Mesenchymal Stem Cells Ameliorate Experimental Autoimmune Uveoretinitis by Comprehensive Modulation of Systemic Autoimmunity

Xiaomin Zhang,1,2 Xinjun Ren,1,2 Guangda Li,1 Chunna Jiao,1 Lei Zhang,3 Shaozhen Zhao,1 Jiantao Wang,1 Zhong Chao Han,3 and Xiaorong Li1

PURPOSE. The authors studied the therapeutic effect of rat mesenchymal stem cells (MSCs) on experimental autoimmune uveoretinitis (EAU) induced in rats by peptide 1169–1191 of the interphotoreceptor retinoid-binding protein (IRBP).

METHODS. The authors intravenously injected syngeneic (isolated from Lewis rats) or allogeneic (isolated from Wistar rats) MSCs into IRBP-induced EAU Lewis rats, either before disease onset (simultaneous with immunization, preventive protocol) or at different time points after disease onset (therapeutic protocol). T-cell response to IRBP 1169–1191 from MSC-treated rats was evaluated, Th1/Th2/Th17 cytokines produced by lymphocytes were measured, and CD4+/CD25+ regulatory T cells (Treg) were detected.

RESULTS. MSC administration before disease onset not only strikingly reduced the severity of EAU, it also delayed the onset of the disease. MSC administration was also effective after disease onset and at the peak of disease, but not after disease stabilization. Clinical efficacy for all treatments was consistent against EAU development.8–15 Recently, the role of the IL-17-producing effector T-cell type, Th17, is now being intensively studied in EAU.16–24 Experiments suggest that Th17 is the other kind of uveitogenic effector T cells, playing a crucial role in the progress of EAU, and Th1 and Th17 are differentially required for EAU.17–19,24 Blockade of the negative regulatory factors of Th17 results in an exacerbation of EAU at later phases with augmented IL-17 production.19 In addition, CD4+/CD25+ regulatory T cells (Treg) play an important role in the regression of EAU.25 The upregulation and adoptive transfer of Tregs ameliorate EAU,26,27 whereas the dysregulation and malfunction of Tregs may contribute to EAU recurrence.28 Mesenchymal stem cells (MSCs) have shown much promise in the past decade as pluripotent cells for use in regenerative medicine.29 These cells are able to differentiate into various cell lineages and preferentially home in on damaged tissues, where they exert anti-inflammatory and tissue-protective effects and support the growth of other cells.30–32 Further clinical interest in MSCs has been raised by the observations that they can exert profound immunosuppression both in vitro and in vivo by inhibiting the proliferation and function of a number of cell types, including T-lymphocytes,33,34 NK cells,35 and dendritic cells.36 The mechanisms underlying these effects are largely unknown but are likely to be mediated by soluble factors.39–41 MSCs have also shown a form of immune privilege that allows allogeneic and xenogeneic transplantation because their immunoregulatory ability is independent of the major histocompatibility complex.42 They are also able to escape from allogeneic immune responses because of their poor immunogenicity.43 In addition, no tissue toxicity of MSCs has been found until now.

E XPERIMENTAL AUTOIMMUNE UVEORETINITIS (EAU) IS A PROTOTYPE T-CELL-MEDIATED AUTOIMMUNE DISEASE THAT TARGETS THE RETINA.1 IT CAN BE INDUCED IN SUSCEPTIBLE ANIMALS BY IMMUNIZATION WITH EVOLUTIONARILY CONSERVED RETINAL PROTEINS, SUCH AS THE INTERPHOTORECEPTOR RETINOID-BINDING PROTEIN (IRBP)2 AND S-ANTIGEN (S-Ag)3 OR BY ADAPTIVE TRANSFER OF ANTIGEN-PRIMED T CELLS.1 EAU CLOSELY RESSEMBLES THE IMMUNOPATHOLOGIC FEATURES OF HUMAN POSTERIOR UVEITIS, A MAJOR SIGHT-THREATENING DISEASE,4 AND IN MANY WAYS IS SIMILAR TO ANOTHER MODEL OF AUTOIMMUNITY, EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS (EAE).5 THEREFORE, DETAILED STUDY OF THERAPEUTIC APPROACHES TO EAU MAY AID THE TREATMENT OF OTHER OCULAR AND AUTOIMMUNE DISEASES.

In EAU, it is generally believed that the Th1 subset of CD4+ T cells, which characteristically produce IFN-γ and IL-2, act as one kind of uveitogenic effector T cells.5 Susceptibility to EAU has been associated with an increased expression of genes of Th1-type cytokines and chemokines,6 whereas Th2 type cells, which produce IL-4 and IL-10, are believed to be protective.8 Upregulation of the Th2 response or downregulation of the Th1 response prevents inflammatory responses and protects against EAU development.8–15 Recently, the role of the IL-17-producing effector T-cell type, Th17, is now being intensively studied in EAU.16–24 Experiments suggest that Th17 is the other kind of uveitogenic effector T cells, playing a crucial role in the progression of EAU, and Th1 and Th17 are differentially required for EAU.17–19,24 Blockade of the negative regulatory factors of Th17 results in an exacerbation of EAU at later phases with augmented IL-17 production.19 In addition, CD4+/CD25+ regulatory T cells (Treg) play an important role in the regression of EAU.25 The upregulation and adoptive transfer of Tregs ameliorate EAU,26,27 whereas the dysregulation and malfunction of Tregs may contribute to EAU recurrence.28 Mesenchymal stem cells (MSCs) have shown much promise in the past decade as pluripotent cells for use in regenerative medicine.29 These cells are able to differentiate into various cell lineages and preferentially home in on damaged tissues, where they exert anti-inflammatory and tissue-protective effects and support the growth of other cells.30–32 Further clinical interest in MSCs has been raised by the observations that they can exert profound immunosuppression both in vitro and in vivo by inhibiting the proliferation and function of a number of cell types, including T-lymphocytes,33,34 NK cells,35 and dendritic cells.36 The mechanisms underlying these effects are largely unknown but are likely to be mediated by soluble factors.39–41 MSCs have also shown a form of immune privilege that allows allogeneic and xenogeneic transplantation because their immunoregulatory ability is independent of the major histocompatibility complex.42 They are also able to escape from allogeneic immune responses because of their poor immunogenicity.43 In addition, no tissue toxicity of MSCs has been found until now.

From the 1Tianjin Medical University Eye Center, Tianjin, China; and the 3State Key Laboratory of Experimental Hematology, Institute of Hematology and Blood Diseases Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Tianjin, China.

1These authors contributed equally to this work presented here and should therefore be regarded as equivalent first authors.

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Corresponding author: Xiaorong Li, Tianjin Medical University Eye Center, 251 Fu Kang Road, Tianjin, China 300384; xiaorli@hotmail.com.

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For these reasons, the use of MSCs has been suggested as an ideal strategy for treating immune-mediated diseases, and their immunosuppressive properties have been exploited in a number of experiments of autoimmune diseases, and organ transplantation. Results from these experiments confirm the therapeutic plasticity of MSCs arising from their capacity to modulate immune system responses and to inhibit inflammation.

In the present study, we investigated the effect of MSCs on EAU development, T cell proliferation, Th1/Th2/Th17 cytokine profiles of T cells, and Tregs. We observed that MSCs could strikingly ameliorate both the clinical symptoms and the pathologic manifestations after their injection either at the beginning of inflammation or during onset and peak phases of the disease. This effect might be caused by inhibition of both the Th1 and Th17 responses and upregulation of the Th2 response and Tregs. These data support the potential use of MSCs as a treatment for ocular autoimmune diseases.

**Materials and Methods**

**Animals**

Male Lewis and Wistar rats (age range, 6–8 weeks; weight range, 160–180 g) were obtained from Vital River (Beijing, China). Throughout the experiment, food and water were provided ad libitum. Animals were housed under 12-hour light/12-hour dark cycles. All procedures involving rats were approved by the Laboratory Animal Care and Use Committee of the Tianjin Medical University and adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

**Reagents**

A truncated form of IRBP peptide 1169–1191 (PTARSVGADGSWEGVGV) was synthesized and purified by Sangon (Shanghai, China). Complete Freund’s adjuvant (CFA) was purchased from Sigma (St. Louis, MO). Mycobacterium tuberculosis strain H37RA was obtained from Difco (Detroit, MI). Pertussis toxin (PTX) was purchased from Sigma. Cell proliferation ELISA kit was obtained from Roche (Basel, Switzerland). ELISA kits for quantitative analysis of interleukin (IL)-2, interferon (IFN)-γ, IL-4, IL-10, and IL-17 levels were from R&D Systems (Minneapolis, MN).

**Isolation and Characterization of MSCs**

MSCs were isolated from Lewis or Wistar rats. Briefly, the rats were anesthetized, the bone marrow was removed, and the gelatinous bone marrow was suspended in phosphate-buffered saline (PBS). A single-cell suspension was then achieved by passage through a 21-gauge needle. The cells resuspended in PBS were laid on the top of a Percoll gradient (d = 1.073 g/mL) in a 50-mL Falcon tube and were centrifuged for 25 minutes at 900 g. The intermediate zone, which was ivory white and rich in MSCs, was collected. A single-cell suspension was then achieved by passage through a 21-gauge needle. The cells resuspended in PBS were laid on the top of a Percoll gradient (d = 1.073 g/mL) in a 50-mL Falcon tube and were centrifuged for 25 minutes at 900 g. The intermediate zone, which was ivory white and rich in MSCs, was collected.

**Clinical Assessment of EAU**

The incidence and severity of EAU were examined with a slit lamp on day 4 during the preclinical stage of the disease and daily thereafter to assess disease development. Clinical signs of inflammation were scored in a masked fashion as grades 0 to 4 according to Caspi. Rats were followed up, unless moribund, for 21 days after immunization.

**Histology**

Animals were killed on day 21. The eyes were prefixed for 1 hour in a vial containing 4% glutaraldehyde/PBS and then were fixed in 10% formaldehyde/PBS at least overnight before they were embedded in paraffin. Sections measuring 4-μm thick were stained with hematoxylin and eosin. At least four sections of each eye, cut at different levels, were prepared. Based on the number and extent of lesions seen in the tissue, histopathologic disease scores were assigned blinded on a scale of 0 to 4 using the criteria of Caspi.

**Lymphocyte Proliferation Assays**

To measure the direct suppressive effect of MSCs on uveitic T cell proliferation, mononuclear cells (MNCs) for proliferation were obtained from the spleens and lymph nodes of either healthy rats or EAU-affected rats 21 days after immunization. Cell suspensions were prepared using 70-μm filters and separating MNCs from Ficoll gradient (Roche). Cells were washed twice in PBS and suspended in medium (Complete RPMI 1640; Gibco BRL, Grand Island, NY) containing 2 mM glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin, 10% fetal bovine serum (HyClone), and 50 μM β-mercaptoethanol (ICN Biomedicals, Irvine, CA). MNC suspensions were seeded into 96-well, flat-bottomed microtiter plates (Corning, Corning, NY) at a concentration of 10^6 cells/well in a final volume of 200 μL/well. Cells were treated with 30 μg/mL IRBP peptide or concanavalin A (ConA, 2.5 μg/mL; Sigma) in medium supplemented with 10% FBS. Syngeneic MSCs were irradiated (35 Gy) and then added to the plates at different ratios (0:1; 0.2:1; and 1:1 for MSCs/T cells). In experiments addressing T-cell inhibition in vivo, MNCs were isolated from the spleens and lymph nodes of IRBP-immunized rats on day 21 and treated with either MSCs or vehicle alone. The cells were plated at a concentration of 10^6 cells/well in a final volume of 200 μL/well in 96-well, flat-bottomed, microtiter plates in the presence of 30 μg/mL IRBP peptide. In every experimental condition, each culture was performed in triplicate, and the plates were incubated in a humidified atmosphere of 5% CO_2 at 37°C for 3 days. T cell proliferation was studied thereafter in medium (Complete RPMI 1640; Invitrogen) by measurement of BrdU incorporation using a cell proliferation ELISA kit according to the manufacturer’s instructions.
MSCs Ameliorate EAU

RESULTS

MSCs Ameliorate EAU

As shown in Figure 1, most of the IRBP-treated rats began to show early signs of uveitis on day 9, including dilated blood vessels in the iris, abnormal pupil contraction, or a hazy anterior chamber (Fig. 1B). A severe illness developed on day 12 that was characterized by an opaque anterior chamber, a dull red reflex or red reflex absent, or an obscured anterior chamber (Fig. 1C) and that eventually diminished by day 21 (Fig. 1D). Peak clinical scores of 3 and 4 were observed on day 12.

In group treatment simultaneous with immunization, MSC infusion strikingly reduced disease severity, and MSC-treated rats showed only slight signs of anterior chamber inflammation, reaching statistical significance from day 6 onward when compared with control rats (Figs. 1E–H, 2A). In IRBP-immunized rats without MSCs, EAU fully developed (grade 3 or more) in 8 of the 10 rats. However, MSC addition nearly completely suppressed the clinical expression of EAU (grade 3 or more) in 8 of the 10 rats. However, MSC addition nearly completely suppressed the clinical expression of EAU (grade 3 or more) in 8 of the 10 rats.

At the end of the experiment (day 21), we collected peripheral blood from IRBP-immunized rats that were treated at the beginning of immunization with either allogeneic MSCs or vehicle alone to determine the number of Tregs. Cells were stained for surface CD4 and CD25 with PE-anti-CD4 and FITC-anti-CD25, fixed with solution (Cytofix/Cytopermer; BD PharMingen, San Diego, CA), and analyzed by flow cytometry (FACScalibur; BD Biosciences, Franklin Lakes, NJ). The ratios of CD4+CD25+ Treg cells/CD4+ T cells were calculated in each group.

FoxP3 Expression Analysis

Total RNA was isolated from the spleens of untreated or allogeneic MSC-treated IRBP-immunized rats at the end of the experiment (day 21) with a purification kit (RNasy Mini Kit; Qiagen, Milan, Italy). RNA was treated with DNase (RNase-Free DNase Set; Qiagen) to avoid contamination of the genomic DNA. We used first-strand cDNA synthesis (SuperScript II First-Strand Synthesis System; Invitrogen, San Diego, CA) to synthesize complementary DNA. Real-time polymerase chain reaction (PCR) was performed using FoxP3-specific primers (forward, 5'-CTCACCCCCACCTAC AGGCC-3'; reverse, 5'-GGCATCCCAAGTGGAAGT-3') and probe (5'-6-FAM-TCTCCAGGACAGCAACTTTGATGATTG-3'). Gene expression levels were measured as the ratio of expression values and internal GAPDH (Rodent GAPDH Control Reagents [VIC-labeled]; Applied Biosystems, Monza, Italy).

Statistical Analysis

Results of EAU clinical scores were assessed by repeated-measures ANOVA using mixed models. Histopathologic disease scores were calculated by the nonparametric Mann-Whitney U test. A two-tailed Student’s t test was used to evaluate the frequency of CD4+CD25+ T lymphocytes, FoxP3 expression, supernatant concentrations of IL-2, IFNγ, IL-10, and IL-4 of the in vitro effect of MSCs on T cells, and the production of IL-17. Cell proliferation rates and supernatant concentrations of IL-2, IFN-γ, IL-10, and IL-4 of the in vivo effect of MSCs on T cells were analyzed by one-way ANOVA. Data are expressed as mean ± SD. P < 0.05 was considered significant.

Cytokine Production

IFN-γ, IL-2, IL-4, IL-10, and IL-17 production levels were measured in supernatants derived from 72-hour cultures of lymphocytes stimulated by IRBP under various conditions using a commercially available ELISA kit according to the manufacturer’s instructions.

Determination of Tregs

At the end of the experiment (day 21), we collected peripheral blood from IRBP-immunized rats that were treated at the beginning of immunization with either allogeneic MSCs or vehicle alone to determine the number of Tregs. Cells were stained for surface CD4 and CD25 with PE-anti-CD4 and FITC-anti-CD25, fixed with solution (Cytofix/Cytopermer; BD PharMingen, San Diego, CA), and analyzed by flow cytometry (FACScalibur; BD Biosciences, Franklin Lakes, NJ). The ratios of CD4+CD25+ Treg cells/CD4+ T cells were calculated in each group.
pupil contraction (grade 1), were primarily found from day 8 (9 of 10 rats), whereas in rats treated early with MSCs, the onset of clinical EAU was delayed to day 11 (13 of 19 rats).

MSC injection at disease onset (days 9–11) and at the peak of disease (days 12–14) halted the disease progression when compared with controls. This resulted in a statistically significant reduction in disease scores from the third day of injection (Figs. 2B, 2C). However, MSC injection from day 16, on disease stabilization, showed no significant clinical amelioration compared with control rats (Fig. 2D).

Consistent with the clinical effect, histologic examination of the retinal sections showed striking IRBP-induced inflammatory cell infiltration and retinal architecture damage in the control group (Fig. 3B), whereas MSC preventive treatment greatly reduced the inflammatory cell infiltration and retinal damage, and the entire structure of the retina was apparently normal (Fig. 3C). Delayed MSC treatment at disease onset and at the peak of disease also led to fewer inflammatory cell infiltrations and less extensive retinal edema and photoreceptor damage compared with the control group (Figs. 3D, 3E). However, MSC injection on disease stabilization showed no significant pathologic improvement (Fig. 3F). The final histologic scores of the rats in the first three treating groups were significantly lower than those in the control group (Fig. 4).

**MSCs Inhibited Proliferation of Pathogenic T Cells In Vitro and Prevented T Cell Response on In Vivo Injection**

Uveitogenic T cells, cultured in the presence of MSCs, showed significantly reduced rates of proliferation when challenged with IRBP 1169–1191 or ConA. This inhibition was dose dependent when the ratios of MSCs to T cells increased from 0.2:1 to 1:1 (Figs. 5A, 5B). To verify whether MSCs affected the capacity of T cells from treated rats after intravenous administration, we tested the IRBP response of T cells from MSC-treated rats and vehicle-treated control rats. The proliferative response of T cells from MSC-treated rats was significantly reduced compared with that of T cells from control rats when challenged with IRBP (Fig. 5C).

**MSC Treatment Reduced Th1 and Th17 but Elevated Th2 Cytokine Secretions in IRBP-Immunized Rats**

We first checked the Th1 and Th2 cytokine expression profiles in supernatants of rechallenged IRBP-primed T lymphocytes in the presence or absence of MSCs at the ratio of 1:1. In vitro, MSCs significantly decreased the production of IFN- \( \gamma \) and increased the production of IL-10 of T lymphocytes from EAU rats, but no significant effect was seen on IL-2 and IL-4 secretions.

**Figure 2.** Effects of MSCs on the development of EAU. In groups treated simultaneously with immunization, the differences between either allogeneic or syngeneic MSC-treated and vehicle-treated conditions were statistically significant from day 6 to day 12 after immunization (A). The groups treated 9 days after immunization showed significant amelioration from day 11 and peaked on day 13 (B). The groups treated 12 days after immunization showed significant improvement on day 14 and peaked on day 15 after immunization (C). No significant difference was found between MSC-treated and control rats with EAU in groups treated 16 days after immunization (D). Values are expressed as mean ± SD. *\( p < 0.05 \).
cantly reduced histopathologic signs and inflammatory cell infiltration (Fig. 4). Allogeneic and syngeneic MSCs also showed a similar immunosuppression potential with regard to T cell proliferation and Th1 and Th2 cytokine secretion (Figs. 5C, 7).

**MSC Treatment Upregulates Tregs**

To explore the possibility that the in vivo immunosuppressant action of MSCs was mediated by activation of a cascade of different cell types (i.e., Tregs), we detected the frequency of peripheral Tregs characterized by the CD4<sup>+</sup>CD25<sup>+</sup> phenotype within peripheral blood cell populations from IRBP-immunized rats treated with MSCs at the beginning of the experiment. We observed that CD4<sup>+</sup>CD25<sup>+</sup> T lymphocytes represented a mean ± SD of 5.42% ± 0.73% of the peripheral blood cell populations of untreated EAU rats, whereas they were upregulated in the peripheral blood of EAU rats receiving MSC treatment to 8.25% ± 1.62% (mean ± SD frequency; P < 0.05; Fig. 9A). We also assessed the expression rate of the forkhead transcription factor FoxP3, a molecular marker characterizing activated cells with an immunoregulatory function.57 Spleen cells from EAU rats treated with MSCs expressed significantly higher levels of mRNA for FoxP3 (mean ± SD relative expression rate, 2.84 ± 0.41) than untreated rats (relative expression rate, 1.18 ± 0.49; P < 0.05; Fig. 9B).

**DISCUSSION**

A therapeutic effect of MSCs in autoimmune disease was first addressed in EAE,44 a murine model of multiple sclerosis (MS) in which the infusion of ex vivo–expanded MSCs resulted in both clinical and histologic improvement. Similar effects have been observed in other subsequent studies of EAE45,46 and in experimental models of other autoimmune diseases, such as rheumatoid arthritis,47,48 diabetes,49,50 colitis,51,52 multorgan autoimmunity,56 and systemic lupus erythematosus.55 Recently, several clinical cases showing the successful application of MSCs in autoimmune diseases have been reported.53-55

In this study, we first determined the therapeutic role of MSCs in different phases of EAU development. MSC therapy remarkably reduced the severity of IRBP-induced EAU when administered before disease stabilization, and it delayed the

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**Figure 3.** Histopathologic changes of posterior segments of the eyes from the rats in different groups. (A–F) MSCs were given to rats. (A) Typical stratiform morphology of the retina of a normal rat eye (grade 0). (B) Retinal detachment, severely damaged photoreceptor layer, and inflammatory cell infiltration in rats with EAU (grade 3). (C) Nearly normal retinal structure with only a few inflammatory cell infiltrations in EAU rats treated with MSCs simultaneously with immunization (grade 0.5). (D) The retina was somewhat damaged and slightly irregular in rats treated 9 days after immunization (grade 1). (E) The retina was mildly damaged and irregular in rats treated 12 days after immunization (grade 2). (F) Similar to control rats, marked retinal inflammation and lesions in rats treated 16 days after immunization (grade 3). V, vitreous; G, ganglion cell layer; BP, bipolar cell layer; PR, photoreceptor layer; C, choroids. Magnification, ×200.

**Both Syngeneic and Allogeneic MSCs Protect against IRBP-Induced EAU**

Both allogeneic and syngeneic MSCs efficiently protected against IRBP-induced ocular inflammation (Fig. 3) and...
onset of the disease if administered preventively. Clinical efficacy for all treatments was demonstrated by a decreased mean maximum score and a cumulative disease score, which were consistent with reduced cellular infiltrates and milder uveal and retinal impairment. Preventive treatment proved to be much better than delayed treatment according to the final histopathologic results. However, in a clinical setting, treatment usually is adopted only after uveitis has developed. There-
fore, despite the observation that preventive treatment with MSCs was more effective, it is of therapeutic importance that MSCs were also effective when administered to animals with established disease. Conversely, we could not detect any improvement of clinical or histopathologic scores when MSCs were injected after the disease reached a chronic phase, possibly because EAU is not a chronic disease but resolves in 3 weeks. At day 16 after immunization, the immunologic mechanisms involved in the disease are not active anymore and MSCs, at that time, have no role in downregulating them.

The beneficial effect of MSCs on IRBP-induced EAU was not restricted to syngeneic systems because allogeneic MSCs were also as efficient in ameliorating both clinical and pathologic signs of EAU. These findings suggest that the immunosuppressive action of MSCs is not restricted by the major histocompatibility complex and that the infused allogeneics are sufficiently well immunotolerated by the host, which facilitates future clinical applications of these cells in uveitis.

Previous reports showed no indication that the therapeutic effects of MSCs were due to tissue repair sustained by the regeneration of damaged cells. Rather, the effects were apparently explained by modulation of the autoimmune attack of the target tissue. The clinical results are consistent with an effect occurring during the early inflammatory phase of the disease, suggesting the possibility that MSCs could affect the generation of effector T cells. For this reason, we addressed whether MSCs can inhibit T-cell responses within the peripheral compartment of the EAU-affected rat. In vitro coculture of uveitogenic T cells and MSCs at different ratios in the presence of IRBP 1169–1191 or ConA showed that both syngeneic and allogeneic MSCs could significantly inhibit uveitic T cell proliferation in a dose-dependent manner. Comparison of the T cell response with IRBP 1169–1191 from treated rats and from EAU-affected control rats showed a striking inhibition of T cell proliferation in the MSC-treated rats, further verifying an in vivo suppressive action of MSCs on the capacity of pathogenic T cells.

The development of EAU in rats is associated with the activation of a Th1-like response during the acute phase. In contrast, the subsequent activation of a Th2-like response is believed to serve a protective function. Th1 and Th2 closely regulate each other: IFN-γ inhibits the generation and function of Th2 cells, whereas IL-4 or IL-10, or both, inhibit the generation and function of Th1 cells. IFN-γ plays a critical role in the attraction of mononuclear cells to the eyes in EAU. In contrast, IL-10 is an important anti-inflammatory cytokine that protects against EAU by inhibiting the activation and effector function of T cells and monocytes/macrophages and by prompting the differentiation and function of some T regulatory cells. In our study, MSC therapy significantly decreased the production of IFN-γ and increased the production of IL-10 of T lymphocytes isolated from MSC-treated EAU rats. In vitro, MSCs had a similar effect on the cytokine profile of IRBP-primed T lymphocytes of EAU rats. These data suggest

**Figure 7.** In vivo effect of MSCs on Th1 and Th2 cytokine production. In groups treated simultaneously with immunization, the production of IL-2 and, especially, IFN-γ was significantly decreased, whereas concentrations of IL-10 and IL-4 were markedly increased compared with the control group. In the group treated 12 days after immunization, the presence of MSCs increased IL-4 and IL-10 production and lowered concentrations of IFN-γ. The decrease in IL-2 production was not statistically significant. Values are expressed as mean ± SD. *P < 0.05 (n = 10).
that MSC treatment might shift the immune balance from Th1 to Th2 dominance, thereby decreasing the incidence or severity of EAU in rats.

However, further study showed that Th2 cells also had the ability to induce uveitis, provided that one used immunodeficient hosts.68 Furthermore, in the past several years, the role of a recently discovered IL-17–producing effector T cell type, Th17, has been intensively studied with respect to autoimmune diseases, including EAU.16–24 Experiments suggest a rather complex relationship exists between Th1 and Th17. Th17 is negatively regulated by the Th1 cytokine IFN-γ and the Th2 cytokine IL-4. Blockade of IFN-γ and IL-4 results in the exacerbation of EAU at later phases with augmented IL-17 production.17–19,24 It is suggested that Th17 might participate primarily in the late phases of EAU and that Th1 and Th17 are differentially required for EAU.19 All these findings further complicate the pathogenic mechanism of uveitis and shed light on the heterogeneity of human disease. It could be hypothesized that Th1, Th17, and Th2 have pleiotropic effects that may be contradictory or consistent at different stages or conditions of the immune response. Thus, it may be the balance between them that determines the outcome of a uveitogenic challenge. In our study, MSCs also showed effects in established EAU, and the cytokine secretion profile of T cells separated from MSC-treated EAU rats after the remission of the disease still showed characteristically low IFN-γ and high IL-10, which hinted that MSCs might also negatively regulate Th17. Interestingly, MSCs have been shown to inhibit Th17 in EAE.69 Therefore, further investigation is being carried out to explore the effect of MSCs on the dynamic balance of Th1/Th2/Th17.

In addition, CD4+CD25+ regulatory T cells (Treg) act to control the self-antigen-reactive T cells in autoimmune diseases. A significantly increased frequency and immunoregulatory action of CD4+CD25+ Treg cells is associated with the development and regression of EAU, suggesting that these cells are induced during EAU and may be involved in its regression.70 In our study, MSC therapy in EAU rats significantly upregulated Treg cells in the peripheral blood and the expression rate of FoxP3 in the spleen, which suggest that MSCs treatment might also activate a cascade of Treg cells, thereby inhibiting EAU in rats.

Taken together, these results suggest that MSCs can effectively prevent and ameliorate EAU. Their action apparently occurs through the inhibition of pathogenic T cell responses, modulation of the balance of Th1/Th2/Th17, and activation of Treg cells, which confirms the therapeutic plasticity of MSCs on immunologic diseases because of their capacity for comprehensively modulating systemic autoimmunity. Further in-depth studies should now explore the mechanism of MSC action in EAU to confirm the long-term therapeutic effects and potential side effects. Given the success of cell therapy with MSCs for the treatment of graft-versus-host disease in humans and for many autoimmune conditions in animal models, we believe that the use of MSCs is an attractive potential therapeutic approach for the treatment of autoimmune disease. However, adequate preclinical experiments are required before these results can be translated to a clinical setting.

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**Figure 8.** Effect of MSCs on Th17. In vitro, MSCs significantly decreased the production of IL-17 of rechallenged IRBP-primed T lymphocytes (A). IL-17 expression of rechallenged T lymphocytes isolated from EAU rats treated with MSCs simultaneously with immunization was also reduced compared with the control group (B). *P < 0.05 (n = 7).

**Figure 9.** Immunophenotype of MSC-educated Tregs. Top: frequency of CD4+CD25+ regulatory T cells isolated from MSC-treated or untreated immunized rats. Results are representative of one experiment. Bottom: FoxP3 expression levels of spleen cells isolated from MSC-treated or untreated IRBP-immunized rats. Expression levels were analyzed by real-time polymerase chain reaction. Values are expressed as mean ± SD. *P < 0.05 (n = 8).
References

37. Spaggiari GM, Capobianco A, Becchetti S, Mingari MC, Moretta L. Mesenchymal stem cell natural killer cell interactions: evidence that activated NK cells are capable of killing MSCs, whereas MSCs can inhibit IL-2-induced NK-cell proliferation. Blood. 2006;107:1484–1490.
40. Meisel R, Zibert A, Laryea M, Gobel U, Daubener W, Dilloo D. Human bone marrow stromal cells inhibit allogeneic T-cell re-


