**ORBITAL FIBROBLASTS FROM GRAVES’ ORBITOPATHY PATIENTS SHARE FUNCTIONAL AND IMMUNOPHENOTYPIC PROPERTIES WITH MESCENHYMAL STEM/STROMAL CELLS**

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**PURPOSE.** In Graves’ orbitopathy (GO), inflammation and expansion of the retrobulbar tissue are the result of a pathophysiologic process in which orbital fibroblasts (GO-Fs) are considered the central cell type. However, in a previous study we observed that GO-Fs expressed some of the consensus surface markers described for mesenchymal stem/stromal cells (MSC). In this study, we further elucidate the stem cell characteristics of GO-Fs by comparing them with orbital fat-derived mesenchymal stem cells.

**METHODS.** We enriched primary human GO-MSCs and GO-Fs simultaneously from the same retrobulbar fat biopsies obtained during decompression surgery of GO patients. The biological characteristics of donor-matched GO-MSCs and -Fs were compared along criteria that define MSC: fibroblast-like growth, MSC surface marker profile, multilineage differentiation potential, and immunomodulatory functions.

**RESULTS.** Application of a standardized isolation and expansion protocol yielded GO-MSCs, which showed plastic adherent fibroblast-like morphology and proliferated and produced hyaluronan similarly to GO-Fs. Both GO-MSCs and GO-Fs expressed orbital fat-derived stem cell surface markers CD29, CD44, CD71, CD73, CD90, CD105, and CD166 and were negative for CD34, CD45, and CD146, and Stro-1 after ex vivo expansion. Further, GO-MSCs and GO-Fs displayed adipogenic, osteogenic, chondrogenic, myogenic, and neuronal differentiation, although GO-Fs with a lower capacity. In addition, when compared to GO-MSCs, the GO-Fs showed reduced T-cell suppression and secreted reduced amounts of IL-6, suggesting a lower immunosuppressive potential.

**CONCLUSIONS.** The in vitro data obtained in this study provide the first experimental evidence that orbital fibroblasts derived from retrobulbar fat of GO patients share biological characteristics with MSCs. These findings provide new insight into the biology of key cells in GO.

Keywords: mesenchymal stem/stromal cells, orbital fibroblasts, Graves’ orbitopathy

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Graves' orbitopathy (GO) is the most common extrathyroidal manifestation of Graves' disease.1 Graves' orbitopathy is a fibroproliferative disease of autoimmune origin characterized by inflammation and by remodeling and expansion of the retroocular connective tissue.2 Resident orbital fibroblasts (GO-Fs) are recognized as the central cell type mediating inflammation and tissue remodeling in GO. A series of observations made in vitro suggested that once GO-Fs were activated during the inflammatory process in response to anti-thyroid-stimulating hormone receptor (TSHR) autoantibodies, immigrated T cells and macrophages, inflammatory cytokines, or some combination of these, GO-Fs reacted with excessive proliferation and adipogenesis, as well as production of hyaluronan and proinflammatory cytokines.3,4 However, GO-Fs were recognized as a heterogenous cell population containing subtypes exhibiting different adipogenic/myogenic differentiation ability, as well as production of inflammatory mediators, and thus may be differently involved in connective tissue remodeling and immune responses in GO.5,6 In a previous study, we recognized that GO-Fs expressed some of the consensus surface markers described for mesenchymal stem cells (MSC); they were positive for CD73, CD90, and CD105 and negative for CD11b, CD19, CD31, CD45, and HLA-DR.7 This surface marker set with the cells’ ability to undergo adipogenic and myogenic differentiation8 makes us wonder whether GO-Fs meet further characteristics of MSCs. Mesenchymal stem cells are fibroblast-like undifferentiated nonhematopoietic progenitor cells that are capable of differentiating into mesenchymal (fat, bone, cartilage, and muscle) and nonmesenchymal lineages (e.g., neuron).9 Recent evidence suggests that MSCs play an important role in both tissue remodeling and immune response.10 Because of their regenerative and immunoregulatory properties, MSCs are an attractive tool for the cellular treatment of inflammation and autoimmunity, which is reflected by an increasing number of clinical trials using allogenic MSCs in graft-versus-host disease, Crohn's disease, or multiple sclerosis.9 Previously, we identified
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METHODS

Patients

During orbital decompression surgeries, surgical tissue explants of orbital adipose tissue were obtained from 12 patients with active, severe GO, as classified by the European Group on Graves’ Orbitopathy. The mean clinical activity score (CAS) was 5.2 (range, 3–10), and the mean NOSPECS classification was 10.1 (range, 6–14). The mean duration of GO was 10.5 years (range, 6–108) months. The mean age of patients was 54.6 years, and 85.3% were female. Patients receive anti-inflammatory treatment (steroids) immediately before decompression, and orbital irradiation was applied to four patients. Decompression was performed as primary therapy because of severe corneal exposure; intravenous steroids were given postoperatively. The study followed the tenets of the Declaration of Helsinki and was approved by the institutional ethics committees. All study participants gave written informed consent.

Isolation and Culture of Cells

After resection, orbital fat tissue samples or control samples from the mucosal tissue of the ethmoid from GO patients were collected aseptically in NaCl (0.9%; Fresenius Kabi, Bad Homburg, Germany). Subsequently, tissues were washed several times with PBS and cut into 1- to 2-mm pieces. Orbital fibroblasts were isolated in accordance with Bahn et al. In brief, the orbital tissue pieces were placed directly in six-well culture plates in standard culture medium (Dulbecco’s modified Eagle’s medium [DMEM] supplement with 10 mM HEPES and 1% fetal bovine serum, 1% penicillin/streptomycin, 1% sodium pyruvate, 100 mM nonessential amino acids) and grown in a humidified atmosphere of 5% CO2 at 37°C. After 2 to 3 weeks the tissue pieces were removed, nonadherent cells were removed by washing with PBS, and the remaining cells were cultivated as described earlier.

Potential mesenchymal stem cells from the orbital fat tissue (GO-MSC) were isolated in accordance with an isolation method for classical adipose-derived stem cells. Briefly, the tissue pieces were digested in PBS/1%BSA solution containing 0.1% collagenase type I (Worthington Biochemical Corporation, Lakewood, NJ, USA) for 60 minutes at 37°C with gentle agitation. Tissues were centrifuged at 300 g for 5 minutes. Tubes were vigorously shaken to release fat cells from the fibrous tissue. The cell suspension was then centrifuged again, and the supernatant was discarded. The pellet containing stromal cells was washed several times and resuspended in standard culture medium. Cells were transferred to 24-well dishes; nonadherent cells were removed by washing with PBS 72 hours later, and fresh culture medium was added over the remaining cells. Potential MSCs were isolated from control samples of the mucosal tissue of the ethmoid from GO patients (GO-ETC) with an MSC protocol as described earlier.

Multiparameter Flow Cytometry

For immunophenotyping of GO-Fs and GO-MSCs, the following antibodies were used simultaneously in multiparameter multicolor flow cytometry, which included all markers within one staining procedure: CD29 (β1-integrin, clone MAR1; BD Bioscience, Heidelberg, Germany), CD31APC-Fluor 780 (PE-CAM1, clone WM59; eBioscience, Frankfurt am Main, Germany), CD54 FITC (My10, clone 581, Class 3; Invitrogen, Karlsruhe, Germany), CD44 PE (clone 515; BD Bioscience), CD45 V500 (leukocyte common antigen, clone H130; BD Bioscience), CD71APC (transferrin receptor, clone AD2; BD Bioscience), CD73 PerCP-Fluor 710 (ecto-5-NT, SH4; clone AD2; eBioscience), CD90 PE-Cy7 (Thy-1; clone 5E10; BioLegend, Fell, Germany), CD105 PE (clone 3A6; BD Bioscience), CD146 Brilliant Violet 421 (ALCAM, clone 3A6; eBioscience), and Stro-1 Alexa Fluor 647 (clone Sro-1; BioLegend). To determine nonspecific signals, isotype controls were used at the same concentration as for the specific antibody. Analysis was performed using a BD FACSCanto II flow cytometer (BD Bioscience) with Diva Software 6.0.

Adipogenic Differentiation

For adipogenic differentiation, 6 × 10^4 cells were seeded on round glass slides in 24-well culture dishes and cultured in 1 mL standard culture medium until reaching 80% confluence. Adipogenic differentiation was induced with Mesenchymal Stem Cell Adipogenic Differentiation Medium (PromoCell, Heidelberg, Germany). In order to examine the generation of oil droplets in the cytoplasm after differentiation, cells were fixed with 10% formalin (Sigma-Aldrich Corp., St. Louis, MO, USA) and stained with Sudan III (Sigma-Aldrich Corp.) for 60 minutes at room temperature. In brief, cells were washed twice in PBS, and 0.03% Sudan III in 10 mL 70% ethanol was added. Hematoxylin was used to counterstain nuclei. Images were generated using an AxioCam MRc microscope camera and Axioscience AxioVision AxioViS40 software (all Carl Zeiss MicroImaging, Göttingen, Germany).

Osteogenic Differentiation

For osteogenic differentiation, 6 × 10^4 cells were seeded on round glass slides in 24-well culture dishes; they were cultured in 1 mL standard culture medium until reaching 95% confluence and were fed every 3 to 4 days with Mesenchymal Stem Cell Osteogenic Differentiation Medium (PromoCell). As a marker for osteogenic differentiation, Alizarin red S staining was performed to detect microcrystalline or noncrystalline calcium phosphate salts. After fixation with formaldehyde, cells were stained with 1% Alizarin red S (Sigma-Aldrich Corp.).

Chondrogenic Differentiation

To induce chondrogenic differentiation, 2.5 × 10^6 cells were transferred into a 15-mL polypropylene tube and centrifuged at 300 g for 5 minutes to form micromass pellets at the bottom of...
the tube. After 48 hours of culture in standard medium, chondrogenic differentiation was induced with standard medium supplemented with dexamethasone: $1 \times 10^{-3}$ M l-proline (Sigma-Aldrich Corp.), 10 ng/mL TGF-β3 (Peprotech, Hamburg, Germany), and 1% BD ITS Culture supplement (BD Bioscience). After 5 weeks of culture, micromass bodies were embedded in Tissue-Tek (Sakura Finetek, Staufen, Germany), cryosectioned at 5-μm thickness, and frozen at $-80^\circ$C. To demonstrate the presence of glycosaminoglycans in specimens, we used Alcian blue 8X (Roth, Karlsruhe, Germany) staining, and Shandon Instant Hematoxylin (Thermo Scientific, Pittsburgh, PA, USA). After 3 weeks of culture, micromass bodies were transferred to 80% DMEM supplemented with dexamethasone: $1 \times 10^{-3}$ M l-proline (Sigma-Aldrich Corp.), 10 ng/mL TGF-β3 (Peprotech), Cells were immunostained for α-smooth muscle actin (α-SMA, 1:200, mouse monoclonal clone 1A4), secondary antibody goat anti-mouse IgG-fluorescein isothiocyanate (FITC) (1:100; Dianova, Hamburg, Germany), and 7-aminoactinomycin (7AAD).

**Myogenic Differentiation**

Cells ($6 \times 10^6$) were seeded on round glass slides in 24-well culture dishes overnight in standard medium. Myogenic differentiation was induced by adding 100 ng/mL TGF-β1 (Peprotech). Cells were immunostained for a-smooth muscle actin (α-SMA, 1:200, mouse monoclonal clone 1A4), secondary antibody goat anti-mouse IgG-fluorescein isothiocyanate (FITC) (1:100; Dianova, Hamburg, Germany), and 7-aminoactinomycin (7AAD).

**Neuronal Differentiation**

To induce neuronal differentiation, $5 \times 10^4$ cells were grown on glass slides in 24-well culture dishes and incubated with 40 μM neuronal inducer TCS 2210 (Tocris, Boston, MA, USA) in standard medium as described previously. Neuronal differentiation was observed by detection of β3-tubulin (1:200, rabbit monoclonal IgG; Cell Signaling Technology, Danvers, MA, USA) in combination with a secondary antibody goat anti-rabbit IgG-fluorescein isothiocyanate (FITC) (1:100; Dianova, Hamburg, Germany), and 7-aminoactinomycin (7AAD).

**T-Cell Suppression Assay**

Responder T cells/monocytes were isolated by anti-CD3 and CD14 microbeads from peripheral blood of healthy volunteers according to the manufacturer’s instructions (Miltenyi, Bergisch-Gladbach, Germany). Isolated responder cells were stained with 5 μM carboxyfluorescein diacetate succinimidyl ester (CFSE) (Invitrogen, Karlsruhe, Germany) and stimulated at 0.25 × 10⁶ cells/mL with Dynabeads human T-Activator CD3/CD28/CD137 (Invitrogen; responder cell to beads ratio 1:10). GO-MSC or GO-F isolated from the same GO patient in the same passage was added to different amounts of T cells (40:1, 20:1, or 10:1 T-cell to GO-MSC/F ratios). The CFSE dilution assays were analyzed after 7 days in a BD FACSCanto II flow cytometer, and the proliferation index was determined with ModFit LT 3.2 software (Verity Software House, Topsham, ME, USA).

**Enzyme-Linked Immunosorbent Assay (ELISA) for Detection of Cytokines**

Constitutive secretion of IL-6, IL-8, and MIF (macrophage migration inhibitory factor) was detected by DuoSet Human Immunoassay (R&D Systems, Wiesbaden, Germany) according to the manufacturer’s protocol. Third-passage GO-MSCs or GO-Fs were washed three times with DMEM and cultured for 24 hours in standard medium at a concentration of $1 \times 10^6$ cells/mL. The supernatant was collected, further cleared from debris by centrifugation, and stored at $-20^\circ$C until measurements.

**Proliferation Assay**

Proliferation of fourth-passage cells was measured with a chemiluminescent immunoassay for quantification of cell proliferation based on the measurement of bromodeoxyuridine (BrdU) incorporation during DNA synthesis (Roche, Mannheim, Germany) as described before.

**Measurement of Hyaluronan**

For determination of hyaluronan production, $1 \times 10^4$ cells were grown for 48 hours in a 96-well plate in 200 μL DMEM supplemented as above. The medium was aspirated, and HA was measured with an enzyme-linked binding protein assay (Corgenix (Broomfield, CO, USA) according to the manufacturer’s instructions as described before.

**Statistical Analysis**

Statistical analysis was performed with 2-tailed Student’s $t$-test with a confidence level greater than 95%. Data are presented as arithmetic means ± SD. Statistical significance was set at the level of $P \leq 0.05$.

**RESULTS**

**Isolation of Mesenchymal Stem Cells From Retrobulbar Fat Tissue of GO Patients**

In the present study, we aimed to investigate the biological characteristics of orbital mesenchymal cells isolated from the diseased orbit along criteria that define MSC. To test whether orbital fat tissue can be a source of multipotent cells, retrobulbar-derived fibroblast-like cells from GO patients were isolated according to an isolation method for adipose tissue-derived stem cells. To compare GO-MSCs with GO-Fs, which are key cells in GO pathology, we isolated GO-Fs simultaneously from the same piece of fat tissue of the respective GO patient with a standard method as described before. As shown in Figure 1A, these putative orbital fat-derived GO-MSCs were of fibroblast-like morphology and expressed the mesenchymal marker protein vimentin and fibroblast surface protein 1 just as did the GO-Fs, indicating a similar morphology of the two cell populations. Because proliferation and hyaluronan production of GO-Fs are a hallmark of GO pathology, we tested hyaluronan secretion of GO-MSCs. We found that the putative GO-MSCs proliferated and produced hyaluronan to the same extent as the GO-Fs (Fig. 1B), implying that GO-MSCs can contribute to pathogenesis of GO.

**Characterization of GO-MSCs and -Fs by Expression of Typical Surface Markers**

To further characterize putative GO-MSCs and -Fs, we analyzed a set of surface markers with flow cytometry. As illustrated in Figure 2, after ex vivo selection and expansion into passage 3, both of the donor-matched cell lines were strongly positive for the consensus MSC markers CD29, CD44, CD71, CD73, CD90, CD105, and CD166 (Fig. 2A). In contrast, GO-MSCs and -Fs lacked expression of a known hematopoietic progenitor marker CD34, a leukocyte marker CD45, and the endothelial marker CD31, as well as the pericyte marker CD146 and Stro-1 (Fig. 2A). Additional analysis of early passage 1 cells revealed expression of CD31 and CD146 (Supplementary Fig. S1), which were downregulated after further expansion (Fig. 2A). Although the GO-MSCs and -Fs predominantly expressed CD90 to high levels
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In the first part of our study we demonstrated that both GO-MSCs and GO-Fs fulfilled the minimal MSC definition criteria of plastic adherence and fibroblast-like growth (Fig. 1), distinct set of surface markers (Fig. 2), and multilineage differentiation (Fig. 3). In the second part of our study we further aimed to investigate typical immunomodulatory function of MSC. As suppression of T-cell proliferation is a functional hallmark of MSCs, we performed polyclonal stimulation of responder T cells in the presence of autologous monocytes. Donor-matched GO-MSCs and GO-Fs were added, and their suppressive potential on T-cell proliferation was compared. As illustrated in Figure 4A, T-cell proliferation is increasingly inhibited by both mesenchymal cell types depending on the T cell to GO-MSC/F ratios. Notably, GO-MSCs exhibited a significant enhanced capacity to suppress polyclonal T-cell activation when compared to GO-Fs at a 40:1 ratio (Figs. 4A, 4B). The obtained data provide evidence that GO-Fs have an immunosuppressive potential, although with lower capacity compared to GO-MSCs.

Further, secretion of cytokines has previously been shown to mediate immunosuppressive ability of tissue-resident MSCs. Therefore, constitutive secretion of IL-6, IL-8, and MIF was determined by ELISA and compared for GO-MSCs and GO-Fs. Both mesenchymal cell lines produced low amounts of IL-8 (<1 ng/mL) and high amounts of MIF (>1 ng/mL). In contrast, GO-MSCs secreted higher levels (P = 0.022) of the inflammatory cytokine IL-6 that easily exceeded 1 ng/mL under standard culture conditions (Fig. 5). The data indicate that GO-MSCs and GO-Fs by secreting inflammatory cytokines could exert an immunomodulatory function in the orbit.

DISCUSSION

In the present study, we successfully identified for the first time MSCs in adipose retobulbar tissue of patients with GO (GO-MSCs) using standard protocols for adipose tissue-derived stem cell isolation and expansion. We use the term mesenchymal stem/stromal cells (MSCs) because we could demonstrate that orbital fat-derived MSCs fulfill the minimal criteria for defining multipotent MSCs together with immunomodulatory function, in agreement with MSC phenotype definition of the Interna-
Importantly, we could demonstrate for the first time that GO-Fs, an accepted in vitro cell model of GO since their first isolation by Bahn and coworkers, exhibit the same morphologic features, as well as a surface marker set and multilineage differentiation potential similar to GO-MSCs (Figs. 1, 2, 3). Interestingly, the GO-MSCs and -Fs expressed the same surface marker as orbital fat-derived stem cells obtained from fat tissue of the intraorbital cavity during blepharoplastic surgery. In addition, the observed reduction of the surface markers CD31 and CD146 during the early passages (Fig. 2A, Supplementary Fig. S1) was found to be a characteristic of mesenchymal progenitor cells derived from orbital adipose tissue. Taken together, the observed surface marker profile supports the notion that GO-MSCs and -Fs isolated from the retrobulbar tissue during decompensation procedure of GO patients represent orbital fat-derived stem cell populations. Indeed, GO-MSCs and GO-Fs shared the most important characteristic of orbital adipose tissue-derived stem cells, namely, the potential to differentiate into adipocytes, chondrocytes, and osteoblasts, and in addition into myogenic and neuronal precursors. These findings demonstrate that our isolation procedure effectively yields the generation of MSCs from retrobulbar fat tissue. In contrast, GO-EthC fibroblasts showed no multilineage differentiation capacity and therefore could not be considered as containing MSC populations although isolated with a MSC protocol. Similarly, fibroblasts isolated from the eyelid skin during blepharoplastic surgeries did not differentiate toward the adipogenic or osteogenic lineage, and they showed less pronounced signs of chondrogenic differentiation even though they expressed a similar set of surface markers as the orbital fat-derived stem cells. Several working groups previously reported differentiation of GO-Fs into adipocytes or myocytes. However, we found that GO-Fs were also able to differentiate along the osteocytic, chondrocytic, and neuronal lineages (Fig. 3), hence shared characteristics of classical adipose tissue-derived stem
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Figure 3. Multilineage differentiation studies. GO-MSCs/-Fs and GO-EthC isolated from three or four GO patients were subjected (first to third passages) to the respective differentiation medium (diff) to induce adipogenic (adipo), osteogenic (osteo), chondrogenic (chondro), myogenic (myo), or neuronal (neuro) lineages. After 2 weeks of incubation, the adipogenic differentiation was observed by staining fatty vacuoles with Sudan III. We demonstrated osteogenic differentiation by Alizarin red S staining of microcrystalline or noncrystalline calcium phosphate salts after 3 weeks of incubation. The chondrogenic differentiation with Alcian blue 8GX staining of glycosaminoglycans was observed after 4 weeks. The myogenic differentiation was analyzed after 48 hours by staining of α-SMA, and neuronal differentiation was observed by staining of β3-tubulin after 72-hour incubation. (A) Representative images obtained with light transmission microscopy from three or four GO-MSCs/-Fs or GO-EthC (adipo, osteo, and chondro at ×100 magnification) or with fluorescence microscopy (α-SMA at ×100 magnification and β3-tubulin at ×200 magnification) are shown. (B) Semiquantitative analysis of differentiation was carried out using ImageJ (http://imagej.nih.gov/ij/; provided in the public domain by the National Institutes of Health, Bethesda, MD, USA) either by determining the colored stained area (%) (adipo, osteo, chondro) or by counting stained cells with changed morphology (myo: cells with α-SMA positive filaments; neuro: cells with β3-tubulin positive dendrites) from three to four fields of view at ×100 magnification of three or four GO-MSCs/-Fs, respectively.

cells that reside in the vascular stroma of adipose tissue as well as in the bone marrow. Because adipose cells are mesodermal in origin, the differentiation of adipogenic stem cells into neural tissue of ectodermal origin seemed to be very unlikely. However, they can take on a neuronal morphology when exposed to different induction agents. While MSCs, adipose-derived stem cells, and most tissue fibroblasts are of mesenchymal origin, the GO-Fs have been hypothesized to be derived from the neuroectodermal origin. Although ocular and orbital components have been shown to be derived from a combination of mesodermal and neural crest cells, the origin of the retrobulbar connective/fat tissue and derived cells remains unclear. Interestingly, it has been reported that in the cephalic region a subset of adipocytes arises from the neural crest. Further, a subpopulation of adipogenic progenitors derived from the neural crest has been identified in adipose stromal cells. Because our in vitro data provide the first experimental evidence that the GO-F population contains progenitors with neuronal differentiation potential, it appears possible that GO-Fs contain neural crest-derived progenitors. However, we found that GO-Fs differentiate and showed multilineage potency, indicating that mesenchymal multipotent progenitor cells were effectively present in the GO-F population. In comparison to GO-Fs, the GO-MSCs showed better cell differentiation ability in terms of adipogenesis, osteogenesis, and myogenesis. These data may suggest that GO-Fs, in contrast to GO-MSCs, represent a more heterogeneous population of fibroblastoïd cells containing more mature differentiated cells and a minority of MSC-like multipotent progenitor cells. Further studies are needed to fully elucidate the functional heterogeneity of orbital progenitor cells.

While CD90 (Thy-1) is consistently expressed on the cell surface of adipose stem cells, we found that GO-MSC and GO-F populations of some GO patients (two out of nine patients) contained considerable amounts of subtypes expressing low levels of CD90 (Fig. 2B). It was reported earlier that the degree of CD90 expression on GO-Fs influences their differentiation capacity. While CD90+ subtypes differentiate into myocytes, CD90– subtypes differentiate into adipocytes. However, it was shown recently that in GO-Fs expressing a high level of CD90, adipogenesis can also be induced in three-dimensional culture and under pressure stress as might occur in the diseased orbit. In contrast, GO-MSCs clearly exhibited overall higher adipogenic and myogenic differentiation capacity than the GO-Fs (Fig. 3B), although CD90 expression level of the two populations was very similar (Fig. 2). This indicates that factors other than CD90 expression additionally impact differentiation capacity of GO-F-like/mesenchymal stem cells. However, constitutive proliferation capacity and hyaluronan production did not differ between undifferentiated GO-MSCs and GO-Fs (Fig. 1). It remains to be investigated whether under stimulation conditions as occur in the diseased orbit, GO-
MSCs and GO-Fs were stimulated to different degrees in terms of proliferation, hyaluronan production, and adipogenic/myogenic differentiation and may differently contribute to pathologic volume enlargement of the orbit in GO. Beyond that, it is completely unknown whether osteocytic, chondrocytic, or neuronal differentiation pathways could be involved in GO. Hence comparative studies including MSCs/Fs derived from retrobulbar tissue of control healthy persons are needed to fully elucidate the role of the fat-derived stem cell population for tissue remodeling and expansion in GO.

An emerging body of evidence shows that in addition to their regenerative properties, MSCs also possess broad immunoregulatory abilities. Recent studies have shown that MSCs can influence both the adaptive and the innate immune cells. Because MSCs release proinflammatory as well as immunosuppressive effects, they are considered to act as sensors and switchers of inflammation. As GO-Fs are recognized as potent regulators of immune cell recruitment and activation, the GO-MSC subtype may represent a thus far underestimated player in local immune regulation. To evaluate the potential role of GO-MSCs in orbital immunology, we tested key features of immunologic cell–cell interactions such as suppression of T-cell proliferation and secretion of inflammatory cytokines. When compared to GO-Fs, the GO-MSCs were more effective in suppression of T-cell proliferation (Fig. 4) and constitutively secreted higher amounts of IL-6 (Fig. 5). A current model of MSC–T-cell/monocyte interaction suggests that constitutively produced IL-6 by MSCs balances the polarization of monocytes into anti-inflammatory M2 macrophages and favors the emergence of regulatory T cells—while in the absence of IL-6, MSCs induce monocyte polarization into proinflammatory M1 macrophages and promote T-cell activation. In accordance with this model, we propose that GO-MSCs represent an anti-inflammatory MSC subtype that could dampen inflammation. Regarding this, it has been shown that topical administration of allogenic orbital fat-derived stem cells inhibits inflammation during cornea injury. Investigation of the immunosuppressive potential of GO-MSCs and control MSCs obtained from healthy persons in response to an inflammatory microenvironment (as occurs in the diseased orbit) could lead to a promising therapeutic approach for GO.

In summary, our data show for the first time the presence of mesenchymal progenitor cells (GO-MSCs) in the retrobulbar fat tissue of GO patients. Importantly, we discovered that GO-Fs share all biological characteristics with orbital MSCs. However, differentiation potential, T-cell suppression, and cytokine release suggest some distinct functional differences between

![Figure 4](image-url)
the same 11 GO patients was quantified as described in Methods.

GO-Fs and GO-MSCs. Further studies including orbital MSCs isolated from healthy control persons are needed to better define mesenchymal progenitor cell populations and their relative contribution to disease pathology in GO.

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