Antiphospholipid Syndrome: Ultrastructure of Microvascular Endotheliocytes in Musculocutaneous Bioptates during Systemic Lupus Erythematosus

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Degeneration and atrophy of the epidermis, disorganization of the connective tissue, focal atrophy of skeletal myocytes, and diffuse vasculopathy are the main pathomorphological signs found in musculocutaneous bioptates during the antiphospholipid syndrome associated with systemic lupus erythematosus. The major morphogenetic disorder is alteration of microvascular endotheliocytes accompanied by the formation of concentric perivascular mononuclear infiltrates.

Key Words: *antiphospholipid syndrome; systemic lupus erythematosus; endothelium; musculocutaneous biopsy; light and electron microscopies*

Antiphospholipid syndrome (APS) is a complex of clinical symptoms that includes thrombotic and/or occlusive damages to vessels associated with overproduction of antiphospholipid antibodies [1,5,9]. APS most often accompanies systemic lupus erythematosus (SLE) [15] and other connective tissue diseases.

Despite considerable clinical and pathogenetic diversity of vascular disorders during APS, pathomorphological changes are not characterized by clear pathognomonic signs, do not correspond to the classic notion of vasculitis, and are named vasculopathies [5,6]. Studies of musculocutaneous bioptates showed that the early stages of APS are characterized by acute hemodynamic disturbances, endothelial alterations, and non-inflammatory thrombotic changes [8]. Reactive vascular proliferation and recanalization of thrombi accompanied by minimal and facultative reactions of the vascular wall appear at later stages of APS [6,13].

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Here we studied pathomorphology of vascular wall cells in musculocutaneous bioptates from patients with SLE and evaluated ultrastructural changes in microvascular endotheliocytes.

MATERIALS AND METHODS

We examined 39 musculocutaneous samples from patients with combined APS and SLE. The diagnosis of APS was based on general clinical signs: recurrent thrombosis, high titers of IgM and IgG antibodies to cardiolipin, and the presence of lupus anticoagulant. SLE was verified by clinical and serological markers, e.g. LE cells and antibodies to native and denatured DNA.

Musculocutaneous samples were studied by light and electron microscopies. Fragments of skeletal muscles were studied by polarization microscopy. For light microscopy, the specimens were fixed in 10% neutral formalin and treated by routine methods. Paraffin slices were stained with hematoxylin and eosin by the van Gieson's method combined with the Perls reaction. Elastic fibers were stained with Weigert's resorcin-fuchsin. Periodic acid-Schiff reaction was performed.

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Fragments for electron microscopy (not more than 1 mm^3) were fixed in 4% paraformaldehyde, postfixed with 1% OsO_4, treated by routine methods, and embedded into Epon-Araldite [2]. Semithin sections were stained with 1% azure II. Ultrathin sections were contrasted with uranyl acetate and lead citrate and examined under a JEM 1010 electron microscope.

RESULTS

Clinical manifestations included various symptoms and syndromes reflecting systemic pathological processes [12]. The disease was characterized by predominance of skin (erythema, photodermatitis, papuloerythematous rash, and reticular livedo) and joint symptoms (arthralgias and polyarthritis), myalgia, and gastrointestinal (hepatitis, cholecystitis, and pancreatitis) and renal disorders.

In the majority of musculocutaneous bioptates, the epidermis had the composition typical of thin skin. The state of cell elements in the epithelium, its width, and the relief of the epidermis-derma interface greatly varied. We found small fragments with normal composition, hyperkeratosis, moderate or severe atrophy of the epidermis, flattened papillae, and (more rarely) focal acanthosis. Atrophy of epidermal cells was manifested in the formation of 2 zones: light empty perinuclear zone occupying nearly all cytoplasm, and dense peripheral zone.

Dermal collagen fibers were characterized by polymorphic structural organization and different staining with dyes. In some cases, these fibers were fuzzy, homogenous, and weakly stained with fuchsin. Disorganization of fibrous structures and major substance of the connective tissue was revealed in some bioptates (primarily in the perivascular zone). Elastic fibers were rarely found.

Structural changes in the vascular network of subcutaneous fat and derma were heterogeneous. Thickening of the vascular wall was related to edema or plasma infiltration accompanied by degeneration and compensatory hyperplasia of endotheliocytes and obstruction of the lumen. Most bioptates were characterized by the presence of perivascular infiltrates around papillary vessels and arterioles of the surface and deep vascular networks and subcutaneous fat. These infiltrates primarily contained pericytes, fibroblasts, mast cells, and lymphocytes; neutrophils and macrophages were not found. Cells in these perivascular infiltrates formed long branching cords (Fig. 1, *a*) [2] and concentric structures containing newly formed collagen fibers. These changes resembled bulbar sclerosis (Fig. 1, *b*), the pathognomonic sign of SLE [11].

Electron microscopy of microvascular endotheliocytes revealed pronounced polymorphism of their ultrastructural organization associated with intensive degenerative processes and changes in pinocytotic activity, which modulated the shape of cell membranes and luminal surface (Fig. 2, *a*). In papillary capillaries and arterioles endotheliocytes nuclei had irregular shape and contained decondensed chromatin, nucleoli were occasionally seen. Electron density of the cytoplasmic matrix varied. We found numerous microfilaments, free ribosomes, single profiles of the granular cytoplasmic reticulum, mitochondria with disorganized cristae, pinocytotic vesicles, and lysosomes. Alteration of membrane cytoplasmic organelles was manifested in the appearance of large polymorphic osmiophilic residual bodies localized subendothelially.

The endothelial lining in venules was characterized by pronounced degenerative changes: sharply increased electron density of the cytoplasmic matrix containing solitary membrane organelles with signs of destruction (Fig. 2, *b*). Endotheliocytes had cylindrical shape; their nuclei contained considerable amounts of the heterochromatin, and intercellular spaces were enlarged.

Multilayered endothelial basal membrane in microvessels indicated repeated desquamation and proliferation of endotheliocytes. Perivascular pericytes had individual cytoplasmic organelles and long processes entering the multilayered basal membrane. Collagen fiber bundles were found. Mast cells containing heterogeneous secretory granules with signs of secretory degranulation, activated fibroblasts, and individual lymphocytes were localized distally.

Thinned skeletal myocytes were heterogeneous by tinctorial properties and glycogen contents (Fig. 1, *c*). Focal changes were manifested in the presence of small regions of myofibril disaggregation (myocytolysis) and residual signs of resorption and organization (agglomerates of mononuclear cells and fibroblasts at short segments along muscle fibers). Atrophic and necrobiotic changes were confirmed by marked activation of satellite cells and signs of intracellular regeneration: increased number of nuclei in some regions of muscle fibers and chains of large elongated nuclei with optically light karyoplasm and firmly dispersed chromatin (Fig. 1, *d*). Intermuscular spaces were swollen and contained thin bundles of collagen fibers and individual lymphocytes. Arteriolar walls were thickened, their endothelium was hypertrophic and hyperplastic, the lumen was narrowed or completely obstructed, and venules were characterized by plethora.

Thus, the major pathomorphological changes in musculocutaneous samples included dystrophy and atrophy of the epidermis, pronounced focal disorganization of the connective tissue derma, focal metabolic damages to skeletal myocytes, and diffuse vasculopa-

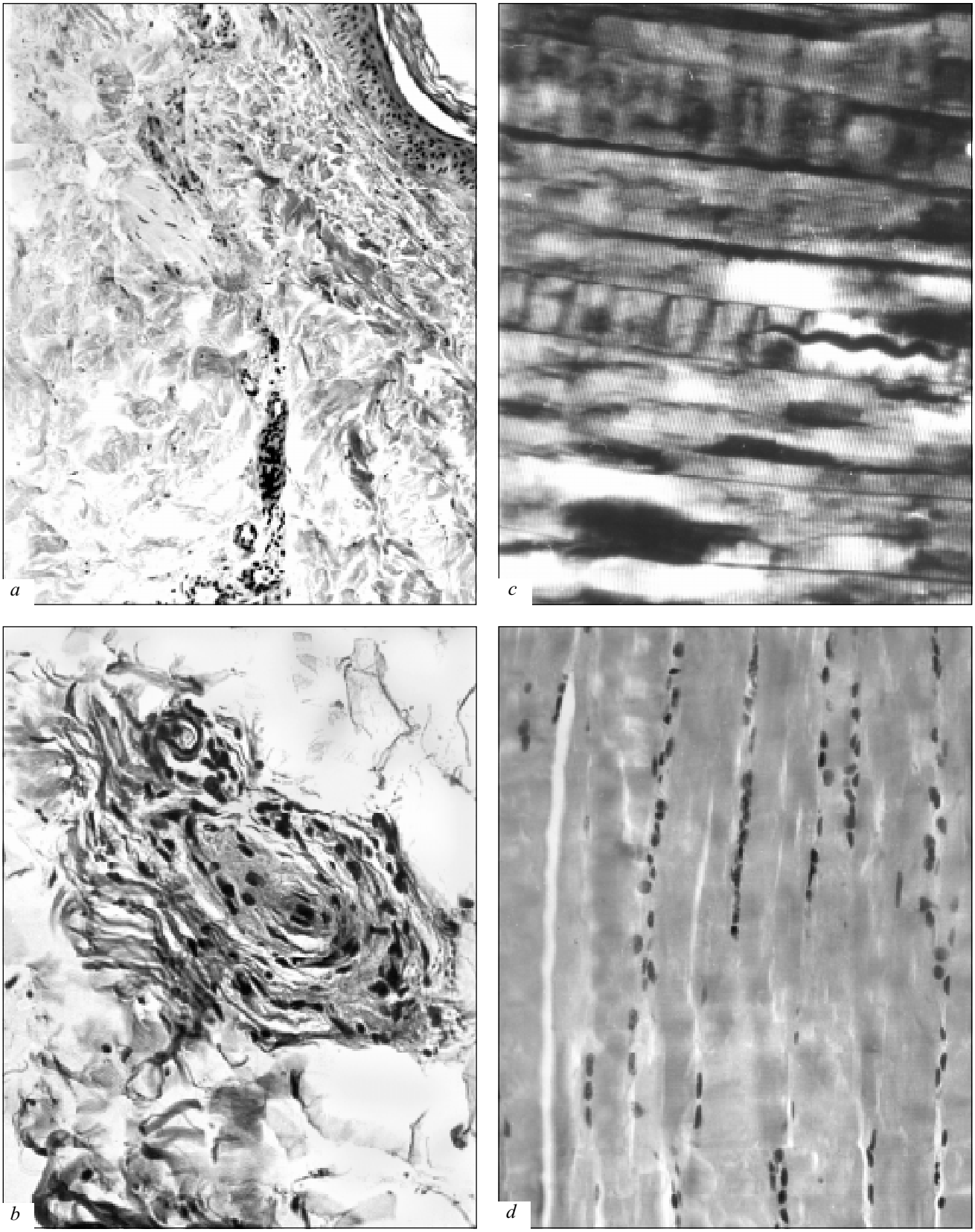

Fig. 1. Light microscopy of musculocutaneous bioptates from patients with antiphospholipid syndrome associated with systemic lupus erythematous: atrophy of the epidermis, hyperkeratosis, and formation of perivascular infiltrates (×180, *a*); formation of perivascular bulbar sclerosis in subcutaneous fat (×280, *b*); atrophy of individual myocytes and preserved myofibril apparatus (polarized light, ×250, *c*); and associates of myocyte nuclei in the region of atrophic muscle fiber (×160, *d*).

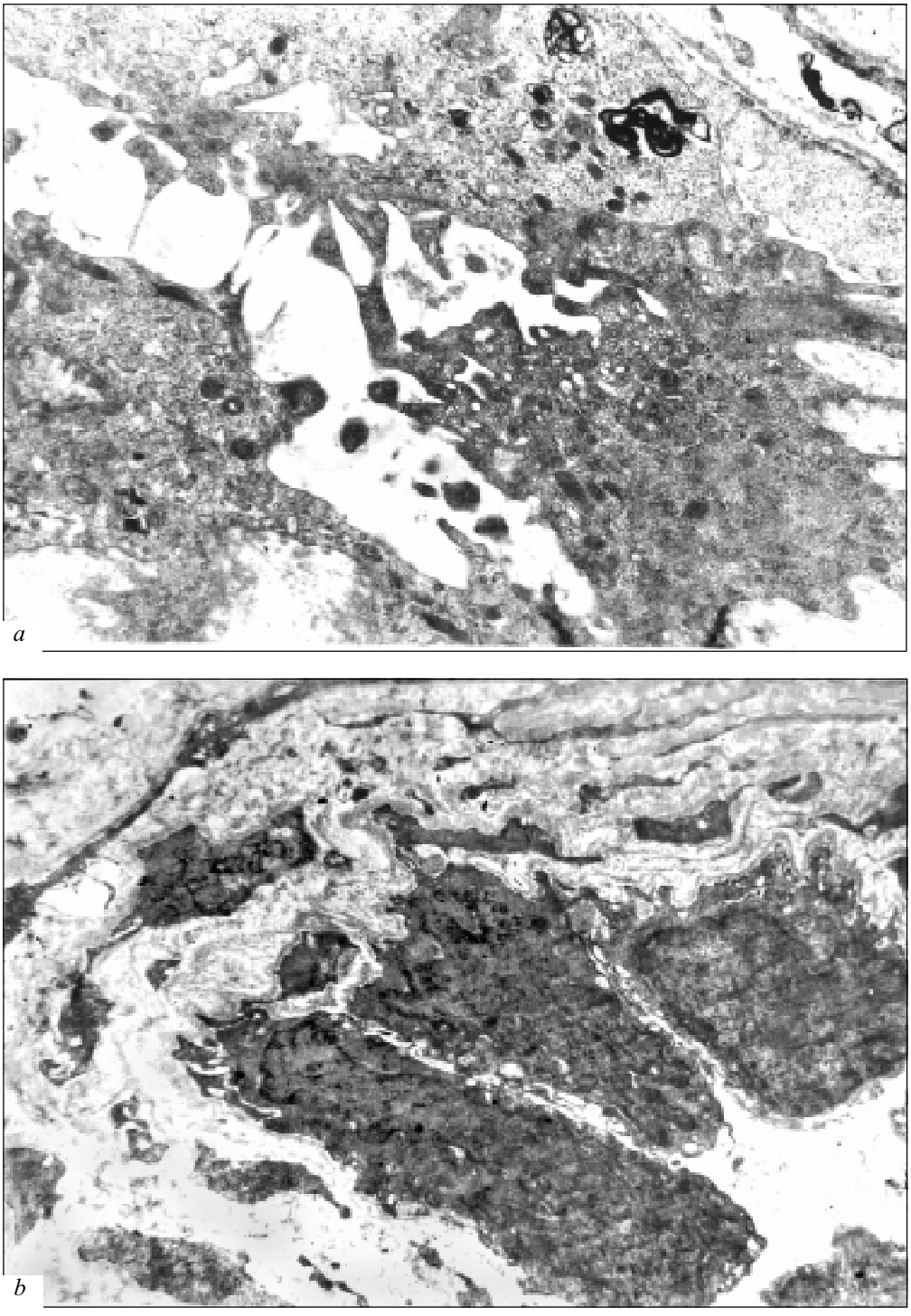

Fig. 2. Ultrastructural organization of endotheliocytes in dermal microvessels during antiphospholipid syndrome associated with systemic lupus erythematous: pronounced heterogeneity of ultrastructural organization of endothelial cells in papillary capillaries and formation of polymorphic residual bodies (×8000, *a*); and high electron density of the cytoplasmic matrix in venular endotheliocytes and numerous perivenular processes of pericytes and fibroblasts (×600, *b*).

thy (edema of the vascular wall, alteration and hyperplasia of endotheliocytes, perivascular mononuclear infiltration, and obstruction of the lumen). It should be emphasized that perivascular infiltrates mainly contained pericytes, fibroblasts, mast cells, and lymphocytes. We revealed observed perivascular concentric structures, which included cells and collagen fibers.

Comparative studies of various bioptates, and examination of endothelial cells in the same sample, indicate that endothelial changes play the major role in

the pathogenesis of vasculopathies during APS associated with SLE, because damages to the endothelium precede thrombosis and perivascular infiltration. Incompetence of endothelial cell associates related to the synthesis of antiphospholipid (including antiendothelial) antibodies and induction of thrombotic processes is probably the major pathogenetic mechanism of APS and its clinical manifestations.

Primary damages to the endothelium determine the simultaneous reaction of perivascular cells, which plays a key role in the relationship between the parenchyma and stroma and the development of general pathological processes [4,7]. In this respect, high proliferative activity of endotheliocytes and perivascular cells is of considerable importance. Changes in functional activity of vascular cells are synchronized with the metabolism and morphogenesis of perivascular cells. It can be hypothesized that under conditions of chronic damages to the endothelium, perivascular cells serve as the source of newly formed stromal elements. These processes also accompany the growth of connective tissue tumors [10].

SLE can be induced by many factors, including viruses. Long-term viremia in patients with SLE, the presence of antibodies, and the increase in the titer of antibodies to Coxsackie viruses A indicate the involvement of viruses into pathogenesis of SLE [3]. It was shown that viruses play an important role in the development of other systemic connective tissue diseases, e.g. systemic scleroderma. Retroviruses and cytomegaloviruses attract much attention [16]. Recent studies identified homologous sequences of some retroviruses and topoisomerase I, the antigenic target for Scl-70 autoantibodies specific for systemic scleroderma [14].

APS is the model of systemic thrombotic vasculopathy, which holds much promise for understanding the interrelations between general pathological processes underlying acute and chronic vascular diseases, atherosclerosis, heterogeneous vasculitis, vasculopathy, homeostatic disturbances, and polymorphic visceral diseases.

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