Pathogenetic Characteristics of Mesenchymal Stem Cells in Hidradenitis Suppurativa

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IMPORTANCE Hidradenitis suppurativa (HS) is a disease of the terminal hair follicle in apocrine gland–enriched skin areas, where immunobiology dysregulation of mesenchymal stem cells (MSCs) may have a key role.

OBJECTIVE To investigate the MSC profile in patients with HS and in healthy controls.

DESIGN, SETTING, AND PARTICIPANTS In this prospective case-control study, patients with HS were recruited from the Dermatological Clinic at the Department of Clinical and Molecular Sciences, Università Politecnica delle Marche, Ancona, Italy. Biopsy specimens were analyzed at the Histology Section of the Department of Clinical and Molecular Sciences. Participants included 11 patients with HS and 9 healthy controls, who were recruited into the study between January 20, 2015, and September 20, 2016, and underwent punch biopsy from axillary skin. None of the participants had received any antibiotics (systemic or topical therapy) within almost 12 weeks before the study.

MAIN OUTCOMES AND MEASURES The immunophenotypic profile of MSCs was characterized following the minimal criteria established by the International Society for Cellular Therapy for the identification of MSCs. Levels of 12 cytokines belonging to helper T-cell subtypes 1, 2, and 17 pathways were examined on the secretome of isolated cells by enzyme-linked immunoabsorbent assay.

RESULTS Skin MSCs were characterized in 11 patients with HS (8 women and 3 men; mean [SD] age, 35.8 [7.9] years) and 9 healthy controls (7 women and 2 men; mean [SD] age, 36.7 [6.9] years). The healthy controls were matched with patients with HS for body mass index. Mesenchymal stem cells isolated from patients with HS (HS-MSCs) and from healthy controls (C-MSCs) met the International Society for Cellular Therapy minimal criteria. Compared with C-MSCs, cytokine analyses of HS-MSCs revealed statistically significant overexpression of interleukin (IL) 6 (median [interquartile range [IQR]], 8755 [7659-10233] pg/mL; P = .008), IL-10 (median [IQR], 29.46 [26.35-35.79] pg/mL vs 21.36 [19.89-23.31] pg/mL; P = .004), IL-12 (median [IQR], 15.25 [13.72-16.25] vs 11.89 [10.73-12.33] pg/mL; P = .03), IL-17A (median [IQR], 15.24 [13.23-17.24] vs 11.24 [10.28-11.95] pg/mL; P = .008), tumor necrosis factor (median [IQR], 42.54 [40.20-44.94] vs 32.55 [31.78-33.28] pg/mL; P = .004), transforming growth factor β1 (median [IQR], 1728.00 [1535.00-1979.00] pg/mL vs 100.80 [465.00-634.00] pg/mL; P = .004), and interferon γ (median [IQR], 11.49 [10.71-12.35] vs 9.45 [9.29-10.01] pg/mL; P = .005).

CONCLUSIONS AND RELEVANCE Mesenchymal stem cells isolated from the skin of patients with HS seem to be activated toward an inflammatory status. The imbalance between proinflammatory and anti-inflammatory activities of MSCs favors the hypothesis of their pathogenic involvement in HS.

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Hidradenitis suppurativa (HS) is a chronic inflammatory and immune-mediated skin disease characterized by painful deep lesions and abscesses that can progress to cause fistula formation, odorous discharge, and dermal fibrosis. It is a disease of the terminal hair follicle and usually appears in apocrine gland–enriched skin areas of the body, most commonly the axillae, inguinal, and anogenital regions, where immunobiology dysregulation of mesenchymal stem cells (MSCs) may have a key role. Hidradenitis suppurativa is also associated with several other diseases and comorbidities, such as obesity, type 1 diabetes, inflammatory bowel disease (especially Crohn disease), and psoriasis, as well as depression and other psychosocial morbidity. It affects between 1% and 4% of the population, often in the postpubertal period, with a female predominance.

It is widely accepted that HS is associated with early plugging and dilation of the pilosebaceous unit, followed by rupture and subsequent involution of this structure. A secondary inflammatory process then occurs, with the involvement of immune cells into the area and cytokine release. Suggestions of an immunity aberrance in HS gradually began to appear in the literature from the 1980s onward. Several studies have aimed to characterize the cytokine profile in HS skin. Chiefly, interleukin (IL) 1β, IL-6, IL-10, IL-12, IL-17, IL-20, IL-22, IL-23, and tumor necrosis factor (TNF) seem to be dysregulated in patients with HS. Despite the extent of the literature, results are often contradictory, with some authors reporting that these cytokines were significantly overexpressed in HS skin compared with healthy skin and other authors finding suppressed levels of the same factors. None of the previous studies investigated the cytokine profile in MSCs of HS skin, although the involvement of MSCs in HS has been suggested and appears likely from the nature of the disease.

Mesenchymal stem cells are undifferentiated, multipotent, self-renewal cells that can be isolated from different tissues, including skin. Great attention has been paid to their role in the onset of other inflammatory diseases. Previous researchers demonstrated that MSCs isolated from patients with skin disease, such as psoriasis or atopic dermatitis, show the typical phenotype that characterizes differentiated cells, suggesting their involvement in the early phase of disease development. Mesenchymal stem cells are able to secrete different soluble factors under the microenvironmental influence, which in turn they contribute to create through a paracrine effect. It is reported that MSCs can sustain or counteract inflammation by secreting different cytokines. In this scenario, our primary concern is can the HS-inflamed microenvironment alter the immunobiology of MSCs, making them a source of inflammatory mediators able to sustain inflammation in turn?

To address this issue, MSCs were isolated and characterized from skin biopsy specimens of patients with HS (HS-MSCs) and healthy controls (C-MSCs). In this prospective case-control study, inflammatory cytokines involved in helper T-cell subtype 1 (TH1), TH2, and TH17 inflammatory pathways were then analyzed to evaluate the MSC immunological profile in HS. Patients with HS were recruited from the Dermatological Clinic at the Department of Clinical and Molecular Sciences, Università Politecnica delle Marche, Ancona, Italy. Biopsy specimens were analyzed at the Histology Section of the Department of Clinical and Molecular Sciences.

**Methods**

**Patients and Skin Sample Collection**

Eleven consecutive patients of white race/ethnicity with HS (8 female and 3 male; mean [SD] age, 35.8 [7.9] years) were recruited between January 20, 2015, and September 20, 2016,
patients with HS for body mass index and who had surgical resection (inflamed and noninflamed), abscesses, and scarring (atrophy, mesh-like, red, hypertrophic, or linear). Hidradenitis suppurativa was active in all patients, as demonstrated by elevated serum levels of C-reactive protein (>5.00 mg/mL in all enrolled patients). Patients were homogeneous for disease severity (Hurley score of 3). All patients were overweight, with a body mass index (calculated as weight in kilograms divided by height in meters squared) of 25 to 29.9, and none showed other significant comorbidities (type 1 diabetes, Crohn disease, or psoriasis).

All enrolled participants had been free of any drug treatment, including antibiotics (oral or systemic), oral and injected corticosteroids, estroprogestinic integration, metformin hydrochloride, and oral isotretinoin, for almost 12 weeks. They had not taken oral cyclosporine for almost 24 weeks, and none had received acitretin, methotrexate sodium, or adalimumab in the past.

To rule out any variation associated with different bacterial resident flora, all 11 patients underwent a 6-mm punch skin biopsy that included active inflammatory lesions at the same site (axillary region). Samples were also collected from the skin of 9 healthy controls of white race/ethnicity (7 female and 2 male; mean [SD] age, 36.7 [6.9] years), who were matched with patients with HS for body mass index and who had surgical removal of cutaneous benign lesions from the axillary area.

Characterization of MSCs

The MSCs isolated from patients with HS and healthy controls met the minimal criteria for the definition of MSCs in an International Society for Cellular Therapy position statement by Dominici et al.24 Levels of 12 cytokines belonging to Th1, Th2, and Th17 pathways were examined on the secretome of isolated cells by enzyme-linked immunoabsorbent assay. Cells at the third passage and at a confluence of approximately 75% were used. For immunophenotyping, cells were stained at 4°C for 30 minutes with fluorescein isothiocyanate–conjugated antibodies (Becton Dickinson) against HLA-DR, CD14, CD19, CD34, CD45, CD73, CD90, and CD105, and flow cytometry was performed with a scanning instrument (FACScan; Becton Dickinson). Data acquisition was carried out with a software program (CellQuest; Becton Dickinson). The differentiation potential in vitro was then tested by using an osteogenesis, chondrogenesis, and adipogenesis differentiation kit (StemPro; Gibco). For the osteoblast and adipocyte differentiation, 5 × 10⁴ cells were seeded in a 2-well chamber slide. For chondrogenesis differentiation, a pellet of 5 × 10⁴ cells was made. Cells maintained in MSC growth medium were used as a negative control. All media were replaced twice a week. After assessing cell morphological changes, osteogenic or adipogenic differentiation was evaluated by alkaline phosphatase reaction and von Kossa staining (osteogenic differentiation [10 days]) and oil red O (adipogenic differentiation [15 days]). For chondrogenic differentiation, the pellet was embedded in paraffin after 30 days of culture, and sections were then stained with safranin O.

Conditioned Medium Preparation

The HS-MSCs and C-MSCs at 4 to 6 passages were seeded at 3.3 × 10⁵ cells in a T25 flask and incubated at 37°C and 5% carbon dioxide with a culture medium (Dulbecco modified eagle medium/F12 supplemented with 10% fetal bovine serum) for 72 hours. Medium was then collected, centrifuged at 1000 revolutions per minute for 5 minutes, and filtered in a 0.22-μM pore membrane. Conditioned medium was stored at −80°C until use.

Enzyme-Linked Immunosorbent assay

Human cytokines belonging to Th1/Th17 and Th12 pathways were analyzed by enzyme-linked immunosorbent assay (ELISA) with a kit (Multi-Analyte ELISAArray; Qiagen) as previously described.25 Briefly, 50 μL of medium conditioned for 72 hours by each sample of HS-MSCs and C-MSCs was used for the test. Samples were dispensed into a 96-well microtiter plate and incubated for 2 hours at room temperature. After washing, avidin and horseradish peroxidase–conjugated antibody was
added to the plate and incubated for 30 minutes. Finally, captured cytokines were detected by the addition of substrate solution. The optical density at 450 nm was determined using a microtiter plate reader (Multiskan GO; Thermo Scientific). Absorbances of sample cytokines were then compared with those of the standard antigens. Tests were performed in triplicate. Data are reported as means (SDs) and expressed in picograms per milliliter.

Statistical Analysis
All data were analyzed using software programs (GraphPad Prism, version 5.0; and QuickCalcs [both from GraphPad Software, Inc]). The distribution of continuous variables was verified with the Kolmogorov-Smirnov test. Because data did not assume a gaussian distribution, the Wilcoxon signed rank test for unpaired variables was used. For all analyses, 2-sided \(P < .05\) was considered statistically significant.

Results
All participants in the study (patients with HS and healthy controls) were similar in age, sex, race/ethnicity, and anthropometric measurements. No statistically relevant differences were found between the HS-MSCs and C-MSCs.

MSC Isolation and Characterization
Mesenchymal stem cells were successfully isolated from biopsy samples from each study participant; cells with the typical fibroblast-like morphology began to be visible near the explants after 7 days of culture (Figure 1). Proliferation was monitored from the first passage to the 12th passage by calculating DT. Cells isolated from HS biopsy specimens had higher DT than those from controls. In both cases, DT was lower for the first 8 passages (mean [SD], 40 [1] hours for HS-MSCs and 19 [2] hours for C-MSCs) and then gradually increased up to passage 12 (mean [SD] and 71 [2] hours for HS-MSCs and 39 [2] hours for C-MSCs). Cells met the 3 minimal criteria for MSCs by Dominici et al.24: (1) plastic adherent in standard culture condition; (2) strongly positive for CD73, CD90, and CD105 and negative for HLA-DR, CD14, CD19, CD34, and CD45 (Table 1); and (3) able to differentiate toward osteogenic, chondrogenic, and adipogenic lineages as revealed by specific stains, including alkaline phosphatase reaction (Figure 2A) and von Kossa staining (Figure 2B) for osteocytes, oil red O (Figure 2C) for adipocytes, and safranin O staining (Figure 2D) for chondrocytes.

Expression of Secreted Cytokines by ELISA
Expression of specific cytokines secreted by MSCs was evaluated by ELISA. Most of the analyzed cytokines were significantly more expressed in HS-MSCs than in C-MSCs. Compared with C-MSCs, cytokine analyses of HS-MSCs revealed statistically significant overexpression of interleukin (IL) 6 (median [interquartile range [IQR]], 8765.00 [7659.00-9123.00] vs 2849.00 [2609.00-3001.00] pg/mL; \(P = .008\)), IL-10 (median [IQR], 29.46 [26.35-35.79] vs 21.36 [19.89-23.33] pg/mL; \(P = .004\)), IL-12 (median [IQR], 15.25 [13.27-16.25] vs 11.89...
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Table 2. Secreted Cytokines From HS-MSCs and C-MSCs by Enzyme-Linked Immunosorbent Assaya

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Median (IQR), pg/mL</th>
<th>HS-MSCs</th>
<th>C-MSCs</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-2</td>
<td>24.58 (22.48-29.57)</td>
<td>25.00 (22.50-30.20)</td>
<td>.91</td>
<td></td>
</tr>
<tr>
<td>IL-4</td>
<td>12.97 (11.68-15.06)</td>
<td>13.15 (12.49-14.58)</td>
<td>.73</td>
<td></td>
</tr>
<tr>
<td>IL-5</td>
<td>13.21 (9.68-14.66)</td>
<td>11.46 (10.82-13.23)</td>
<td>.50</td>
<td></td>
</tr>
<tr>
<td>IL-6</td>
<td>8765.00 (7659.00-9123.00)</td>
<td>2849.00 (2609.00-3001.00)</td>
<td>.008</td>
<td></td>
</tr>
<tr>
<td>IL-10</td>
<td>29.46 (26.35-35.79)</td>
<td>21.36 (19.89-23.33)</td>
<td>.004</td>
<td></td>
</tr>
<tr>
<td>IL-12</td>
<td>15.25 (13.27-16.25)</td>
<td>11.89 (10.73-12.33)</td>
<td>.03</td>
<td></td>
</tr>
<tr>
<td>IL-13</td>
<td>35.56 (34.57-35.72)</td>
<td>35.91 (35.12-36.62)</td>
<td>.18</td>
<td></td>
</tr>
<tr>
<td>IL-17A</td>
<td>15.24 (13.23-17.24)</td>
<td>11.24 (10.28-11.95)</td>
<td>.008</td>
<td></td>
</tr>
<tr>
<td>TNF</td>
<td>42.54 (42.20-43.94)</td>
<td>32.55 (31.78-33.28)</td>
<td>.004</td>
<td></td>
</tr>
<tr>
<td>TGF-β1</td>
<td>1728.00 (1535.00-1979.00)</td>
<td>500.80 (465.00-635.50)</td>
<td>.004</td>
<td></td>
</tr>
<tr>
<td>IL-10</td>
<td>11.49 (10.71-12.35)</td>
<td>9.45 (9.29-10.01)</td>
<td>.005</td>
<td></td>
</tr>
<tr>
<td>IL-6</td>
<td>45.37 (41.25-52.96)</td>
<td>41.27 (39.82-42.97)</td>
<td>.15</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: C-MSCs, mesenchymal stem cells isolated from controls; G-CSF, granulocyte-macrophage colony-stimulating factor; HS-MSCs, mesenchymal cells isolated from patients with hidradenitis suppurativa; IFN, interferon; IL, interleukin; IQR, interquartile range; TGF, transforming growth factor; and TNF, tumor necrosis factor.

a The HS-MSCs were compared with those isolated from C-MSCs.

Discussion

Hidradenitis suppurativa is a chronic relapsing inflammatory disease characterized by follicular occlusion leading to boils, sinus discharge, fistulae, and scarring. The etiopathogenesis of HS is not fully understood, although it is known that several genetic, environmental, endocrine, and microbiological factors variably contribute to its onset.31 Three decades ago, dysregulation of the immune system was found to have an important role in the onset of HS.6 In particular, levels of some specific cytokines seem to be altered in skin and serum of patients with HS. These cytokines are mainly produced by several cells of the immune system, as well as by affected MSCs, and in turn influence the microenvironment.20 Mesenchymal stem cells can be considered a paradigm of the “giantis bifrons” cell model. Under selective inflammatory stimulus, MSCs isolated from skin can modify their phenotype and anti-inflammatory intrinsic properties, becoming a source of several proinflammatory cytokines on their own, thus promoting and sustaining the inflammatory status in the tissue from which they were isolated.25,26 It is reasonable that the early inflammatory status in HS could promote the upstream milieu of Th1 to Th17 cytokines, activating MSCs from a quiescent toward an inflammatory status, as assessed in other inflammatory diseases of the skin.16-18,27,28 In this regard, MSCs could be more than a passive spectator in the inflammatory process of HS, becoming an active source of several mediators able to amplify the inflammatory disintegration of infundibular keratinocytes and sebaceous gland homeostasis.

To evaluate the potential involvement of MSCs in HS, we isolated and characterized HS-MSCs and evaluated their immune profile. For comparative purposes, C-MSCs were used, as already done in similar analyses investigating the MSC immunophenotype in psoriasis and atopic dermatitis.16,17 Compared with C-MSCs, HS-MSCs showed no significant differences in satisfying the minimal criteria for MSC identification by Dominici et al.24 Both groups were plastic adherent with a stem-like immunophenotype; strongly positive for CD73, CD105, and CD45; and able to differentiate toward osteogenic, chondrogenic, and adipogenic lineages. Significant differences between groups were noted in the secretion of 7 cytokines (IL-6, IL-10, IL-12, IL-17A, TNF, TGF-β1, and IFN-γ) that were differentially involved in inflammation.

Tumor necrosis factor is a proinflammatory cytokine. It has been reported that TNF levels are increased in HS skin compared with control skin.29 The successful treatment of patients with HS with TNF antagonists suggests a key role for this factor in the pathogenesis of the disease.30 Our results herein suggest that this seems to be evident also for MSCs, with HS-MSCs secreting higher levels of TNF than C-MSCs.

For 2 other proinflammatory cytokines, IL-12 and IL-17A, it is reported that messenger RNA expression and protein expression are enhanced in HS-differentiated cells compared with control skin.31 A similar finding has been observed herein in undifferentiated cells, with HS-MSCs secreting increased levels of IL-12 and IL-17A compared with C-MSCs.

Interleukin 12 is involved in many inflammatory conditions through its ability to induce the production of IFN-γ.32 The role of IFN-γ in HS is still unclear, and previous studies have found contradictory results. Compared with healthy skin, van der Zee et al.33 reported decreased levels of IFN-γ in HS skin, whereas Wolk et al.34 reported increased levels of IFN-γ in HS skin. Our findings support the observation by Wolk et al., with greater expression of IFN-γ herein by HS-MSCs than by C-MSCs.

Interleukin 10 has an anti-inflammatory role,35 suppressing the development of Th1, Th12, and Th17. A positive correlation between IL-10 expression and HS severity has been observed.31 In the present study, the upregulation of IL-10 seen...
in differentiated HS cells reflects the increased levels observed in HS-MSCs compared with C-MSCs. In addition, as reported for differentiated cells, secretion of IL-10 in HS-MSCs herein reached higher levels than those observed in psoriatic MSCs.

Interleukin 6 is a pleotropic cytokine, and its role in the pathogenesis of HS is still debated because IL-6 levels in HS skin are variable. It is secreted by neutrophils and macrophages via inflammatory stimulus (eg, stimulation of Toll-like receptor 4 by lipopolysaccharide, IL-1, and TNF). Furthermore, IL-6 is directly involved in expression of serum C-reactive protein levels, with serum C-reactive protein levels correlating with inflammatory activity of HS and potentially representing a biomarker for evaluating the severity of HS. As for IFN-γ, there is evidence of both enhanced and suppressed levels of IL-6 in HS skin compared with healthy skin. In our model, HS-MSCs expressed higher amounts of IL-6 than C-MSCs.

The TGF-β1 cytokine is directly involved in all phases of wound healing by regulating cell proliferation, differentiation, and extracellular matrix production and by modulating the immune response. In our series, TGF-β1 was expressed to a greater extent in HS-MSCs than in C-MSCs. These results are not surprising because HS is associated with scar formation. Furthermore, expression of the TGF-β1/mammalian target of rapamycin (mTOR) signaling pathway in scar fibroblasts is significantly increased, suggesting that this may be an important mechanism in scar formation. This is in line with the upregulation observed herein for the other TH1 cytokines.

**Limitations**
The main limitation of this study was the absence of a group of patients with inflammatory skin disease with a TH2 cytokine pathway for control purposes. This would have allowed us to evaluate and compare specific differences in immunophenotypes of MSCs.

**Conclusions**
Taken together, these preliminary results seem to confirm the activation of MSCs in HS toward an inflammatory phenotype characterized by the involvement of TH1 to TH17 cytokines. The imbalance between proinflammatory and anti-inflammatory activities of MSCs favors the hypothesis of their pathogenic involvement in HS. Hidradenitis suppurativa is an immune-mediated skin disease whose inflammatory milieu is basically TH1 and TH17, as in psoriasis. The physiopathogenetic data herein indicate that MSCs may have an early phenotype early activated toward this inflammatory pathway and opposite to the TH2 pathway that is typical of atopic dermatitis, as demonstrated previously. Further research is needed to enhance our understanding of MSC involvement in HS, with the goal of novel therapeutic strategies.

**ARTICLE INFORMATION**
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Levels of cytokines isolated from patients with hidradenitis suppurativa (HS-MSCs) were compared with those isolated from healthy controls (C-MSCs). IFN indicates interferon; IL, interleukin; TGF, transforming growth factor; and TNF, tumor necrosis factor.

* $P < .01.$

**$P < .005.$

$P < .05.$
Pathogenetic Characteristics of Mesenchymal Stem Cells in Hidradenitis Suppurativa

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