Clinical Case Report

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Successful mesenchymal stem cell treatment of leg ulcers complicated by Behcet disease

A case report and literature review

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Abstract

Rationale: Behçet disease (BD) is a recurrent vasculitis characterized by oral and genital mucous membrane ulcers, uveitis, and skin lesions but only rarely leg ulcers. To our knowledge, no efficacious therapy has been described for BD patients with complicating, destructive leg ulcers.

Patient concerns: Here, We report the case of a 55-year-old woman with generalized erythema nodosum-like, papulopustular lesions, recurrent oral and genital ulcers accompanied with recurrent leg ulcers and trouble walking.

Diagnoses: Based upon the patient's clinical feature and positive pathergy test , BD was confirmed.

Interventions: Conventional immunosuppressive therapy and anti-tumor necrosis factor inhibitors, adalimumab and etanercept, had no demonstrable clinical effect. Mesenchymal stem cell (MSC) injection combined with low-dose prednisone and thalidomide, however, completely ameliorated the ulcers on one leg, significantly improved ulcers on the other leg, and returned normal function to both legs.

Outcomes: The ulcerative lesions remained in remission, and the affected leg functioned normally after 34 months' follow-up.

Lessons: Our experience suggests that MSC infusion might be a potentially successful therapy for intractable drug-resistant BD patients with concomitant leg ulcer.

Abbreviations: ANCA = anti-neutrophil cytoplasmic antibodies, AZA = azathioprine, BD = Behçet disease, CTX = cyclophosphamide, ISG = International Study Group, MSC = mesenchymal stem cell, MTX= methotrexate, RA = rheumatoid arthritis, SLE = systemic lupus erythematosus, SSc = systemic sclerosis, TNF = tumor necrosis factor.

Keywords: Behçet disease, leg ulcer, mesenchymal stem cell transplantation, therapy

1. Introduction

Behçet disease (BD) is a systemic vasculitis characterized by recurrent oral and/or genital aphthosis, uveitis, retinal vasculitis, and variable skin lesions.[1] The etiology of BD remains unknown, and its treatment depends upon clinical presentation and organ involvement.[2,3] Jung et al[4] reported that leg ulcers are rare in BD patients, generally associated with vasculitis or deep vein thrombosis, and are refractory to conventional immunosuppressive therapy. To date, available evidence has suggested that tumor necrosis factor (TNF) inhibitors may be effective for treatment of leg ulcers.[5,6] Mesenchymal stem cells (MSCs), mainly isolated from bone marrow and some other sources such as umbilical cord blood, possess unlimited self-renewal and pluripotential capacity.[7] Several studies have documented the immunosuppressive and anti-inflammatory effect that MSC may exhibit in different diseases.[8,9] For example, MSC treatment has been reported to be a new, effective therapeutic strategy for severe, refractory autoimmune diseases including systemic lupus erythematosus (SLE),[10] rheumatoid arthritis (RA),[11] and systemic sclerosis (SSc).[12–14] In the present case report, we describe a BD patient with leg ulcers who did not respond to anti-TNF-α or conventional immunosuppressive therapy, but did achieve sustained, successful therapeutic response when MSC injection was used in combination with low-dose conventional immunosuppression. To our knowledge, this case report is the first documented evidence for the potential benefit of MSC transplantation in the treatment of leg ulcers associated with BD.

2. Case report

A 47-year-old woman with generalized erythema nodosum-like, papulopustular lesions, recurrent oral and genital ulcers, and positive pathergy test was diagnosed with BD (Table 1). The diagnosis was consistent with International Study Group (ISG) recommendations,[1] and the recently developed International

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Table 1

Behçet diagnosis[*].

Patient's sign/symptom[**]	Point score
Ocular lesions	0
Genital aphthosis	2
Oral aphthosis	2
Skin lesions	1
Neurological manifestations	0
Vascular manifestations	1
Positive pathergy test	1
Total[***]	7

[*] International Criteria for Behçet Disease point score system: a score of great than≥4 indicates Behçet diagnosis.
[**] Date: 3/2009.
[***] Patient total point score = 7, almost certainly BD.

Criteria for Behçet Disease (ICBD)[15]; the patient's ICBD score would have been 7 at the time of diagnosis. An ICBD score of 4 is sufficient for BD diagnosis. The patient was initially treated with oral prednisone (35 mg qd), cyclosporine A (75 mg bid), colchicine (0.5 mg qd), and thalidomide (100 mg qn). Symptoms including oral and genital ulcers were partially improved (Table 2). One year later, the patient developed multiple painful and destructive leg ulcers with biopsy confirmed leukocytoclastic vasculitis (Fig. 1). Cyclosporine A was then replaced with cyclophosphamide (1 g qm) with some subsequent improvement in clinical symptoms. Treatment was suspended after 2 months because of an infection. Two years later, when the patient was 50 years' old, she received treatment with etanercept (25 mg biw) for 1 month, but with no clinical improvement. Replacement of etanercept with adalimumab yielded no clinical benefit. During the following 3 years, the patient received several additional therapies, including mycophenolate mofetil and hydroxychloroquine (Table 2); however, the leg ulcers persisted and were exacerbated.

When admitted in our hospital at age 53, physical examination revealed wide spread papulopustular lesions, oral and genital ulcers, multiple scars, and a positive pathergy test. Her right lower leg ulcers were located between the knee and ankle, with diffuse swelling (Fig. 2A). Her left lower leg lesion was a painful and destructive ulcer with irregular margin and a ragged overhanging edge (approximately 6 × 5 cm) (Fig. 2B). Laboratory results were negative for rheumatoid factor, antinuclear antibodies, anti-double stranded DNA antibody, p-anti-neutrophil cytoplasmic antibodies, and anti-cardiolipin antibodies. Other

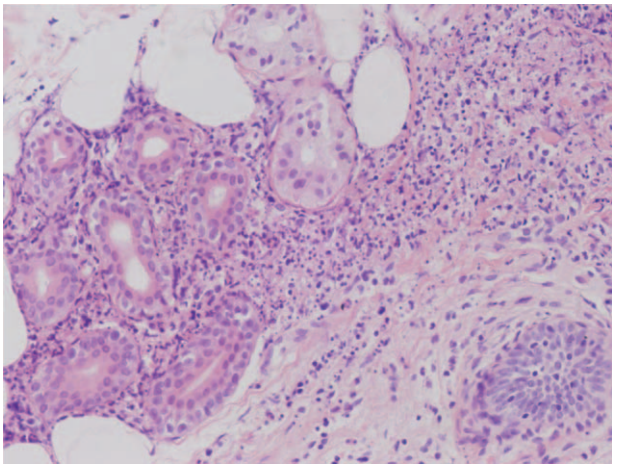

Figure 1. Leg Ulcer biopsy. Small vessel leukocytoclastic vasculitis (H&E, 20×).

laboratory test results were as follows (normal range in parentheses): C-reactive protein of 9.26 mg/L (<5 mg/L), erythrocyte sedimentation rate of 32.0 mm/h (<43 mm/h), IgG of 5.25 g/L (8–15 g/L), IgA of 686.00 mg/L (836–2900 mg/L), IgM of 392.00 mg/L (700–2200 mg/L), and IgG4 of 0.424 g/L (0.035–1.5 g/L). The results of Doppler ultrasound on both legs were normal.

Based upon the patient's clinical history (Tables 1 and 2), characterized by persistence and exacerbation of leg ulcers, poor response to conventional treatment, and our ongoing clinical experience with MSC therapy for SSc (in preparation), we decided to treat this patient with MSC infusions therapy. This decision was approved by the West China Hospital Institutional Research Committee in accordance with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The patient also provided informed consent to receive the MSC infusion therapy. The patient was intravenously infused with 50 mL, 10^6 cells/mL, pooled human umbilical cord MSCs (HUC-MSCs) (Kangjing Biotechnology, Chengdu, PR China) 3 times per month, in the first week of the month and at 7 days' intervals, for 3 months, for 9 total infusions. Prednisone (8 mg/day) and thalidomide (75 mg/day) were administrated to the patient during the same 3-month period. Two months after initiating MSC and low-dosage immunosuppression therapy, the right leg ulcer was markedly improved, and the left ulcer had

Table 2

Therapeutic History.

Date	Sign and symptom	Drugs	Effect of BD
3/2009	BD diagnosis	Prednisone, cyclosporine A, colchicine and thalidomide	Partially improved
12/2010	BD + leg ulcers	Cyclophosphamide pulse, prednisone, colchicine, thalidomide	No improved
2/2011	BD + leg ulcers + infection (fever)	Prednisone, thalidomide	No improved
7/2012	BD + leg ulcers	Prednisone, thalidomide, anti-TNF-α (etanercept or adalimumab), and hydroxychloroquine	No improved
1/2013	BD + leg ulcers	Prednisone, mycophenolate mofetil and hydroxychloroquine	No improved
3/2015	BD + leg ulcers	Low-dose prednisone and thalidomide,[*] MSC[**]	Significantly improved
6/2015	BD + leg ulcers	Low-dose prednisone and thalidomide,[*] MSC[***]	Significantly improved
7/2016	No BD + no leg ulcers	Low dose prednisone	

BD = Behçet disease, MSC = mesenchymal stem cell, TNF = tumor necrosis factor.
[*] Prednisone, 8 mg/day; thalidomide, 100 mg/day.
[**] MSC injections: 3/2015–5/2015 monthly, 3 times per month, beginning the first week of the month at 7-day intervals.
[***] MSC injections: 6/2015–7/2016 every other month 3 times injection.

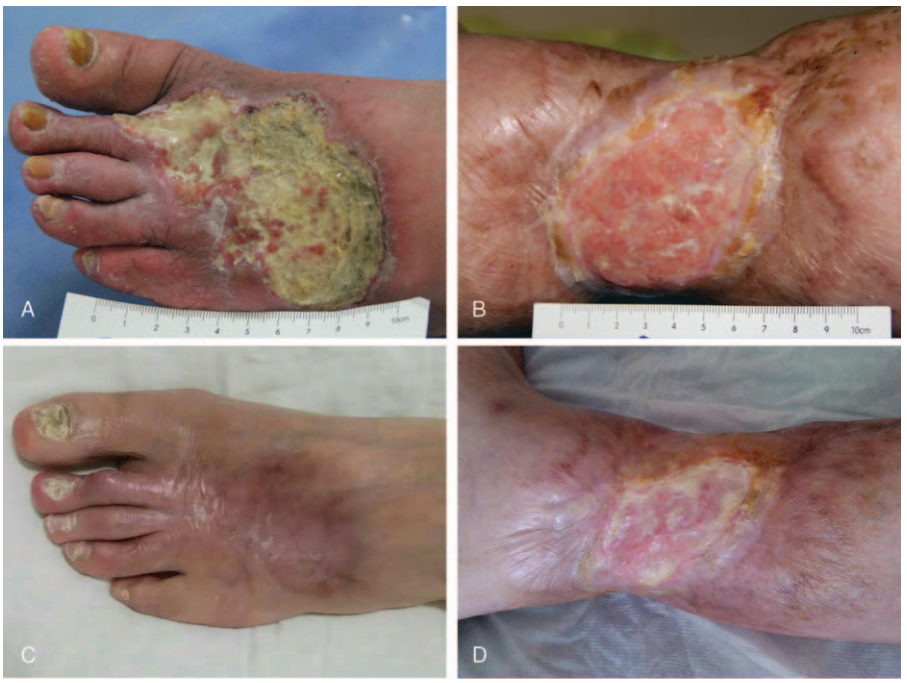

Figure 2. Leg ulcers of patient. (A) Left leg ulcer before treatment with mesenchymal stem cell (MSC) infusion. (B) Right leg ulcer before treatment with MSC infusion. (C) Left leg ulcer after 2 months' treatment with MSC infusion. (D) Right leg ulcer after 34 months' treatment with MSC infusion.

completely healed (Fig. 2C). Three months after initiating MSC therapy, clinical signs of BD, including papulopustular lesions and oral and genital ulcers, were markedly improved upon clinical examination. MSC infusion therapy was continued with 3 monthly infusions, as above, but only every other month for 14 months, an additional 21 infusions. After 34 months, all symptoms of BD have resolved, the left leg remains ulcer free, and the right lesion has continued to improve (Fig. 2D). The patient has continued a maintenance dosage of 4 mg/day of prednisone. None of the potential side effects commonly associated with MSC transplantation, such as vascular occlusion, fibrosis, or malignancy, were apparent from clinical evaluation during the 17-month period of MSC infusions and 34-month follow-up to present. The patient provided informed consent for her laboratory and clinical results to be included in this manuscript.

3. Discussion

BD is an autoimmune, chronic inflammatory disease with unknown etiology.[16] BD is characterized clinically as a chronic, relapsing vasculitis with oral and genital ulcers, cutaneous inflammation, uveitis, and gastrointestinal and central nervous system manifestations.[17] In 1990, an ISG attempted to consolidate diagnostic criteria for BD.[1] The ISG criteria for BD, diagnosis required the presence of oral ulceration plus any 2 of the following: genital ulceration, typical defined eye lesions, typical defined skin lesions, or a positive pathergy test. Although the ISG criteria were simpler and had improved discriminatory performance compared to predecessors, the ISG criteria were less than optimal. The more recently adopted ICBD has been documented to have the highest sensitivity for BD diagnosis.[15] For ICBD classification of BD, ocular lesions, oral aphthosis, and genital aphthosis are each assigned 2 points, whereas skin lesions, central nervous system involvement, and vascular manifestations, 1 point each. A positive pathergy test is also assigned 1 point. An ICBD score ≥ 4 is diagnostic for BD, and when the pathergy test is included, the sensitivity of the ICBD criteria is 95% to 98% with specificity of 92%. Criteria for BD diagnosis in Chinese patients are similar.[18,19] Although BD has moderate association with inheritance of HLA B*51 (odds ratio 5.8),[15] HLA B*51 is not included in the ICBD criteria.[15] Worldwide, 41% to 97% of BD patients have skin lesion such as aphthous stomatitis, genital ulcers, erythema nodosum-like lesions, and papulopustular lesions,[20] whereas leg ulcers were rare.[4] Leg ulcers in BD patients were associated with vasculitis or deep vein thrombosis, were recurrent, and refractory to conventional treatment. The BD diagnosis for the patient in the present case was based upon generalized erythema nodosum-like and papulopustular lesions and recurrent oral and genital ulcers, criteria similar to ISG, and including the positive pathergy test, would have had an ICBD score of 7. HLA genotype was not determined.

The morbidity and mortality of BD are relatively high, and maintaining remission and improving the patients' quality of life are the main goals of therapy. Appropriate treatment strategy of BD is chosen based on the organs involved and clinical presentations.[2,3] Nonsteroidal anti-inflammatory drugs and colchicine are sufficient for mild manifestations in BD, such as mucocutaneous involvement, but corticosteroids, cyclophosphamide (CTX), azathioprine, and/or cyclosporine A are recommended for the treatment of BD patients with complicating acute, large deep vein thromboses.[2,21] In our case, immunosuppressive drugs including corticosteroids, colchicine, cyclosporine A, CTX, mycophenolate mofetil, and thalidomide were prescribed but did not improve upon the refractory, relapsing, and destructive presentation of the leg ulcers.

Improvement toward understanding of the molecular basis for pathogenic mechanisms in chronic inflammation has contributed

to the emergence of immunosuppressive biological therapeutics that target TNF-α. Few studies have indicated that any of the 3 anti-TNF-α agents, infliximab, adalimumab, or etanercept, have therapeutic benefit for chronic mucocutaneous lesions.[5,6] Although adalimumab combined with MTX was reported to successfully treat a patient with vasculitic leg ulcers,[22] neither adalimumab nor etanercept was effective in treating the patient's BD or BD-associated leg ulcers in our case.

Because of their demonstrative immunomodulatory and anti-inflammatory properties and regenerative potential, MSCs have emerged as a new treatment for refractory and severe autoimmune diseases.[8,9] In a multicenter clinical study, 40 SLE patients with active and refractory disease were treated with umbilical cord-derived MSC transplantation.[10] In that study, 32.5% of the patients achieved a significant clinical response, and 27.5% of patients achieved partial clinical response. In a separate study with 136 active RA patients who were refractory to conventional antirheumatic drugs alone, MSC transplantation combined with antirheumatic drugs induced a significantly clinical improvement.[11] Perturbations of T cell homeostasis that correlate with disease exacerbation have been reported recently in BD patients and include elevated TH1 cytokines[23] and promotion of TH17 responses with suppression of regulatory T cell (Treg) expansion.[24] Within this context, MSC transplantation can lead to reduced TH1-derived interferon-γ production, Treg cell expansion and upregulated Treg capacity[25–27] and reduced TH17 proliferation and cytokine production.[9,28,29] A high level of circulating angiostatin was correlated with disease activity in BD patients.[30] MSC transplantation can return normal homeostatic function to injured tissues including the secretion of factors that suppress inflammation and improve angiogenesis.[31–33] Finally, MSC transplantation induced significant healing of ulcers and necrotic skin lesions in SSc patients that had otherwise not responded to conventional therapy.[12–14] Within the context of several years of failed conventional immunosuppressive therapy and the extensive documentation for the immunosuppressive and anti-inflammatory function of transplanted MSC in the treatment of autoimmune and chronic inflammatory diseases including SSc skin lesions, MSC treatment was provided to our patient with rapid and dramatic therapeutic effect. The left leg ulcers disappeared, and the right ulcer was dramatically improved within 2 months of initiating MSC transplantation and, there have been no detrimental side effects commonly associated with MSC transplantation in follow-up to present.

4. Conclusion

In conclusion, MSC therapy for refractory, progressive BD skin lesions has not been reported to date. The present patient had BD with complicating leg ulcers that were refractory to conventional immunosuppressive therapy, including adalimumab and etanercept, for 6 years. Within 2 months of initiating MSC infusions, both BD-associated leg ulcers and other BD-associated lesions showed marked, continuous improvement. The patient's BD remains in remission with no post-infusion complications at 34 months of follow-up. Our experience suggests that MSC infusion might be a potentially successful therapy for intractable, drug-resistant BD patients with concomitant leg ulcer.

Author contributions

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