

Correspondence

Behçet's disease and nitric oxide production

Behçet's disease (BD) is a chronic multisystem disease characterized by oral and genital aphthae, ocular, gastrointestinal, neurologic and vascular manifestations, and pustular vasculitic cutaneous lesions. Although the pathogenesis of BD remains obscure, immune factors, infectious agents, and effector mechanisms have been implicated. It is considered to be a systemic vasculitis of unknown origin. Previous publications have reported an increase in cytokine and superoxide production by neutrophils and macrophages in BD.^{1,2}

Nitric oxide (NO), a highly reactive free radical with a very short half-life, is a critical biologic mediator with physiologic and pathophysiologic roles in nearly every organ system, including the skin. NO is generated by three isoforms of specific nitric oxide synthases (NOS). These three distinct isoforms of NOS include nNOS, eNOS and iNOS.³ NO is generated by eNOS/nNOS in keratinocytes, melanocytes, fibroblasts and endothelial cells, and by iNOS in keratinocytes, Langerhans cells, fibroblasts and endothelial cells.³ The second source of NO in plasma is the diet.⁴

The purpose of this study was to examine the potential role of NO, via its metabolite nitrate, in the mechanism leading to endothelial damage in BD.

Twenty-seven Turkish patients in the active phase of the disease (17 women, 10 men; age range, 14–50 years; mean age, 28.85 ± 1.75 years) and 13 treated patients with BD (six women, seven men; age range, 23–49 years; mean age, 31.46 ± 2.6 years; treated with colchicine, azathioprine, prednisolone, pentoxifylline), fulfilling the criteria of BD set by the International Study Group for BD (ISG-BD),⁵ and 10 healthy controls (six women, four men) were studied. All patients were examined by dermatologists, ophthalmologists, neurologists, rheumatologists, and internal medicine doctors. Serum specimens were obtained after a 12-h fast and stored at -70°C . The clinical data of the patients are summarized in Table 1. The amount of total nitrate in the test samples was determined by a modification of the procedure described by Braman and Hendrix,⁶ using the purge system of a Sievers Instruments Model 280A Nitric Oxide Analyzer. Samoles nitrate levels were calculated using the standard curve. Serum nitrate levels were evaluated in 27 patients with active BD, 13 patients treated for BD and 10 healthy controls. The following mean results were obtained: $12.96 \pm 0.51 \mu\text{M}$ (range, 10–21 μM), $8.24 \pm 0.20 \mu\text{M}$ (range, 6.5–9.2 μM), and $7.61 \pm 0.36 \mu\text{M}$ (range, 6–9 μM), respectively. The Mann–Whitney *U*-test was used for statistical analysis of the results obtained from these three groups. Patients with active BD

had significantly higher levels than those with treated BD and healthy controls ($P < 0.05$).

Ohashi *et al.*⁷ measured the levels of NO in the saliva of 39 patients with oral mucosal disease (oral lichen planus and recurrent aphthous ulceration), and found that they were significantly increased compared with those of healthy subjects. They believed that this increase was a consequence of cell damage. As 100% of our active BD patients (fulfilling the criteria of ISG-BD) also had recurrent aphthous ulceration, our results can be considered to be in agreement with those of Ohashi *et al.* Özkan *et al.*⁸ investigated nitrite plus nitrate levels in 25 patients with symptomatic BD and 19 patients with asymptomatic BD; the levels in patients with symptomatic BD were significantly higher than those in patients with asymptomatic BD. Örem *et al.*⁴ investigated plasma nitrite, nitrate, and nitrite + nitrate levels in 21 patients and 20 healthy controls, and found them to be significantly lower in BD patients. They reported that decreased NO production in BD patients may be a consequence of the endothelial dysfunction in BD. Our results are in agreement with those of Özkan *et al.*⁸, but not those of Örem *et al.*⁴ In inflammatory processes, the regulation of leukocyte trafficking involves a complex interplay of adhesion molecules and chemokines, with cytokines produced by both leukocytes and endothelial cells. There is increasing evidence indicating that iNOS expression and a concomitant increase in NO production in endothelial cells modulate lymphocyte adhesion as well as leukocyte trafficking.³ Circulating immune complexes and neutrophils play an active part in the pathogenesis of mucocutaneous lesions in

Table 1 Clinical manifestations in patients with active Behçet's disease and those treated for Behçet's disease. There was no statistically significant difference between the two groups using chi-squared and Fisher's exact test ($P > 0.05$)

Clinical data	Active BD (%) (n = 27)	Treated BD (%) (n = 13)
Oral ulcers	100	100
Genital ulcers	92	92.3
Ocular disease	66.7	53.8
Cutaneous lesions	53.8	38.5
Positive pathergy test	59.3	53.8
Arthropathy	25.9	38.5
Gastrointestinal symptoms/disease	3.7	7.7
Venous disease	22.2	15.4
Central system involvement	7.4	7.7
Pulmonary involvement	–	–

BD. Biopsy samples show endothelial swelling, leukocytoclasia, and neutrophilic vascular reactions in early lesions.¹ NO can produce inflammation in cell walls, immunomodulation, and oxidative damage to cells and tissue, and can give rise to a picture of systemic vasculitis.^{2,9,10}

In the present study, we demonstrated that mean serum nitrate (the end product of NO) levels were significantly higher in patients with active BD than in controls and those treated for BD. After eliminating dietary factors and taking the relatively high levels of nitrate into consideration, this could reflect the increased activity of iNOS in active BD. It would not be surprising to find increased levels of NO (metabolites) in BD because of the oxidative stress and vasculitic processes in the pathogenesis of the disease. Furthermore, the lower nitrate levels of treated BD than active BD patients suggest the inducible/treatable phenomenon of NO in BD. We believe that excessive production of NO may be associated with endothelial cell damage. The role of NO in BD should be further investigated in the same patients over time in different stages of their disease. This will provide more information on the pathophysiology of BD, and will aid in the development of new treatment modalities.

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Congenital self-healing Langerhans cell histiocytosis:

The need for a long term follow up

In Volume 38 (1999) of the *International Journal of Dermatology*,¹ our report on 12 cases of congenital self-healing histiocytosis (Hashimoto-Pritzker) was published.

Langerhans cell histiocytosis (LCH) is a generic term that identifies several clinical diseases characterized by proliferation of distinctive histiocytes that contain Birbeck granules in their cytoplasm.

Congenital self-healing Langerhans cell histiocytosis (CSHLCH) is an uncommon form of LCH, initially seen at birth or in the neonatal period, and characterized by generalized papules, vesicles, or nodules on the skin without the involvement of other organs.² Some typical features of CSHLCH are: congenital skin lesions, an otherwise healthy infant, histopathology showing Langerhans cell infiltrates, and spontaneous involution of skin lesions.³

At the time of our report, patients had not presented any relapses in routine follow-up visits. However, we emphasized the need for a careful evaluation of systemic involvement and long-term follow-up for evidence of relapse or progression of the disease.¹ The last patient to be included into our report never came back for her control visit. We have recently found out that she died 3 months before her scheduled visit, when she was 11 months old.

We have gathered some information on what happened to her, and consider it important to report this in order that conclusions drawn from our experience can be applied to similar cases.

The patient was a healthy new-born girl, who had been carried to term during pregnancy without complications. At birth, she showed a large number of 2-5-mm papulovesicles with a generalized distribution, including the upper surface of the tongue. There was no organomegaly or lymphadenopathy, and all other signs following physical examination, laboratory tests, abdominal ultrasound, and chest, skull and bone X-rays were normal.

Light microscopy with hematoxyline and eosine stains showed a lichenoid infiltrate composed of many histiocytes and a few neutrophils in the papillary dermis with epidermotrophism; immunomarking with S100 and CD1 were both positive. Our diagnosis was congenital Langerhans cell histiocytosis. The skin and mucosal lesions disappeared when the baby was 3 months old, and the laboratory work-up was completely normal, so we concluded that her diagnosis

was congenital self-healing Langerhans cell histiocytosis of the Hashimoto-Pritzker type. She never came back for routine follow-up, which was scheduled for 1 month later.

When the baby was 8 months old, she was hospitalized at La Plata Children's Hospital with anemia, thrombocytopenia, lung involvement, hepatosplenomegaly and severe systemic involvement, with purpuric lesions on trunk and groin. Cutaneous and bone marrow biopsy were performed with hematoxyline and eosine stains and immunomarking with S100 and CD1. The diagnosis was LCH.

The baby died at 11 months of age with a diagnosis of systemic LCH.

We believe that this case should serve as a warning that, although visible lesions may disappear, it is important to follow up patients with a diagnosis of CSHLCH because of the possibility of misdiagnosis of a congenital systemic histiocytosis.

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Atopy patch test reactivity to house dust mites in patients with scabies

As they are related phylogenetically, house dust mites (HDM: *Dermatophagoides pteronyssinus* = Dp, and *Dermatophagoides farinae* = Df) and the scabies mite (*Sarcoptes scabiei* = ss) have many physical and antigenic similarities.¹ It is well known that circulating immunoglobulin E (IgE) antibodies against scabies mite antigens show cross-reactivity with HDM antigens. In the literature, there are studies reporting that 35 to 75% of scabies patients showed skin prick test (SPT) and/or specific IgE positivity to HDM antigens.^{2–5} These results suggest that specific T cells developed against ss antigens in scabies patients might be cross-reactive with HDM antigens

Our aim was to detect HDM-induced, cell-mediated hypersensitivity with the atopy patch test (APT) in patients with scabies. We also investigated the relationship of the cell-

mediated immune response developed against HDM to the immediate-type immune response [SPT or intradermal test (IDT) reactivity] against HDM in scabies.

Thirteen patients with active scabies aged between 20 and 24 years (mean: 20.92 years), who had no past history of scabies, enrolled in the study. For all patients, the diagnosis of scabies was verified microscopically. The presence of atopic dermatitis (AD) was regarded as an exclusion criterion. Each patient received detailed information regarding the study and written informed consent was obtained.

Disease severity was graded according to the following criteria⁴: mild = skin lesions in a few areas (< 20% of body surface); moderate = skin lesions in many areas (20–50% of body surface); severe = generalized eruption (> 50% of body surface). Severity of itching was assessed on a visual analogous scale (0 = absent ... 10 = very severe).

SPT with standardized HDM "mixed" extracts (5000 AU/mL Dp + 5000 AU/mL Df) (Center Laboratories) was performed for all patients. SPT results were evaluated as positive if the HDM extract-induced weal was at least 3 mm larger in diameter than that of the negative control. For SPT– patients, the IDT procedure was carried out using a 1/1000 dilution of the HDM "mixed" extract. A sterile diluent containing 0.9% physiologic saline was used as a negative control. Weals at least 4 mm larger in diameter than those of the negative control with or without pseudopods were regarded as positive in IDT.

All patients were treated with antiscabies agents (permethrin 5% cream) after SPT/IDT evaluation. On the next day, APT with Dp and Df allergens in petrolatum (Stallergenes, France) was applied to uninvolved skin on the backs of patients after 10 tape-strippings. Pure petrolatum was used as a (negative) control. Results were evaluated after 48 h according to the following criteria⁶:

- Negative
- ? Only erythema; questionable
- 1+ Erythema; infiltration
- 2+ Erythema; few papules (≤ 3)
- 3+ Erythema; ≥ 4 papules
- 4+ Erythema; many papules or spreading papules
- 5+ Erythema; vesicles

Table 1 shows the clinical features and skin test results for the patients. Four of them had allergic rhinoconjunctivitis (ARC) characterized by sneezing, rhinorrhea, nasal itching, nasal blockage and itching of the eyes. Nine out of 13 patients (69%) showed APT positivity against HDM (Dp and/or Df). In seven of these patients, APT was reactive to both mites (Dp and Df). There was a good correlation between SPT/IDT and APT: eight out of nine APT+ scabies patients had positive SPT and/or IDT results against HDM (Dp and/or Df). Similarly, just one APT+ patient had negative results for SPT and IDT. Three out of four ARC+ scabies patients also showed APT positivity against HDM. Two ARC+ scabies patients (nos 6

Table 1 Clinical features and skin test results for the patients

Patient no.	Sex (M/F)/age (years)	Grading	Duration of scabies (days)	Itching (0–10)	Associated respiratory atopy	SPT	IDT	APT		
								Dp	Df	Control
1	M/24	Mild	15	6	–	–	+	–	–	–
2	M/22	Mild	30	8	–	+	ND	–	–	–
3	M/21	Moderate	30	8	AR	–	+	–	–	–
4	M/21	Mild	30	8	–	–	+	–	–	–
5	M/20	Moderate	30	6	–	–	+	3+	3+	–
6	M/20	Mild	21	8	AR	+	ND	5+	3+	–
7	M/20	Moderate	30	4	–	–	+	–	2+	–
8	M/21	Moderate	14	6	–	–	–	1+	1+	–
9	M/20	Moderate	14	5	–	–	+	1+	1+	–
10	M/21	Mild	21	7	AR	+	ND	4+	3+	–
11	M/20	Severe	45	8	–	+	ND	2+	3+	–
12	M/21	Moderate	45	4	AR	–	+	–	2+	–
13	M/21	Moderate	45	7	–	–	+	3+	3+	–

ND, not done.

and 10) had the most severe APT reactivities. There seemed no correlation between APT reactivity and grading of severity, duration of infestation, or itching severity. Nevertheless, as we studied only 13 patients, it is not possible to draw firm conclusions about these correlations.

The results of this study cast doubt on the commonly held opinion that the patch test reaction to aeroallergens is specific for some cases of AD, and does not occur in healthy individuals or in patients with respiratory atopic diseases [ARC and/or allergic bronchial asthma (ABA)].⁷ To the best of our knowledge, this is the first study demonstrating positive APT results against HDM antigens in atopic or nonatopic scabies patients in the absence of atopic dermatitis. The negative control patch test with pure petrolatum on uninvolved skin excluded the possibility that APT reactivity against HDM in scabies patients might have resulted from heightened cutaneous reactivity typically seen in scabies.

Although van Voorst Vader *et al.*⁸ found that all APT+ AD patients had concomitant respiratory allergic disease, it seems that, even if they have no associated AD, ARC and/or ABA, patients with active scabies or those treated for scabies recently may show APT positivity against HDM extracts. We therefore suggest that active allergic airway disease is not a prerequisite for APT positivity against HDM antigens in AD patients; instead, active or recently treated scabies infestation may result in a positive APT reaction.

Changes in immunological parameters in scabies patients (SPT and/or Dp/Df-specific IgE reactivity, and increases in total IgE and blood eosinophil count associated with APT positivity against HDM) suggest that scabies infestation might cause a transient “atopy” or “AD-like” state. In several studies, some patients who had previously had scabies were found to be SPT or IDT positive against HDM for 6 weeks to

several months.^{4,5,9} However, most of the patients who were successfully cured of scabies eventually lost immediate-type HDM reactivity. While concomitant ARC in four scabies patients may be regarded as the cause of HDM positivity in SPT or IDT, it cannot explain APT positivity.

Because the post-treatment follow-up period is not over, it is too early to draw firm conclusions regarding the duration of APT positivity against HDM in scabies patients.

Whether APT+ scabies patients will derive greater benefit from HDM elimination procedures to prevent the persistence of itching (postscabietic pruritus) remains unclear. However, we suggest that the existence or absence of scabies should be taken into consideration before performing the APT procedure. In our opinion, another question should be added to those asked during the evaluation of a patient for APT: “Have you been treated for scabies recently?”

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