-well plates (1.0 × 10^5 cells/well). SHR from light-injured rats (ISHR) and normal rats (NSHR) were added to the culture medium for 24 h (200 μL/well). Rat fibroblasts (ATCC, USA) were cultured in DMEM/F-12 containing 10% FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin. Fibroblasts (FB) were set as sham cells in the co-culture system.

MSCs, CX3CL1-MSCs, CX3CL1-blocked MSCs (anti-CX3CL1-MSCs) or FB were resuspended and plated at 1.0 × 10^5 cells/well on 6-well Transwell polycarbonate membrane inserts, with a 0.4 μm pore size (Corning, NY, USA). After 12 h adhesion, these inserts were placed into the wells with microglia described above to set up a co-culture system.

Western Blot Analysis

Total protein was extracted from the cells and concentration was determined using a BCA kit (Pierce, Rockford, IL, USA). The proteins were separated by electrophoresis on a 10% SDS-polyacrylamide gel and then transferred to a high-quality polyvinylidene difluoride (PVDF) membrane. The membrane was blocked with 5% non-fat dry milk in Tris-buffered saline (TBS) solution and incubated with primary antibodies overnight at 4ºC. The following antibodies were used (all from Santa Cruz Biotechnology, Santa Cruz, CA, USA): rabbit anti-CNTF (1:200); rabbit anti-BDNF (1:500); rabbit anti-CX3CR1 (1:300); rabbit anti-IL-1β (1:200); rabbit anti-TNF-α (1:100); and rabbit anti-GAPDH (1:1000). After washing, each blot was incubated with horseradish peroxidase-conjugated secondary antibody (goat anti-rabbit IgG-HRP, Santa Cruz, CA, USA) for 1 h and visualized with Enhanced Chemiluminescence (Amersham, UK). The quantitation of each band was carried out by Quantity One software 4.6 (Bio-Rad Laboratories, Hercules, CA, USA), and was normalized against its own internal control.

Cell Proliferation Assay

Microglial proliferation was evaluated using the Cell-Light™ EdU DNA Cell Proliferation Kit (RiboBio, Guangzhou, China) according to the manufacturer’s instructions. Briefly, microglia after co-culture were exposed to 50 μM EdU for 2 h at 37ºC and then fixed with 4% paraformaldehyde for 30 min. The cells were permeabilized in 0.5% Triton X-100 for 10 min at room temperature and washed with PBS. Each well was incubated with 500 μL 1 × Apollo® reaction cocktail for 30 min. Nuclei were counterstained with 5 μg/mL Hoechst 33342 for 20 min and imaged under a fluorescent microscope (Olympus, Tokyo, Japan). EdU-labelled cells (red cells) and Hoechst 33342-labelled cells (blue cells) were counted using Image-Pro Plus (IPP) 6.0 software (Media Cybernetics, Bethesda, MD, USA), and the percentages of EdU positive cells were calculated.

Microglial Chemotaxis Assay

The in vitro migration of microglia was evaluated using 24-well Boyden chambers (Corning) containing polycarbonate membranes (8 μm pore size) that allowed for migration of cells from the upper chamber into the bottom chamber. Briefly, microglia were digested by 0.25% trypsin-EDTA after co-culture, resuspended with DMEM/F-12 containing 2% FBS at a concentration of 5 × 10^5 cells/mL, and then 100 μL of cell resuspension solution was seeded into the upper chamber; 600 μL of DMEM/F-12 containing 20% FBS was added to the bottom chamber.

After incubation for 12 h, the inserts were removed from the apparatus, and the cells that did not migrate and stayed on the upper membrane surface were removed with a cotton swab. The migrated cells on the bottom surface of the membrane were fixed in 4% paraformaldehyde for 10 min and were stained using 0.5% crystal violet. The number of migrated cells was counted in six randomly chosen fields under ×200 magnification and averaged.

Phagocytosis Assay

After 24 h co-culture, 500 μL of microglia (2 × 10^6 cells/mL in DMEM/F12) were added to the polypropylene tubes, and then 10 μL of carboxylate-modified polystyrene latex beads (10^8 particles/mL, L4655, Sigma) were added to the cultures for 30 min at 37ºC. The phagocytosis was stopped by adding 2 mL of ice-cold PBS. The cells were washed three times with ice-cold PBS, and then analyzed by flow cytometry. Briefly, the cells were detected in the FL-1 channel. The phagocytic activity is represented by mean fluorescent intensity (MFI). At least three independent experiments were performed.

Exogenous Administration of Soluble CX3CL1

The rat recombinant soluble CX3CL1 protein (5 ng/mL, R&D, USA) was directly added to microglia culture upon ISHR stimulation for 24 h, and the protein expression of CNTF, BDNF, CX3CR1, IL-1β, and TNF-α was detected by Western blot. The capabilities of proliferation, migration, and phagocytosis of ISHR-stimulated microglia were also measured, as described above.
Statistical Analysis

Data are presented as means ± SD. Statistical analysis was performed by one-way ANOVA with the Tukey–Kramer multiple comparison test performed to estimate differences between groups (SPSS 16.0; SPSS Inc., Chicago, IL, USA). A p value of <0.05 was considered statistically significant. Error bars indicate SD.

RESULTS

Evaluation of CX3CL1 Production from MSCs

ELISA analysis showed that secretion of CX3CL1 from CX3CL1-MSCs was significantly higher than that from GFP-MSCs (p < 0.05; Figure 1). Compared with isotype control antibody, CX3CL1 neutralizing antibody could downregulate CX3CL1 secretion in MSCs in vitro (p < 0.05). There was no significant difference among normal MSCs, GFP-MSCs and the control treated with isotype antibody (p > 0.05).

Supernatants of Homogenized Retina from Light-injured Rats (ISHR) Activated Retinal Microglia

Primary retinal microglia exhibited either rounded, bipolar, or multipolar appearance. Purity of microglia was (85.42 ± 2.31)% assessed by flow cytometry using a CD11b antibody.

Western blot analysis indicated that initial expression of TNF-α and IL-1β protein was low in primary retinal microglia. As shown in Figure 2A, the expression of TNF-α and IL-1β protein in microglia was significantly upregulated by ISHR compared to the levels observed in NSHR-treated and normal microglia (p < 0.05). The percentage of EdU-labelled cells in ISHR-stimulated group was higher than that in NSHR-stimulated group and unstimulated group (p < 0.05; Figure 2B). ISHR induced increased retinal microglial migration compared with the control and NSHR-treated group (p < 0.05; Figure 2C). Moreover, the phagocytosis of microglia was estimated by latex beads engulfment. Microglia incubated with ISHR showed higher mean fluorescence intensity (MFI) value compared to the control and NSHR-treated group (p < 0.05; Figure 2D), indicating that phagocytic activity was enhanced.

MSCs Inhibited the Expression of Proinflammatory Factors, Upregulated the Expression of Neurotrophins and CX3CR1

To evaluate whether MSCs could affect the activation state of microglia, we set up a microglia-MSCs co-cultured system. Microglia were stimulated by ISHR for 24 h, and then co-cultured for another 24 h with MSCs, CX3CL1-MSCs, anti-CX3CL1-MSCs, or FB.

We found that the protein expression of two proinflammatory factors, TNF-α and IL-1β, was downregulated in ISHR-treated microglia facing either normal MSCs or CX3CL1-MSCs, compared with stimulated microglia after 24 h of co-culture (p < 0.05; Figure 2A). Compared with MSCs treatment, the expression was further reduced when co-cultured with CX3CL1-MSCs. To further confirm the role of CX3CL1 in regulating function of activated microglia, CX3CL1 neutralizing antibodies were used to block membrane-bound CX3CL1 on MSCs (anti-CX3CL1-MSCs). We observed no statistically significant difference among ISHR-activated group, FB-co-cultured group and anti-CX3CL1-MSCs-co-cultured group (p > 0.05). Exogenous CX3CL1 mimics the effect of CX3CL1-MSCs on the protein expression of TNF-α and IL-1β by activated microglia.

As shown in Figure 3B, the protein expression of CNTF and BDNF in microglia was markedly increased in CX3CL1-MSCs group, which was higher than that in MSCs group or CX3CL1 group (p < 0.05). Blockage of CX3CL1 on MSCs resulted in the downregulation of CNTF and BDNF. The protein expression of CX3CR1 have the similar changes to that of neurotrophins (p < 0.05).

MSCs Suppressed the Proliferative and Migratory Function of Activated Microglia, but Promoted the Phagocytic Capability

In order to assess whether CX3CL1 on MSCs has a major impact on modulating microglia function upon activation, we examined the proliferation, migration and phagocytosis of microglia after co-culture.

As shown in Figure 4, the percentage of EdU-labelled cells in MSCs group was decreased compared with ISHR-
FIGURE 2. ISHR can activate retinal microglia in vitro. Initial expression of TNF-α and IL-1β protein in primary retinal microglia was low, ISHR induced upregulation of protein expression of TNF-α and IL-1β (A, n = 6, *P < 0.05). The percentage of EdU-positive cells in ISHR-stimulated group was higher than NSHR-stimulated group and unstimulated group (B, n = 3, *P < 0.05, **P < 0.01). Compared with the control and NSHR-treated groups, ISHR stimulated microglia exhibited stronger migration (C, n = 8, *P < 0.05) and phagocytosis (D, n = 3, *P < 0.05). Yet there was no statistically significant difference between unstimulated and NSHR stimulated microglia. Values are mean ± SD. Scale bars indicate 100μm. Abbreviations: NSHR, supernatants of homogenized retina from normal SD rats; ISHR, supernatants of homogenized retina were harvested from light-damaged SD rats.

FIGURE 3. MSCs coculture affects the protein expression of cytokines by activated microglia. Western blot analyses showed that cocultured with MSCs inhibit the expression of TNF-α and IL-1β by activated microglia (A). Notably, CX3CL1-MSCs induced most significant down-regulation after 24 hours coculture. Similar to CX3CL1-MSCs, exogenous CX3CL1 treatment could also suppress the expression of TNF-α and IL-1β. We found no statistically significant difference among ISHR-activated group, FB-cocultured group and anti-CX3CL1-MSCs-cocultured group. Soluble CX3CL1 mimics the effect of CX3CL1-MSCs on the expression of TNF-α and IL-1β. MSCs coculture could increase protein expressions of CNTF, BDNF and CX3CR1, which was higher in CX3CL1-MSCs group than that in MSCs group or CX3CL1 group (B). A significant difference was observed between MSCs-treated and CX3CL1-MSCs-treated groups. Anti-CX3CL1-MSCs reverted the upregulation in the expression of CNTF, BDNF and CX3CR1. The changes of CX3CR1 expression on activated microglia was accompanied with the changes of CX3CL1 expression on MSCs. The expression levels of the cytokines were normalized to GAPDH levels. Values are mean ± SD. n = 6, *P < 0.05 Abbreviations: ISHR, supernatants of homogenized retina were harvested from light-damaged SD rats; CX3CL1-MSCs, CX3CL1 over-expressing MSCs; anti-CX3CL1-MSCs, CX3CL1 blockage on MSCs by a neutralizing antibody; FB, Fibroblasts; CX3CL1, exogenous soluble CX3CL1.
stimulated group ($p < 0.05$), which indicated MSCs inhibited microglial proliferation in vitro. As anticipated, the percentage of EdU-labelled cells was lower in CX3CL1-MSCs group than MSCs group ($p < 0.05$). We also observed that soluble CX3CL1 led to inhibition of microglial proliferation. Accordingly, CX3CL1 blockage on MSCs accelerated the proliferation of microglia. There was no significant difference between ISHR-stimulated group and anti-CX3CL1-MSCs group ($p > 0.05$).

We observed a significant decrease of microglial migration when co-cultured with MSCs or CX3CL1-MSCs. CX3CL1-MSCs treatment induced least microglial migration ($p < 0.05$; Figure 5). Instead, anti-CX3CL1-MSCs reverted the suppressed migratory function. Compared with ISHR-stimulated group, soluble CX3CL1 induced stronger migratory activity of microglia ($p < 0.05$).

Microglia co-cultured with MSCs in Transwell showed MFI value of $670.61 \pm 33.61$, which indicated...
the phagocytosis was enhanced compared to that of stimulated microglia \((p < 0.05)\). Microglia co-cultured with CX3CL1-MSCs showed MFI value of 742.80 ± 7.928 and activated microglia in presence of CX3CL1-blocked MSCs showed MFI value of 590.48 ± 4.454 \((p < 0.05); \text{Figure 6}\). No significant difference was observed between CX3CL1-MSCs group and soluble CX3CL1 group \((p > 0.05)\).

**DISCUSSION**

The signaling pathways involved in microglial activation in degenerative retina are complicated and not fully understood. In the current study, we found that MSCs have the capability to modulate the secretion of various neurotrophins and proinflammatory factors on retinal microglia activated by light-induced lesion, accompanied with beneficial changes of microglial functions. These effects were reinforced by CX3CL1-MSCs, while weakened by anti-CX3CL1-MSCs. Microglial response to injurious stimulus is decisive for maintaining homeostasis in the retina. In the early stage of RD, the retina respond to various stress that initiate microglial activation, and evoke retinal remodeling programs. Nevertheless, excessive activation induce a set of cell signals that lead to irreversible impairment of retinal cells. Our results confirm that light-induced retinal injury could activate retinal microglia in vitro, leading to upregulation of proinflammatory factors, enhanced proliferative, migratory, and phagocytic capacities. Light-induced damage to the retina shares several characteristics with other types of RD, including dysfunction and death of retinal cells, migration of resident retinal microglia, and clearance of apoptotic bodies by activated retinal microglia and infiltrating macrophages.\cite{20,21}

The immunomodulatory properties of MSCs translate into anti-inflammatory effects in numerous animal studies.\cite{22,23} Inflammation has recently been recognized to contribute to the pathogenesis of RD.\cite{20,24,25} We observed that interaction with MSCs reduced the increase of the expression of proinflammatory factors by activated microglia in vitro, and induced a significant upregulation of neurotrophic factors. Meanwhile, co-cultured with MSCs, was accompanied with upregulated expression of CX3CR1 on activated microglia. Furthermore, these effects could be enhanced by upregulated expression of CX3CL1 on MSCs, and inhibited by CX3CL1-blocked MSCs. Our results illustrate that MSCs might affect the expression of cytokines on activated microglia via CX3CL1/CX3CR1 signaling. It was generally accepted that CX3CL1/CX3CR1 signaling exert neuroprotective effect in vitro. Consistent with our results, Cardona et al. indicated that CX3CL1 controls the degree of microglial activation and ensuing neurotoxicity. The upregulation of CX3CR1 expression protects against microglial neurotoxicity.\cite{26} However, a neurotoxic role for CX3CL1/CX3CR1 signaling has been demonstrated in studies using CX3CR1-deficient mice, which may be due to compensatory mechanisms and not be the direct result of CX3CR1 deficiency.\cite{10,15}

Upon activation, microglia are able to enter the cell cycle, proliferate and mediate a neurotoxic response. It has previously been demonstrated that MSCs co-culture could restore the percentage of microglia at S and
In the present study, we found that the anti-proliferative effect would be augmented by either CX3CL1-MSCs or soluble CX3CL1, and inhibited by anti-CX3CL1-MSCs. This effect might be closely related to the levels of proinflammatory factors produced by retinal microglia. The structural changes are accompanied by dysregulated inflammation, which in turn produce additional proinflammatory chemokines and cytokines.25,30 CX3CL1 exists either as a static membrane-bound glycoprotein mediating cell adhesion or a soluble chemokine after proteolytic cleavage.2,8,9 CX3CL1-CX3CR1 signaling is involved in microglial migration. The latter could be suppressed in CX3CR1-deficient mice, which led to primary subretinal microglia accumulation.32 We demonstrated that soluble CX3CL1 induced an increased number of migratory microglia. Conversely, our results showed that MSCs treatment inhibited the migratory function of activated microglia. CX3CL1-MSCs co-culture could further decline the number of mobilized microglia, corresponding with better functional preservation. The superior anti-migratory effect of microglia was abolished by CX3CL1-blocked MSCs. That might be related to membrane-bound CX3CL1 in MSCs that could function as an inhibitor to regulate microglial migration induced by Müller cells.33,34

Interaction of MSCs and microglia might be a possible way that promote endogenous repair. We have also shown that MSCs enhanced this phagocytic capability of stimulated microglia. After exposure to CX3CL1-MSCs or soluble CX3CL1, microglia engulfed much more latex beads as compared with other groups, contributing to remove cellular debris and apoptotic cells. Conversely, blockade of CX3CL1 on MSCs resulted in a striking inhibition of the phagocytic function. Our present results are in line with the current evidence that MSCs, through the release of CX3CL1, may bind to CX3CR1 in microglia, thus exerting neuroprotective and immunomodulatory effects.34,35 Either membrane-bound CX3CL1 or soluble CX3CL1 might enhance homeostatic role of microglia in the maintenance of a healthy retina.

Most importantly, this is the first study to our knowledge to induce microglial activation with SHR from light-injured rats, which was commonly triggered by lipopolysaccharide (LPS) or interferon-γ (IFN-γ).34 Meanwhile, this study has several limitations: (1) CX3CL1/CX3CR1 downstream signaling have not been extensively investigated; (2) whether other signaling pathways involve in regulation of retinal microglia by MSCs was not evaluated. Even so, our results could partly interpret the mechanism of transplanted MSCs in RD treatment.

In conclusion, MSCs might attenuate inflammation in light-injured retina, and instruct retinal microglia toward a beneficial phenotype mainly through CX3CL1/CX3CR1 signaling pathway. Accordingly, maintaining microglial homeostatic functions by MSCs appears to be effective in treating RD.

DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the article.

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