Morphological Changes in the Skin during Experimental Allergic Encephalomyelitis and Multiple Sclerosis: Bases for New Diagnostic Test

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Skin specimen from the medial malleolar area of patients with multiple sclerosis and guinea pigs with experimental allergic encephalomyelitis were examined by light and electron microscopy. Demyelination, inflammation, and dystrophic changes in peripheral nerves and skin correlating with clinical manifestations were revealed in both sclerosis and encephalomyelitis. Maximum changes were revealed in nerve-associated cells (Merkel cells), Langerhans cells reflecting inflammation intensity, and fibroblasts responsible for nerve integrity and resistance against external stimuli.

Key words: *multiple sclerosis; experimental allergic encephalomyelitis; skin biopsy; peripheral nerves; Merkel cells; Langerhans cells*

Multiple sclerosis (MS) is one of the most severe neurological diseases. Despite numerous experimental studies on different models of MS including experimental allergic encephalomyelitis (EAE), many aspects of MS remain unclear because of the absence of universal concept on the etiology and pathogenesis of this pathology and reliable criteria for its diagnostics. It was previously believed that MS foci cannot penetrate from the central nervous system (CNS) to the periphery through transitional zones, where oligodendroglia and astrocytes are replaced by Schwann cells and fibroblasts [6].Recent studies demonstrated the presence of analogous changes in the peripheral nervous system [1,9]. However, changes in peripheral nerves, in particular, demyelination and, sometimes, axonal damage, were found only in autopsies from cachectic patients at advanced stages of MS. Dystrophic changes were explained by insufficient nutrition [10].

The aim of the present morphological study was to define the nature and topography of demyelination in the peripheral nervous system during relatively mild remittent MS and experimental EAE.

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MATERIALS AND METHODS

Skin specimen from 3 patients aged 24-35 with 1-5-year history of MS were examined. The severity of MS estimated using Kurtzke scale [7] was 1-6. One patient had remittent and 2 chronic progressive MS. Skin specimen were obtained from the medial surface of the malleolus under local anesthesia (0.5% novocaine). Skin biopsies from 3 surgical patients without neurological diseases and from 3 patients suspected for MS served as the control. We also examined skin specimens from 8 outbred adult male guinea pigs with EAE (in cooperation with Yu. A. Zhitnukhin and I. V. Litvinenko) and 5 control animals. For modeling EAE the animals were treated with myelin basic protein suspended 1:1 in complete Freund adjuvant [3]. The animals were anesthesized with ether. The material for morphological study was taken from the crus at the peak of the disease. Light microscopy was performed using routine neurohistological methods, some preparation were stained with osmium by the method of Marki and impregnated with $AgNO_3$ according to Bielschowsky—Gross for estimation of changes in the skin nerves. Nissl-stained semithin sections (1 μ) prepared from the material embedded for electron mi-

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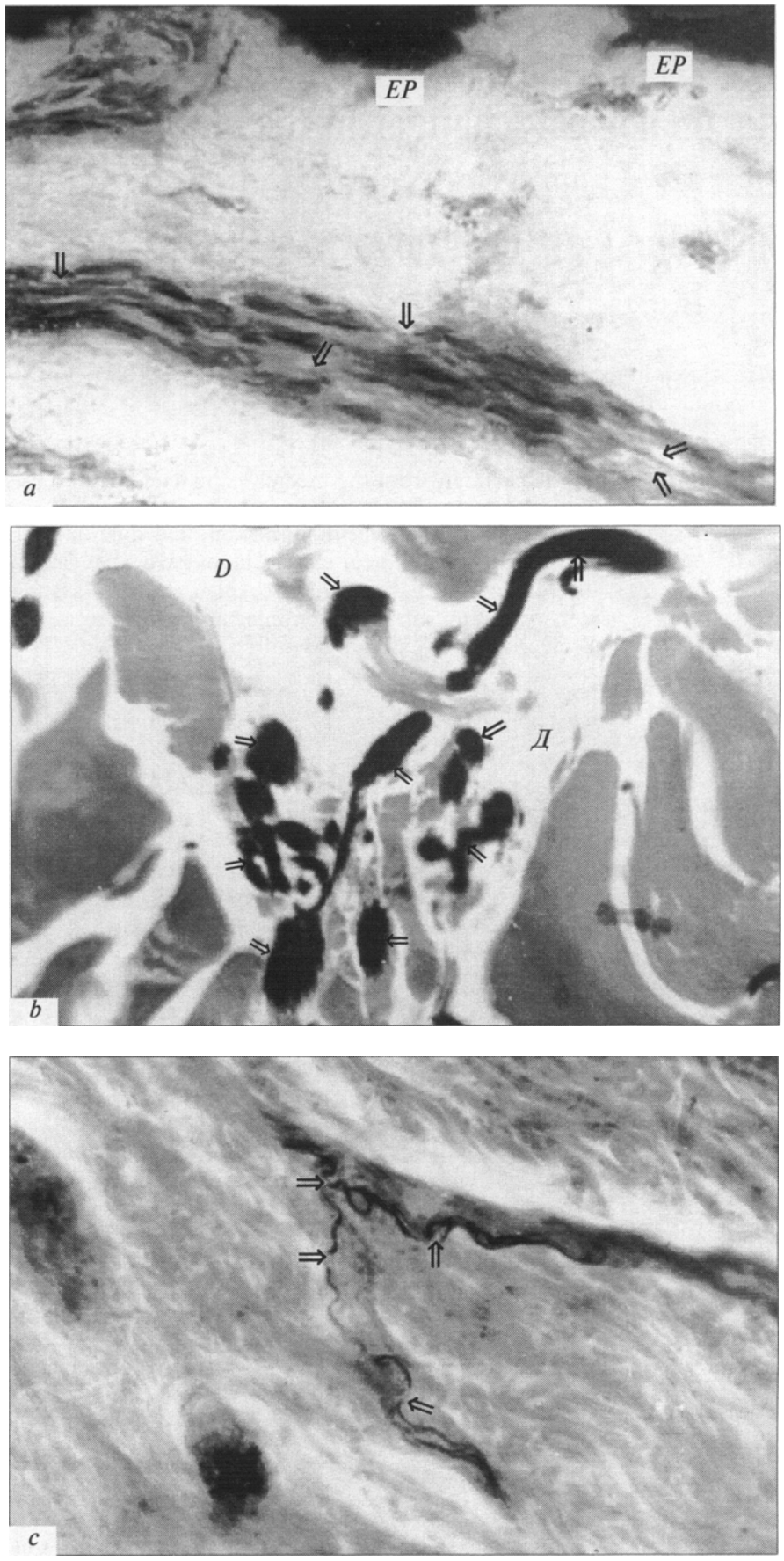

Fig. 1. Skin specimens from patient P. (early manifestations of multiple sclerosis, *a*), patient M. (acute state, *b*), and guinea pig with experimental encephalomyelitis (*c*). Light microscopy. *a*) nerve fibers are fragmented, arrowheads indicate the damaged regions; the structure of derma is relatively intact. *EP*: epidermis. Cajal impregnation, ×250; *b*) homogeneously stained fragments of poorly differentiated collagen and myelin fibers (arrowheads) are surrounded by swollen derma (*D*). Semithin section, Nissl staining, ×400; *c*) nerve fibers (arrowheads) are thin and fragmented. Cajal impregnation, ×160.

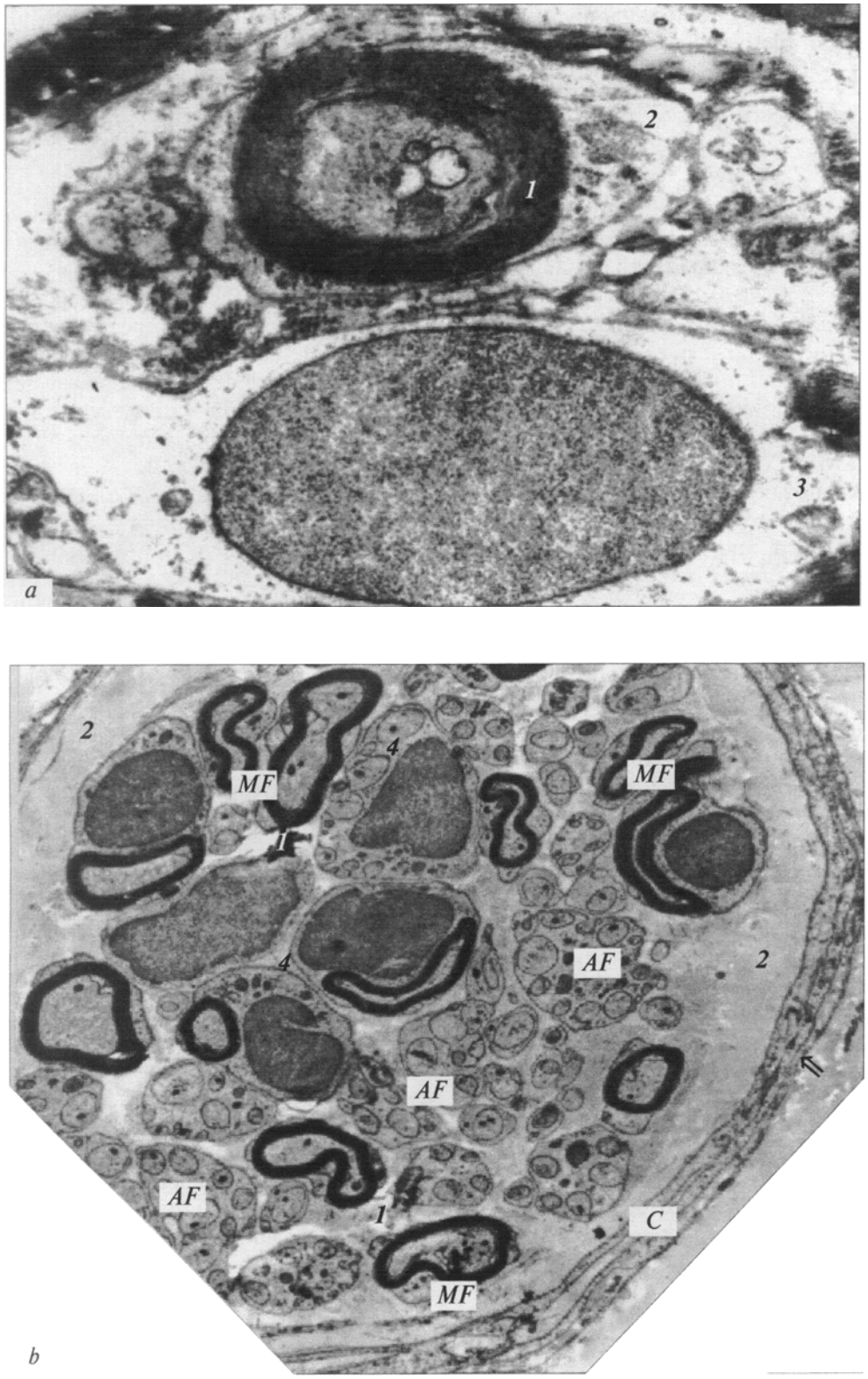

Fig. 2. Electron microscopy of skin biopsy from patient P. with early multiple sclerosis (*a*) and subcutaneous nerve of a guinea pig with experimental allergic encephalomyelitis (*b*). *a*: *1)* myelinated fiber with altered axon and swollen myelin, *2*) Schwann cell around myelin fiber is also swollen, cytoplasm is practically deprived of organelles, *3*) swollen fibroblast, ×13,000; *b*: *1*) endoneurium, *2*) perineurium, *3*) epineurium, *4*) Schwann cells, *MF*: myelinated fibers, *AF*: unmyelinated fibers, *C*: nerve capsule, ×10,000.

croscopy were also examined under light microscope. Material for electron microscopy was prepared routinely.

RESULTS

Pathomorphological examination of skin specimens from MS patients and animals with EAE revealed demyelination of peripheral nerves and sensory receptors accompanied by swelling, unclear outlines, and cluster enlargements (Fig. 1, *a, b*). Typically, perivascularly inflammatory monocyte and lymphocyte infiltration was found mainly at the early stages or in mild forms of MS and EAE, but not at the peak of the disease. On the contrary, the degree of demyelination of skin nerves increased proportionally to clinical features of EAE and MS. Cell density in the derma and subepidermal layer also decreased (Fig. 1, *c*).

Electron microscopy revealed considerable structural changes in the skin and nerves in MS patients and EAE animals. Thus, axons in unmyelinated fibers were almost electron transparent. Axons of myelinated fibers were also electron transparent or had normal structure, but myelin sheath was always altered. Highly osmiophilic regions with irregular internal structure alternating with regions with regular lamellar structure were seen. In specimens from EAE animals, Schwann cells near myelinated and unmyelinated fibers contained round transparent nuclei with homogenous chromatin surrounded by thin cytoplasmic rim with electron transparent vacuoles (enlarged cisterns of the endoplasmic reticulum, EPR, and swollen mitochondria) and large dense osmiophilic structures of irregular shape (phago- and lysosomes).

During MS and EAE, endoneuronal collagen around myelin fibers and Schwann cell processes often disappeared, perineural space became empty and swollen, while in control specimens it always contained collagen (Fig. 2, *a, b*). Cytoplasm of collagen-synthesizing perineural fibroblasts was sharply vacuolated due to changes in mitochondrial ultrastructure and enlargement of EPR cisterns. Hypertrophy and moderate enlargement of EPR were sometimes observed. These changes are typical of morphofunctional strain; however, against the background of collagen disappearance from endo- and perineurium they attested to dystrophic changes in fibroblasts and disturbances of collagen production (Fig 3, *a*). Moreover, sometimes flat fibroblast processes in nerve capsule did not form dense contacts, and fluid from the epineural space could penetrate the perineurium. Dermal edema could result from inflammatory changes in capillaries and large vessels located close to the nerve and was clearly seen under both light and electron microscopes. Changes in vessel ultrastructure manifested as swelling of endotheliocyte cytoplasm and increased number of pinocytotic vesicles and processes in the vessel lumen. Contacts between endotheliocytes were loosened and basal membrane became swollen or stratified.

Solitary Merkel cells with one or several processes rarely contacting with unmyelinated fibers were found. These cells showed signs of hypofunction. Osmiophilic nucleus contained dense karyoplasm, sometimes, external karyolemma formed large transparent perinuclear vacuoles. Cytoplasm was vacuolized due to enlargement of EPR cisterns and mitochondrial swelling. These cells contained no specific granules with dense content (Fig. 3, *b*) [5]. It should be emphasized that Merkel cells were never found in severe MS and EAE.

Langerhans cells with typical homogenous nuclei and cytoplasm enriched with polysomes and EPR profiles (Fig. 3, *c*) were more often seen in specimens from MS patients and EAE animals than in control. The cytoplasm of Langerhans cells also contained vacuoles formed by swollen mitochondria, dense osmiophilic granules and typical for these cells tennis racket-shaped structures [5], markers of high functional activity of these cells. Accumulation of Langerhans cells in the derma of MS patients and EAE animals point to activation of phagocytosis and immune reactions [8,11]. The number of Langerhans cells decreased with augmentation of clinical signs of MS and EAE, which correlates with light microscopy data pointing to less pronounced changes in the blood-tissue barrier in these cases.

Thus, demyelination, inflammation, and dystrophic changes revealed in the skin and skin nerves of patients with MS and animals with EAE clearly correlate with clinical manifestations of these diseases. In the derma, nerve-associated tactile cells and Langerhans cells reflecting the intensity of inflammation changed qualitatively and quantitatively. Inflammatory vascular reaction was most pronounced in remittent MS and mild EAE. Our findings suggest the presence of a systemic pathogenic process destroying peripheral nervous system. Simultaneous and combined damage to the central and peripheral nervous systems was also demonstrated in EAE, the model of MS [1].

The revealed changes can be used in questionable cases for vital diagnostics of MS in skin specimen by morphological methods (light microscopy).

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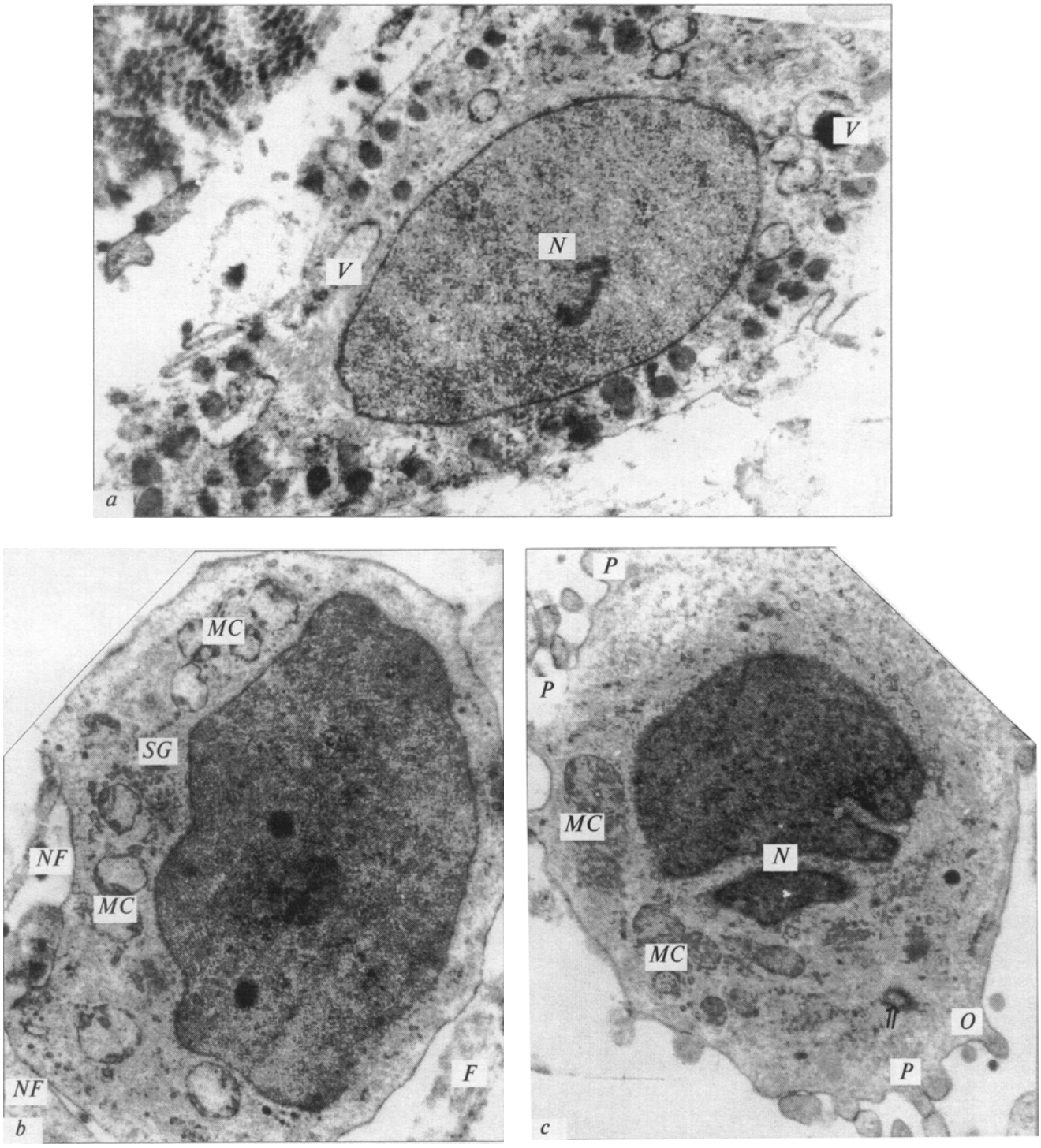

Fig. 3. Electronogram of skin biopsy specimen from patient P. *a*) fibroblast nucleus (*N*) with homogenous chromatin distribution. Numerous vacuoles (*V*) and osmiophilic mast cell-like granules are seen in the cytoplasm, ×18,000; *b*) functionally suppressed Merkel cell. *MC*: vacuolated mitochondria, *SG*: specific granules, *NF*: nerve fibers, *F*: fibroblast process, ×17,000; *c*) active Langerhans cell with multiple processes (*P*). *N*: nucleus, *MC*: mitochondria with dense cristae, arrow indicates specific tennis racket-shaped structure, ×8,000.

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