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# An Immunoelectron Microscopic Study of Biopsy Samples from Erythema Nodosum-like Lesions of Behçet's Disease Using the Sera of Patients with Behçet's Disease

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To find the presence of antigenic source in lesional tissues of Behçet's disease reacting with their own serum, erythema nodosum (EN) -like lesions of Behçet's disease patients were examined on the morphological and immunological aspects by immunoelectron microscopy using protein A-gold. All of the EN-like lesions treated with patients serum showed positive immuno-staining results. Electron-dense gold particles were clearly observed on fat droplets, collagen fiber, and within the lysosomes of macrophages and neutrophils. The present study suggested that fat droplets resulting from the degeneration of fat cells may be an important antigenic source which provokes an immunologic reaction.(Ajou Med J 1997; 2(2): 108~111)

**Key Words:** *Immunoelectron microscopy, Behçet's disease*

## INTRODUCTION

The etiology of Behçet's disease is unclear[1]. To understand the pathogenesis of Behçet's disease, the initial ultrastructural and immunological changes caused by the disease have tc be elucidated. However, it is very difficult to obtain suitable specimens with which we can look into these initial changes, because most lesions have been already ulcerated or altered by secondary infections by the time of observation.

The most frequent skin lesions in our cases were erythema nodosum (EN) - like lesions and papulopustular eruptions. EN-like skin lesions can be a very useful specimens, because biopsy specimens can be obtained from early lesions with no ulceration or secondary infection.

From series of electron microscopic studies on EN-like lesions, we previously reported microvascular changes and lymphocyte-mediated fat cell lysis[2,3].

In this study, biopsy specimens from 8 patients with Behçet's disease were examined by immunoelectron microscopy using protein A-gold.

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## MATERIALS AND METHODS

The diagnosis of the eight patients with Behçet's disease included in our study was based on the criteria of International Study Group for Behçet's disease[4]. All patients manifested at least three of the four cardinal signs of Behçet's disease: oral ulceration, genital ulceration, uveitis, and EN-like lesions. The two male and six female subjects ranged from 27 to 40 years in age.

The biopsy specimens were obtained within one to four days after the initial clinical manifestation of erythematous tender nodules on the lower extremities. As controls, normal tissues were obtained from the opposite, uninvolved sites in 4 of the 8 patients. Immunocytochemistry was performed by minor modification of Silver and

**Table 1.** Clinical characteristics of patients

| Patient | Age(yr)/Sex | Duration of each major symptom(yr) | | | | Site of skin biopsy |
|---|---|---|---|---|---|---|
| | | Oral | Genital | Skin | Ocular | |
| 1. | 40/F | 7.0 | 5.0 | 4.5 | 6.0 | Leg |
| 2. | 30/M | 9.0 | 7.0 | 7.0 | 5.0 | Leg |
| 3. | 27/F | 4.0 | 4.5 | 5.0 | − | Leg |
| 4. | 33/F | 18.0 | 0.5 | 6.0 | − | Leg |
| 5. | 38/M | 10.0 | 8.0 | 8.0 | − | Leg |
| 6. | 33/F | 10.0 | 2.0 | 5.0 | − | Leg |
| 7. | 27/F | 4.0 | 4.0 | 4.0 | − | Leg |
| 8. | 31/F | 1.0 | 1.0 | 0.1 | − | Leg |

* The skin symptom was erythema nodosum-like lesions.

**Table 2.** Summary of protein A-gold immunostaining in erythema nodosum-like lesions of Behçet's disease and in normal tissues

| Patients | Erythema nodosum-like lesions of Behçet's disease | | Normal tissue | |
|---|---|---|---|---|
| | Patient's serum | Normal Serum | Patient's serum | Normal Serum |
| 1. | + | − | − | − |
| 2. | + | + | − | − |
| 3. | + | − | − | − |
| 4. | + | − | | |
| 5. | + | − | | |
| 6. | + | − | | |
| 7. | + | − | | |
| 8. | + | − | | |

Hearn's previously described method[5].

The specimens were immediately fixed for 10 minutes in periodate-lysine-paraformaldehyde fixative solution. After washing in cacodylate buffer, pretreatment with 1% carnation and 0.05% Tween 20 in cacodylate buffer was performed for 5 minutes. The specimens of erythema nodosum were incubated for 2 hours with patient's or normal human serum. Each specimen was treated with each patient's own serum. Five nanometer gold granules (Janssen) labeled with protein-A was used for tagging. Incubation lasted for 1 hour. After washing in cacodylate buffer, specimens were incubated for 30 minutes in Karnovsky's fixative solution, containing 2% paraformaldehyde and 2.5% glutaraldehyde, as a prefixation. The postfixation was performed at room temperature for 20 minutes with 1.3% osmium tetroxide in cacodylate buffer. The specimens were dehydrated in alcohol series, substituted in propylene oxide and embedded in epoxy medium. As a control, the specimens of normal tissue were incubated for 2 hours with normal human serum. The specimens were observed with a Phillips CM 10 electron microscope after staining with lead citrate and uranyl acetate.

## RESULTS

All of the EN-like lesions in 8 cases, treated with patient's serum, revealed positive immunostaining. When the lesions of patients were treated with normal human serum, five patients showed negative and three positive. However, when normal tissue was treated with patient's


**Fig. 1.** Electron micrographs showing the deposition of gold particles on the surfaces and inside of fat droplets (fd1, fd2)
**Fig. 2.** Gold particles are observed at the surface (arrow) of a fat droplets.

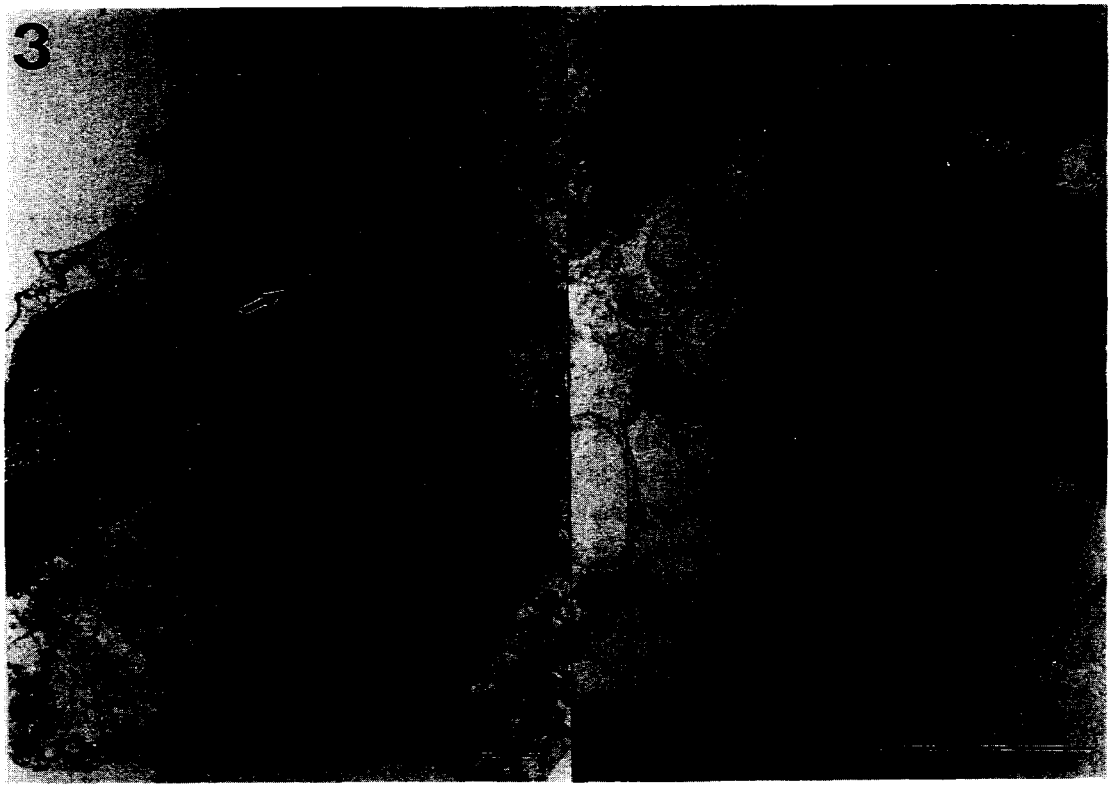


**Fig. 3.** Gold particles are observed within the lysosome (arrow) of a neutrophil.
**Fig. 4.** Higher magnification of the cytoplasm of neutophil.
All electron micrographs show the lesions of patients treated with patient's serum.

serum or with normal serum, all normal tissue showed negative immunostaining.

In the positive-stained tissues, electron-dense gold particles were clearly observed on the surface and inside the fat droplets (Fig. 1, 2), and also in the lysosomes of neutrophils (Fig. 3, 4).

## DISCUSSION

The present study supports our previous morphologic observations and hypothesis that fat cells are important in the pathogenesis of the EN-like lesions of Behçet's disease[2]. In our previous report[2], light microscopic finding revealed predominant lymphocytes and macrophages infiltrated around blood vessels in panniculum of erythema nodosum-like lesion. A few fat cells are seen in perivascular connective tissue of interlobules. Fat cells became detached from the basal laminar in the early stage of the development of the EN-like lesions, and that this detached area was first infiltrated by lymphocytes followed by macrophages, leading to lysis of the fat cells. The early specific degeneration of endothelial cells and vascular stenosis was associated with the delayed-type hypersensitivity reaction.

The present results suggested that fat droplets derived from the degeneration of fat cells might be an important antigenic source which provokes an immunologic reaction.

There are a few possible explanations for the positive immunostaining in three patients when their EN-like lesions were treated with normal human serum. One possibility is that the tissue of lesions locally change and react to normal human serum as an autoantigen. Alternatively, there may have been some factor in the experimental normal human serum which caused a false positive reaction.

Further studies are in need to clarify the true nature of the antibody present in the serum which reacts with the mixture of fat droplets in EN-like lesions in patients with Behçet's disease.

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