Ultrastructure of Vascular Changes in Cutaneous Manifestations of Behçet's Disease

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We observed small dermal blood vessels embolized by thrombus at the sites of needle-prick reaction as well as erythema nodosum-like lesions in 4 patients with Behçet's disease, by means of electron microscopy. The ultrastructural finding associated with overproductive proliferation of the endothelial cells leading to obliteration of the vascular lumen was the sporadical appearance of degenerated cells among proliferating endothelial cells in both cutaneous manifestations. These degenerated cells showed contraction changes and may be involved in thrombogenesis. *Key words: needle-prick reaction; degenerated endothelial cell; thrombus formation.* (Received May 8, 1987.)

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The skin of patients with Behçet's disease is known to be hyperreactive to non-specific minimal trauma such as needle-prick. The needle-prick reaction, or inflammatory reaction induced by pricking with a needle, is useful for diagnosis of this disease (1–3). However, the pathogenesis of this phenomenon, as of other symptoms, still remains unknown. As for the relationship between the hyperreaction following needle-prick and the circulating immune complexes, attempts to immunohistochemically demonstrate deposits of specific immunoglobulin classes and complements have not been successful (4). On the other hand, papulo-pustular manifestations of needle-prick reaction have been noted histologically to resemble erythema nodosum-like lesions (5), one of the cardinal manifestations of Behçet's disease, in that they both show typical of proliferating and/or obliterating vasculitis accompanied by thrombotic phenomena with partial recanalization of the lumen (1).

In our previous report (6), we described proliferation and degeneration of endothelial cells in the dermal and subcutaneous microvasculature in the erythema nodosum-like lesions of Behçet's disease and suggested a close relationship between these phenomena and the pathogenesis of vascular changes in this lesion. In this study, we observed dermal vessels embolized by thrombi at the site of needle-prick reaction in patients with Behçet's disease under electron microscopy, and presented a hypothesis concerning this embolus formation.

MATERIALS AND METHODS

Biopsies of papulo-pustular manifestations induced by needle-prick test and erythema nodosum-like lesions were performed in 4 patients with Behçet's disease. All patients manifested at least three of the four cardinal signs of Behçet's syndrome: aphthous stomatitis, genital ulceration, uveitis, and erythema nodosum-like lesions. Biopsies were obtained from a positive reaction site on the patients'

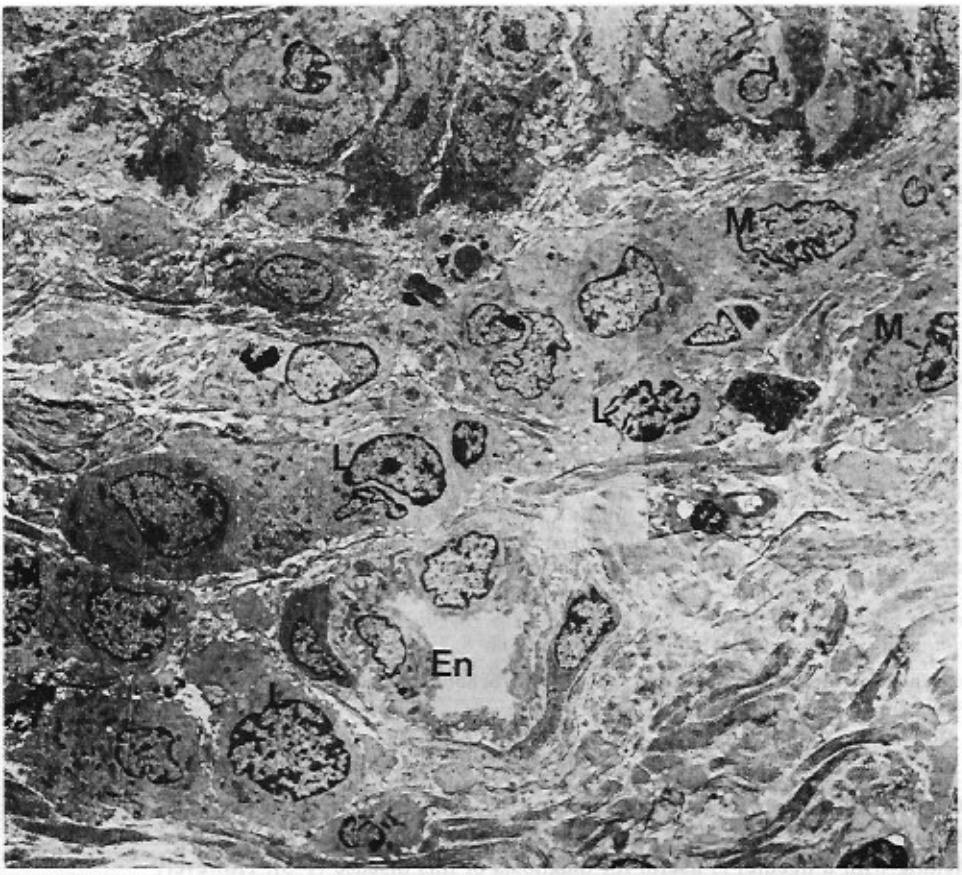

Fig. 1. Papulo-pustular manifestation of the forearm induced by needle-prick. Perivascular cellular infiltrate of a capillary (*En*) in the upper dermis. Perivascular infiltrate chiefly consisting of lymphocytes (*L*) and macrophages (*M*). ×2025.

flexor forearm at 24 h after needle-prick test and also from the most recently developed tender erythematous nodule on their lower extremities.

Specimens were fixed for 2 h in a chilled solution of 2.5% glutaraldehyde in 0.1 M phosphate buffer (ph 7.4). The tissues were then postfixed in 1% osmic acid buffer solution (pH 7.4) at 4°C for 2 h. Dehydration was performed using a graded ethanol series, and the specimens were embedded in epoxy resin (Epon 812). Samples for transmission electron micrographs were prepared by cutting silver to pale gold ultra-thin sections and staining them with uranyl acetate and lead citrate. The ultra-thin sections were examined in an electron microscope.

RESULTS

Twenty-four hours after needle-prick, lymphocyte–mononuclear phagocytic infiltrations are seen around the smaller vessels in the corium, followed by subepidermal edema at the papillary level (Fig. 1). This finding is accompanied by a productive reaction in the form of abnormal proliferation of the endothelial cells leading to obliteration of the vascular lumen.

Enlargement of cytoplasm and mitoses of endothelial cells

The cytoplasm of the endothelial cells of small vessels that constitute subcutaneous microcirculation was enlarged to a great extent and frequently protruded into the vascular

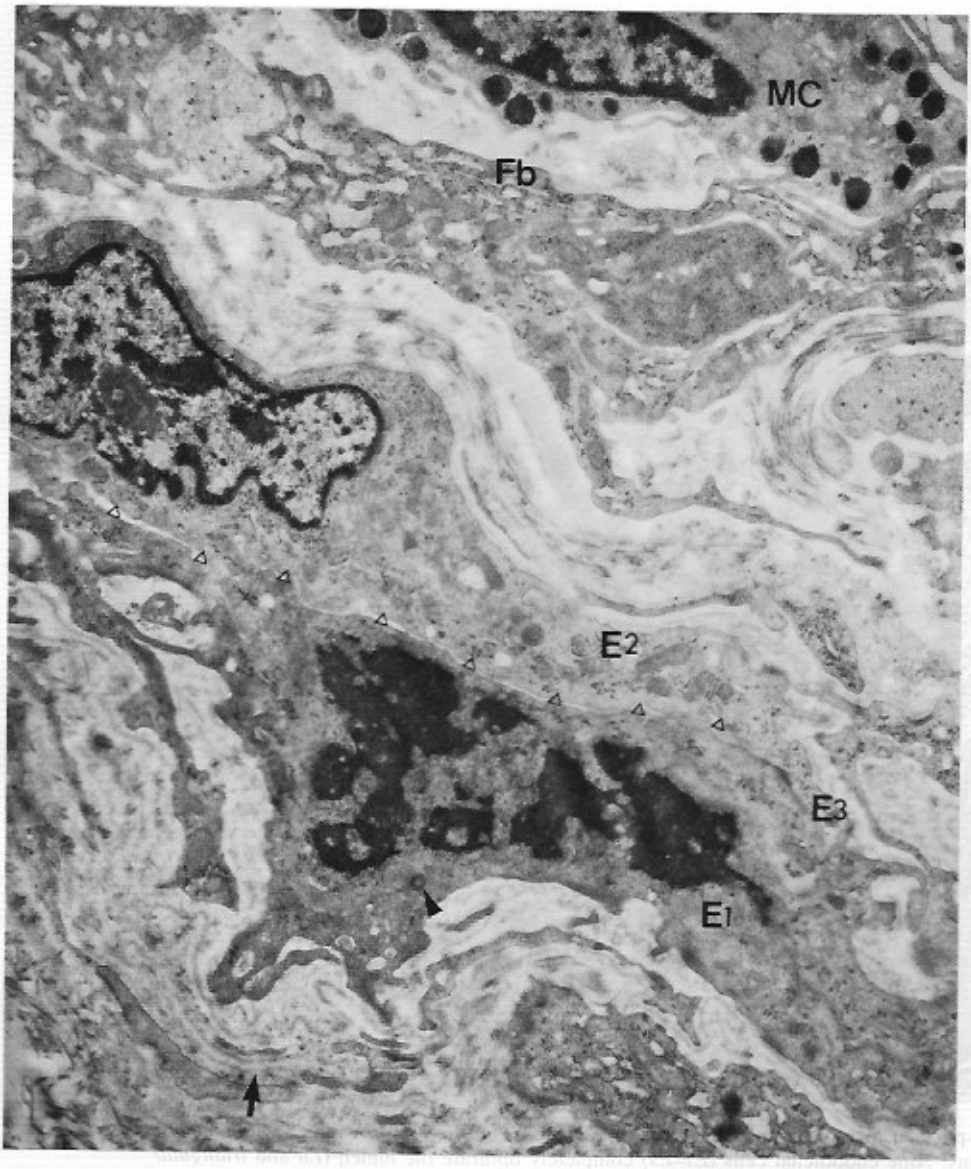

Fig. 2. Papulo-pustular manifestation of the forearm induced by needle-prick. A venule with mitotic figure. Three endothelial cells (*E1–E3*) are obturating the lumen (*triangular marks*). The mitotic endothelial cell (*E1*) contains centrioles (*arrowhead*). The basal lamina is duplicated or multiplicated (*arrow*). Fibroblasts (*Fb*) and a mast cell (*MC*) seen in the perivascular area. ×9000.

lumen. Such endothelial cells showed increased microfilaments and well developed organelles in their enlarged cytoplasm. In rare cases, venous endothelial cells exhibited characteristics of mitosis and caused stenosis of the vascular lumen (Fig. 2).

Proliferation and degeneration of endothelial cells

Other changes in small vessels included complete obstruction of the vascular lumen by proliferation of endothelial cells. The most interesting ultrastructural finding associated

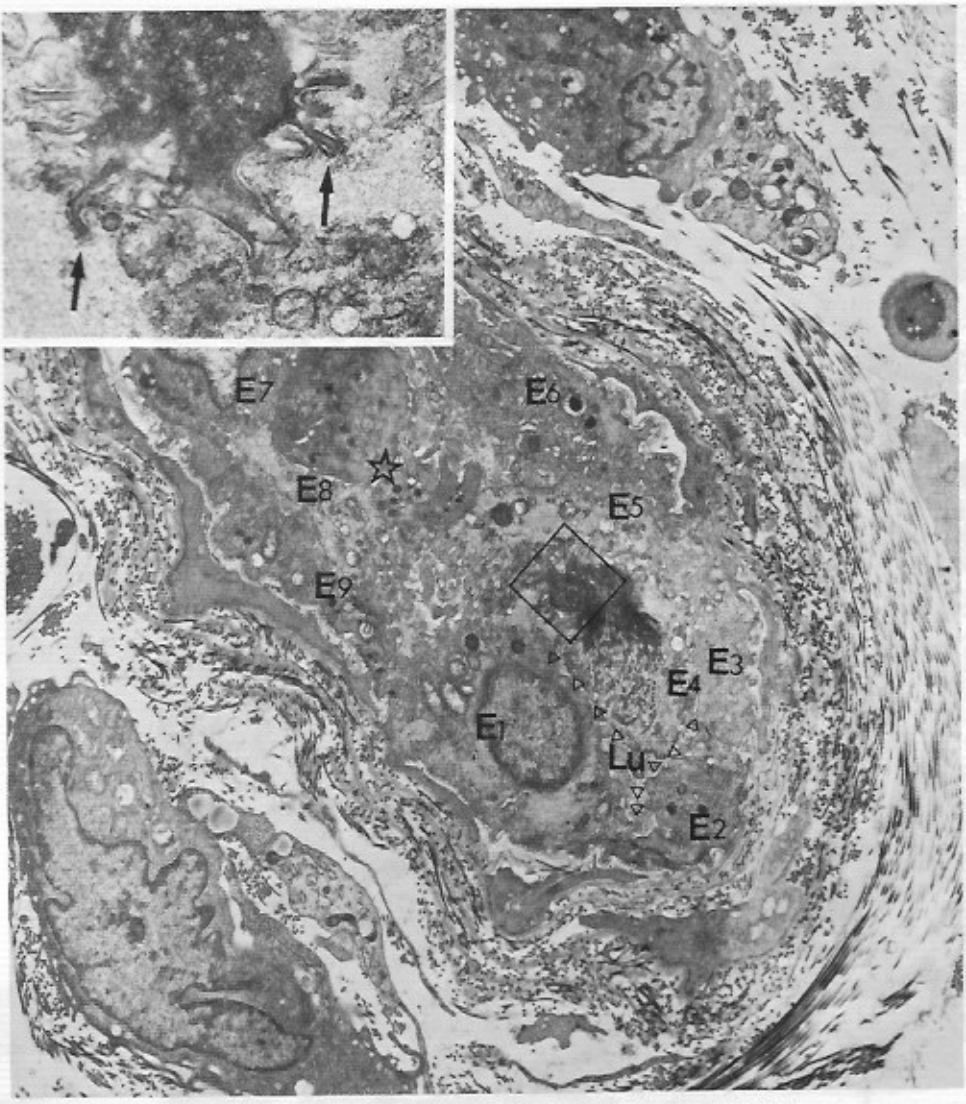

Fig. 3. Papulo-pustular manifestation of the forearm induced by needle-prick. A venule with embolus and/or thrombus. Nine endothelial cells (*E1–E9*) completely obturate the lumen (*Lu* and *triangular marks*). One cell (*E4*) in the lumen shows degenerated changes including nuclear condensation. Platelets (*star*) are also seen in the lumen. ×4500. *Inset:* High-power view of rectangular area indicated in Fig. 3. The degenerated endothelial cell (*E4*) forms gap junctions (*arrows*) with neighbouring endothelial cells. ×18000.

with this phenomenon was the sporadic appearance of degenerated cells among proliferating endothelial cells (Fig. 3). These cells showed contracted degeneration identical with that observed in activated endothelial cells of dermal and subcutaneous blood vessels in erythema nodosum-like lesions (Fig. 4). These degenerated cells—or their nuclei—frequently showed contracted changes, but the gap junctions that connected these cells with adjacent normal endothelial cells were maintained. Thus, the degeneration of endothelial cells was localized in the vascular wall, in which retention of platelets was often observed,

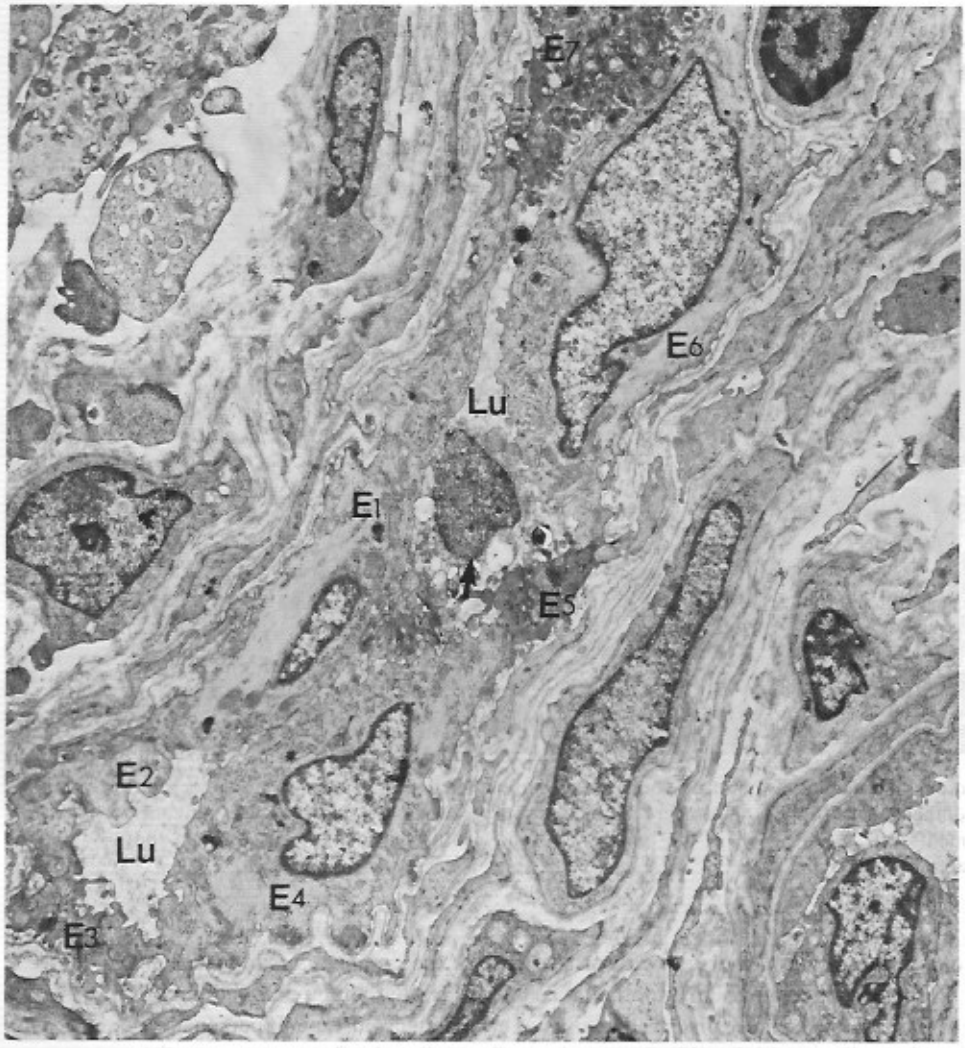

Fig. 4. Nodular lesion of the leg. A venule with embolus. Among seven endothelial cells (*E1–E7*), two cells (*E5* and *E7*) show degenerated changes including cytoplasmic condensation. Cellular fragments (*arrow*) seen in the lumen (*Lu*). ×5400.

but no deposit of fibrin were noted (Fig. 3). Occasionally, marked edema was observed but slight infiltration of not only lymphocytes but also neutrophilic leukocytes was seen in connective tissues surrounding the endothelium showing necrotic configuration with homogeneous condensation of endothelial cells (Fig. 5).

DISCUSSION

It has commonly been agreed that perivascular predominantly lymphocytic infiltration constitutes an early histological feature of the needle-prick reaction as well as erythema nodosum-like lesions in patients with Behçet's disease (1, 7, 8). These blood vessels showing lymphocytic infiltration have also been observed to often become obstructed by

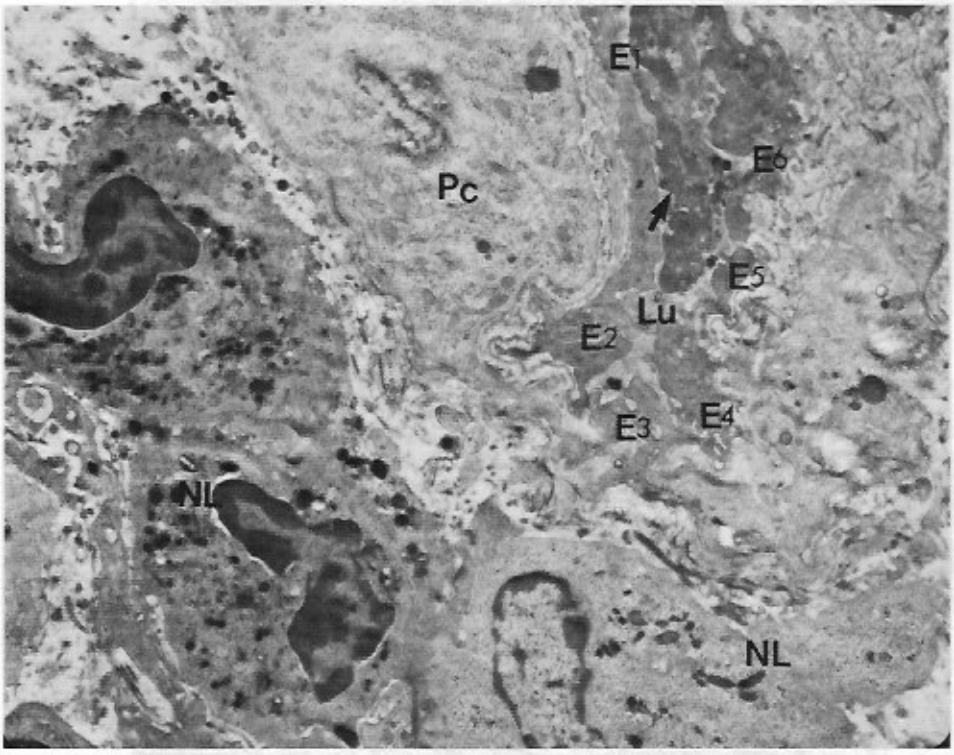

Fig. 5. Papulo-pustular manifestation of the forearm induced by needle-prick. A venule with necrotic figure. All endothelial cells (*E1–E6*) show necrotic changes, but a pericyte (*Pc*) shows an intact figure. Cellular fragments (*arrow*) are seen in the lumen (*Lu*), and neutrophilic leukocytes (*NL*) in the perivascular area. ×5400.

endothelial cell proliferation associated with progression of the cutaneous manifestations (1, 9). Such morphological changes in endothelial cells, however, are not a pathognomonic of Behçet's disease but are widely observed at sites of typical delayed-type hypersensitivity reaction in tuberculin sensitivity (10), immunologically mediated inflammatory reaction induced by peroxidase (11), chronically inflammatory reaction (12) and cell-mediated immunological reaction such as allograft rejection and contact allergy (13, 14). Nevertheless, these changes in endothelial cells are considered to be closely related to the pathogenesis of Behçet's disease, since they are commonly observed in organs or tissues exhibiting symptoms and signs of the disease (7).

On the other hand, we previously carried out an electron microscopic investigation of endothelial cell proliferation and vascular obstruction in erythema nodosum-like lesions (6), and noted the appearance of cells showing characteristic degeneration among endothelial cells. These degenerated cells were distinct from adjacent cells with normal morphology in that the entire cells were condensed and shrunk. We also noted similar degenerated cells in the dermal blood vessels under electron microscopy at the sites of papulo-pustular manifestations induced in the forearm of patients with Behçet's disease 24 h after pricking with a needle. The needle-prick reaction in patients with Behçet's disease may be essentially non-specific inflammation induced by stimulation with needle-prick. However, considering the fact that little signs of local inflammation remain in normal individuals 24 h after the stimulation with needle-prick (unpublished data), the responsiveness to the

stimulation if considered to be enhanced in patients with Behçet's disease (5). Endothelial cell proliferation in blood vessels may be a histological manifestation of the enhanced responsiveness. The endothelial cell proliferation is accompanied by the appearance of degenerated endothelial cells which drop into the vascular lumen during removal of these cells, and results in the construction of endothelium embolized by thrombus formation. It seems that the appearance of degenerated endothelial cells indicates selfregulation of endothelial cell proliferation for the necessity of maintaining homeostasis of the endothelium (15). At any rate, such degenerated endothelial cells may be involved in thrombogenesis. This process of thrombogenesis is completely different from thrombus associated with endotoxin-induced endothelial injury or mechanical injury of the endothelial surface leading to platelet adherence and fibrin formation (16). Thrombus formation and consequential embolus probably cause secondary vasculitis (17), leading to necrosis of the tissues supplied by the affected blood vessels and possibly promoting of local neutrophilic accumulation.

In conclusion, stimulation of the skin with needle-prick induces local non-specific inflammatory reaction and triggers overproductive proliferation of endothelial cells in patients with Behçet's disease. An increase in the number of endothelial cells causes not only vascular stenosis but also the sporadic appearance of the degenerated cells, showing contraction changes during this process.

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