

## Reactivation of Anti-Human Alpha-Enolase Antibody-Positive Behçet's Disease by Carbon Dioxide Laser Treatment

Sung Bin Cho\*, Jung U Shin\*, Keun-Jae Ahn, Mi Ryung Roh, Dongsik Bang, Kwang Hoon Lee and Ju Hee Lee

Department of Dermatology and Cutaneous Biology Research Institute, Yonsei University College of Medicine, 250 Seongsanno, Seodaemun-gu, Seoul, 120-752, Korea. E-mail: juhee@yuhs.ac

\*The first two authors contributed equally to this work and should be considered as first authors.

Accepted November 30, 2009.

We report here a case of a patient with anti- $\alpha$ -enolase antibodies-positive Behçet's disease (BD), which was reactivated as a pathergy reaction after carbon dioxide laser therapy.

### CASE REPORT

A 60-year-old woman presented with multiple encrusted, erythematous ulcerations on her face. Her medical history was significant for BD, including recurrent oral ulceration, erythema nodosum- and Sweet's syndrome-like skin lesions, and a positive pathergy test (1). Pathergy testing was performed by the intracutaneous injection of 0.1 ml isotonic salt solution using a 20-gauge needle on the patient's volar forearm, with pustule formation observed within 48 h at the injection site. Screening for genital ulceration and ocular involvement, such as iritis and uveitis, was performed; however, active disease involvement was not observed. The patient had been treated with colchicine and had been maintained in an inactive disease state for over a year.

One week prior to presentation, the patient underwent carbon dioxide laser treatment for multiple seborrheic keratoses and lentigines at an outside clinic. However, 3 days after the procedure all of the treated sites became inflamed and ulcerated (Fig. 1a). In addition, multiple oral ulcerations, as well as several erythema nodosum-like erythematous tender nodules and a blister on the forearm and dorsum of hand were evident (Fig. 1b). The patient was afebrile, and laboratory blood tests revealed leukocytosis of 10,030/ $\mu$ l with neutrophilia (80.8%), elevated erythrocyte sedimentation rate of 40 mm/h, and positive C-reactive protein of 9.480 mg/dl (normal range 0–8 mg/l). Bacterial cultures of the ulcerated wounds were negative. Skin lesions were biopsied, and histology revealed septal infiltration of lymphohistiocytes and neutrophils with vasculitis, consistent with the findings of erythema nodosum (Fig. 2a, b).

After obtaining informed consent, we collected serum samples from the patient, which were stored at  $-70^{\circ}\text{C}$ . The anti- $\alpha$ -enolase antibody test was performed according to the method described in a previous report (2). In an enzyme-linked immunosorbent assay (ELISA) for the anti- $\alpha$ -enolase antibody, the mean optical density (OD) ( $\pm$  1 SD) for serum samples from 23 healthy controls was  $0.139 \pm 0.0682$  (2). An OD exceeding this value by  $\geq 3$  SD was defined as positive reactivity. The patient's sample was positive, with an OD of 0.6485. Immunohistochemical analysis of a biopsy specimen was also performed using a rabbit anti-human  $\alpha$ -enolase antibody (Acris Antibodies, Acris Antibodies GmbH, Herford, Germany) in accordance with a previous report (3). The cytoplasm from lymphohistiocytes, neutrophils and endothelial cells were stained brown, whereas cell nuclei were counterstained blue (Fig. 2c, d).

The patient was treated with systemic low-dose prednisolone and colchicine. One month following this treatment, skin lesions on both the face and forearm demonstrated improvement, leaving residual post-inflammatory hyperpigmentation and prominent depressed scars, without any evidence of recurrence over a 3-month follow-up period.

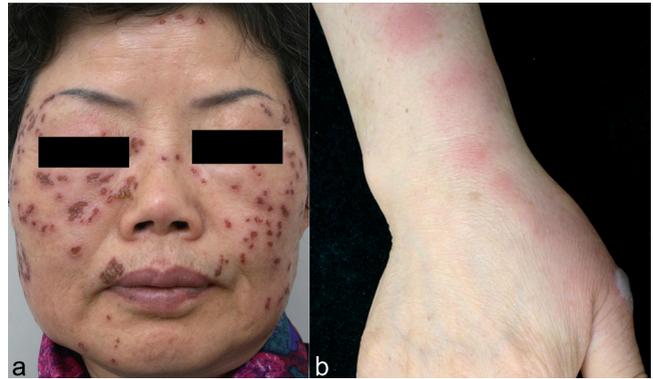


Fig. 1. (a) Multiple erythematous and encrusted ulcerations on the face following carbon dioxide laser therapy, and (b) several tender erythematous subcutaneous nodules on the forearm and a bulla on the base of the thumb.

### DISCUSSION

BD is a chronic, relapsing, multi-system vasculitis. The diagnosis relies on the presence of recurrent oral ulceration plus any two of the following: recurrent genital ulceration, characteristic ocular lesions, typical cutaneous lesions, or a positive pathergy test.

While the pathergy reaction, defined as an induction of an inflammatory process following skin trauma, is not a pathognomonic sign, it is a typical feature of BD (4). Aside from its association with BD, the pathergy reaction can also occur in patients with several conditions, including pyoderma gangrenosum, rheumatoid arthritis, Crohn's disease, genital herpes and Sweet's syndrome (1, 5, 6). A hypersensitivity reaction following trauma has also been reported in patients with BD, including long-lasting severe erythema and induration at the surgically-incised site, as well as acute exacerbations of arthritis following synovial biopsies (7, 8). In the present case, we conclude that the extensive carbon dioxide laser treatment induced a pathergy reaction, which consequently resulted in the activation of BD symptoms. According to a recent report, argon laser photocoagulation can induce skin hyper-reactivity in patients with BD (9).

Melikoglu et al. (10) reported that following a needle prick, patients with BD displayed increased influxes of cellular components, such as mature dendritic cells, monocytes, and lymphocytes, and increased expression of cytokines, including interferon (INF)- $\gamma$ , interleukin (IL)-12 p40, and IL-15, chemokines, such as macrophage inflammatory protein-3 $\alpha$ , IFN-inducible

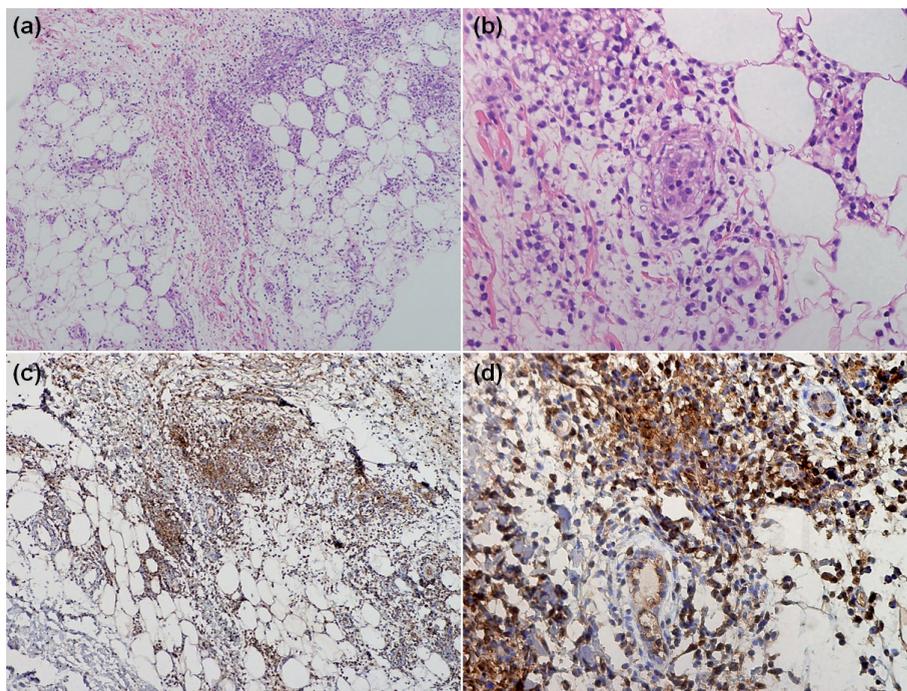


Fig. 2. (a–b) Biopsy specimen obtained from the skin lesions on the forearm demonstrating septal infiltration of lymphohistiocytes and neutrophils with vasculitis consistent with the findings of erythema nodosum (haematoxylin and eosin stain, original magnification, a;  $\times 100$ , b;  $\times 400$ ). (c–d) Immunohistochemistry of a biopsy section with rabbit anti-human  $\alpha$ -enolase antibody showing expression of  $\alpha$ -enolase in the cytoplasm of lymphohistiocytes, neutrophils, and endothelial cells (original magnification, c;  $\times 100$ , d;  $\times 400$ ).

protein-10, monokine induced by INF- $\gamma$ , and interferon inducible T-cell chemoattractant (ITAC), and adhesion molecules, including intercellular adhesion molecule-1 and vascular cellular adhesion molecule-1 compared with normal controls. This immune response ultimately leads to an exaggerated lympho-helper (Th)1-type response and clinically induces papules and pustules on an erythematous base.

The glycolytic enzyme  $\alpha$ -enolase is typically located in the cytoplasm, but can be expressed in the cell membranes of eukaryotic cells, including monocytes, T cells, B cells, neuronal cells, and endothelial cells, following an inflammatory stimulus through unknown mechanisms (2, 11, 12). Testing for antibodies to  $\alpha$ -enolase has been proposed as a diagnostic tool or a biological marker for various conditions, including BD, Kawasaki's disease, rheumatoid arthritis, and severe asthma (2, 11, 12). In the present case, we identified serum reactivity against human  $\alpha$ -enolase as well as expressions of  $\alpha$ -enolase in the cytoplasm of lymphohistiocytes, neutrophils, and endothelial cells from the biopsy specimen, demonstrating the histological findings of erythema nodosum. According to a previous report (2), serum anti- $\alpha$ -enolase antibodies in BD can be associated with vascular system involvement. However, the association between serum anti- $\alpha$ -enolase antibodies and the risk of developing pathergy reaction has not been proven.

The authors declare no conflict of interest.

## REFERENCES

1. Criteria for diagnosis of Behçet's disease. International Study Group for Behçet's Disease. *Lancet* 1990; 335:

- 1078–1080.
2. Lee JH, Cho SB, Bang D, Oh SH, Ahn KJ, Kim J, et al. Human anti- $\alpha$ -enolase antibody in sera from patients with Behçet's disease and rheumatologic disorders. *Clin Exp Rheumatol* 2009; 27: S63–66.
3. Kinloch A, Tatzler V, Wait R, Peston D, Lundberg K, Donatien P, et al. Identification of citrullinated alpha-enolase as a candidate autoantigen in rheumatoid arthritis. *Arthritis Res Ther* 2005; 7: 1421–1429.
4. Ozarmagan G, Saylan T, Azizlerli G, Ovul C, Aksungur VL. Re-evaluation of the pathergy test in Behçet's disease. *Acta Derm Venereol* 1991; 71: 75–76.
5. Bi XL, Gu J, Yan M, Gao CF. A case of Sweet's syndrome with slack skin and pathergy phenomenon. *Int J Dermatol* 2008; 47: 842–844.
6. Awan F, Hamadani M, Devine S. Paraneoplastic Sweet's syndrome and the pathergy phenomenon. *Ann Hematol* 2007; 86: 613–614.
7. Bozkurt M, Torin G, Aksakal B, Ataoglu O. Behçet's disease and surgical intervention. *Int J Dermatol* 1992; 31: 571–573.
8. Giacomello A, Taccari E, Zoppini A. Marked synovial sensitivity to pricking in Behçet's syndrome. *Arthritis Rheum* 1980; 23: 259–260.
9. Sayarlioglu M, Calka O, Cinal A, Sayarlioglu H, Akdeniz N, Topcu N, et al. Comparison of argon laser photocoagulation-induced cutaneous inflammation and skin pathergy test in Behçet's disease. *Clin Rheumatol* 2010; 29: 59–63.
10. Melikoglu M, Uysal S, Krueger JG, Kaplan G, Gogus F, Yazici H, Oliver S. Characterization of the divergent wound-healing responses occurring in the pathergy reaction and normal healthy volunteers. *J Immunol* 2006; 177: 6415–6421.
11. Lee KH, Chung HS, Kim HS, Oh SH, Ha MK, Baik JH, et al. Human alpha-enolase from endothelial cells as a target antigen of anti-endothelial cell antibody in Behçet's disease. *Arthritis Rheum* 2003; 48: 2025–2035.
12. Pancholi V. Multifactorial  $\alpha$ -enolase: its role in diseases. *Cell Mol Life Sci* 2001; 58: 902–920.