Successful mesenchymal stem cell treatment of leg ulcers complicated by Behcet disease
A case report and literature review

Yanhong Li, MD, PhD, Zhongming Wang, MD, Yi Zhao, MD, PhD, Yubin Luo, MD, PhD, Wangdong Xu, MD, PhD, Tony N. Marion, MD, PhD, Yi Liu, MD, PhD.

Abstract
Rationale: Behcet 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 comitant leg ulcer.

Abbreviations: ANCA = anti-neutrophil cytoplasmic antibodies, AZA = azathioprine, BD = Behcet 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: Behcet disease, leg ulcer, mesenchymal stem cell transplantation, therapy

1. Introduction
Behcet 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,[11] and the recently developed International
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 (2.5 mg biw) for 1 month, but with no clinical improvement. Replacement of etanercept with adalimumab yielded no clinical response. 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 widespread papulopustular lesions, oral and genital ulcers, multiple scars, and a positive pathergy test. Her right lower leg ulcer was markedly improved, and the left ulcer had

| 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.

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 infusions therapy. The patient was intravenously infused with 50 mL, 10<sup>6</sup> 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

![Figure 1. Leg Ulcer biopsy. Small vessel leukocytoclastic vasculitis (H&E, 20×).](image-url)
Fig. 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.

3. Discussion

BD is an autoimmune, chronic inflammatory disease with unknown etiology. BD is characterized clinically as a chronic, relapsing vasculitis with oral and genital ulcers, cutaneous inflammation, uveitis, and gastrointestinal and central nervous system manifestations. In 1990, an ISG attempted to consolidate diagnostic criteria for BD. 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.

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. Although BD has moderate association with inheritance of HLA B*51 (odds ratio 5.8), HLA B*51 is not included in the ICBD criteria. Worldwide, 41% to 97% of BD patients have skin lesion such as aphthous stomatitis, genital ulcers, erythema nodosum-like lesions, and papulopustular lesions, whereas leg ulcers were rare. 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. 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. 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.\(^{[15,34]}\) Although adalimumab combined with MTX was reported to successfully treat a patient with vasculitic leg ulcers,\(^{[21]}\) 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.\(^{[1,35]}\) 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\(^{[25]}\) and promotion of Th17 proliferation and cytokine production.\(^{[9,28,29]}\) A high level of circulating angiotatin was correlated with disease activity in BD patients.\(^{[10]}\) 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

Conceptualization: Yanhong Li.
Data curation: Yanhong Li.

Formal analysis: Yanhong Li, Yubin Luo.
Funding acquisition: Yi Liu.
Investigation: Yanhong Li, Zhongming Wang, Yubin Luo.
Methodology: Yanhong Li, Zhongming Wang, Yi Zhao, Wangdong Xu.
Software: Yi Zhao.
Validation: Yi Zhao.
Writing – original draft: Yanhong Li, Yi Liu.
Writing – review & editing: Yanhong Li, Tony N. Marion, Yi Liu.

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