Ultrastructural Modification of the Endothelium in Placental Insufficiency and Microangiopathies

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 147, No. 5, pp. 583-588, May, 2009
Original article submitted October 10, 2008

Structural reorganization of endotheliocytes was studied on models of various pathological processes: placental dysfunction, glomerular pathology, vibration syndrome, antiphospholipid syndrome, and diffuse angiokeratoma, all of these characterized by endothelial insufficiency. Universal modification of endothelial associations was revealed. It included a chain of stereotypical reactions: from degeneration alternating with compensatory hypertrophy to subsequent atrophy and death of endotheliocytes. The time course of the process was confirmed by the results of light microscopy in combination with ultrastructural examination and by evaluation of the biosynthetic reactions by *in vitro* radioautography.

Key Words: *placenta; endothelium; microangiopathy; electron microscopy*

Endothelial cells possess congenital mechanisms of adaptation and resistance to systemic and paracrine effects, responding to aggressive factors by modification of functional activity, subsequent dysfunction, and eventually by cell damage or death. Endotheliocyte activation and/or injury play the key role in the development of a wide spectrum of adaptive and pathological processes, which, combined with endotheliocyte alteration and death, have the worst prognosis [7,14].

Placental abnormalities and the role of infections in the development of placental insufficiency are now extensively studied [1,3,4,13]. The structural organization of the placenta is aimed at protection of the fetus from xenobiotics, infections, and maternal diseases [6,8]. At the same time, it was shown that the placenta is involved in vertical transmission of viruses, such as cytomegalovirus and human immunodeficiency virus [9,10]. Congenital viral infections lead to a number of severe consequence including fetal abnormalities and death, intrauterine and persistent postnatal infections. The routes of virus passage through the placenta and structural equivalents of its viral damage attracted much recent attention. For example, different level of receptor expression in the course of the cytotrophoblast differentiation and migration was demonstrated. The involvement of oxidative stress in placental endothelial damage was also hypothesized [5,7,11, 12,15].

We studied polymorphism of ultrastructural reorganization of endotheliocytes in placental failure caused by infection and in microangiopathies.

MATERIALS AND METHODS

Placental failure of infectious origin. Specimens of 56 placentas after late (22-27 weeks) miscarriages were studied. Persistent herpesvirus infection was detected in 85% patients, cytomegalovirus was detected in 68% of these; combinations of other viral and bacterial infections were detected in 33% cases.

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0007-4888/09/1475-0650

Glomerular pathology. Biopsy specimens of 30 patients (19 men and 11 women aged 17-53 years) were analyzed. Of these, 15 patients presented with markers of HCV infection, 12 with markers of HBV infection, and 3 with mixed HCV+ HBV infection; analysis of 8 nephrobiopsy specimens (6 women and 2 men aged 26-40 years) showed secondary antiphospholipid syndrome (APS) concomitant with systemic lupus erythematosus (SLE). In addition, 35 specimens of the musculocutaneous flap (31 women and 4 men aged 23-63) from patients with APS were examined.

Vibration syndrome (systemic microangiopathy) caused by exposure to local and total vibration: 83 cases (65 men and 18 women aged 36-63 years). Of these, 58 cases with vibration gastropathy and 25 with vibration cystopathy. Gastro- and cystoscopy were carried out with emphasis on the microcirculatory disorders. Biopsy specimens of the mucosa from the fundal and antral compartments of the stomach and from the neck and right lateral wall of the urinary bladder were collected.

Diffuse angiokeratoma (Fabry disease) is an extremely rare genetically determined enzymopathy with the involvement of the endothelium; skin biopsy specimens were studied. Biomicroscopy of the ocular bulbar conjunctiva showed pronounced microcirculatory disorders; in addition, a drop of the leukocytic α-galactosidase activity pathognomonic in Fabry disease was detected.

Light and electron microscopy and autoradiography were carried out as described previously [2]. Light microscopy was carried out using Leica DM 4000B universal microscope with Leica DFC 320 digital camera. Ultrathin sections were examined under a JEM 1010 electron microscope.

RESULTS

Diffuse polymorphic cell infiltration of all layers of the placenta (Fig. 1, *a*) involving all villous generations (Fig. 1, *b*) was seen in placental failure of infectious origin. Hypertrophy of some stem villi was paralleled by atrophy of the adjacent villi; blood vessels in the majority of these villi were in a state of spasm or secondary paresis, in many cases with pronounced intra- and perivascular sclerosis. Capillary plethora in intermediate villi was paralleled by pericapillary edema and sometimes by perivascular tissue fibrosis (Fig. 1, *c*). Hemodynamic disorders were most pronounced in the terminal villi (hyperemia, thrombosis, edema, and foci of fibrinoid necrosis). In addition, there were villi with reduced capillary network and with a significant compensatory increase in the number of capillaries. Microvessels of the decidual membrane were characterized by predominantly degenerative changes in endotheliocytes (Fig. 1, *d*).

Devastation of the cytoplasmic matrix because of intracellular edema (Fig. 1, *e*), cytoplasm vacuolation, degradation of protein-producing organelles, sometimes with a compensatory increase in the number of cytoplasmic protrusions and formation of microfilaments, predominated in the ultrastructure of the majority of placental capillary endotheliocytes. Lytic changes in cytoplasm organelles augmented during active infectious process, this promoting necrobiosis and death of endotheliocytes (Fig. 1, *f*), violation of the integrity of the endothelial association, followed by the development of perivascular edema and hemorrhages. Erythrocytic and mixed clots formed in the capillary lumen in severe destructive changes in the villi.

Degenerative changes in the endothelium, pronounced hemodynamic disorders with plethora, edema, and hemorrhages, paralleled by alteration and death of capillary endotheliocytes in all compartments of the placenta, predominated in chorioamnionitis of infectious (viral) origin with the key role of herpesvirus infection.

Two endotheliocyte populations were clearly distinguished among the glomerular and periglomerular endotheliocytes in glomerular pathologies of infectious (viral) origin, irrespective of the intensity and length of the pathological process. The majority of endotheliocytes exhibited signs of degeneration, others were hyperplastic and hypertrophic, which reflected the wave-like course of the alteration and compensation process. Degenerative changes in endotheliocytes (Fig. 2, *a*) manifested in increased heterochromatin content in the nuclei, often by karyopyknosis, disappearance of protrusions in the lumen, drop of pinocytic activity, condensation of cytoplasmic matrix, reduction and alteration of organelles and in many cases desquamation of the endothelium. In compensatory hypertrophy, the endotheliocytes on the surface of the lumen had long polymorphic pseudovilli often forming arcades and plexuses with lesser numbers of fenestrae (Fig. 2, *b*).

The earliest signs of glomerulocyte alteration were also detected in endotheliocyte population in the nephrobiopsy specimens from patients with APS concomitant with SLE (Fig. 2, *c*), which was paralleled by compensatory proliferation of mesangiocytes, hyperproduction of the mesangial matrix, and podocyte metaplasia. Intimal hyperplasia paralleled by occlusion of vessels caused by endotheliocyte degeneration and proliferation (Fig. 2, *d*) developed in the periglomerular arterioles.

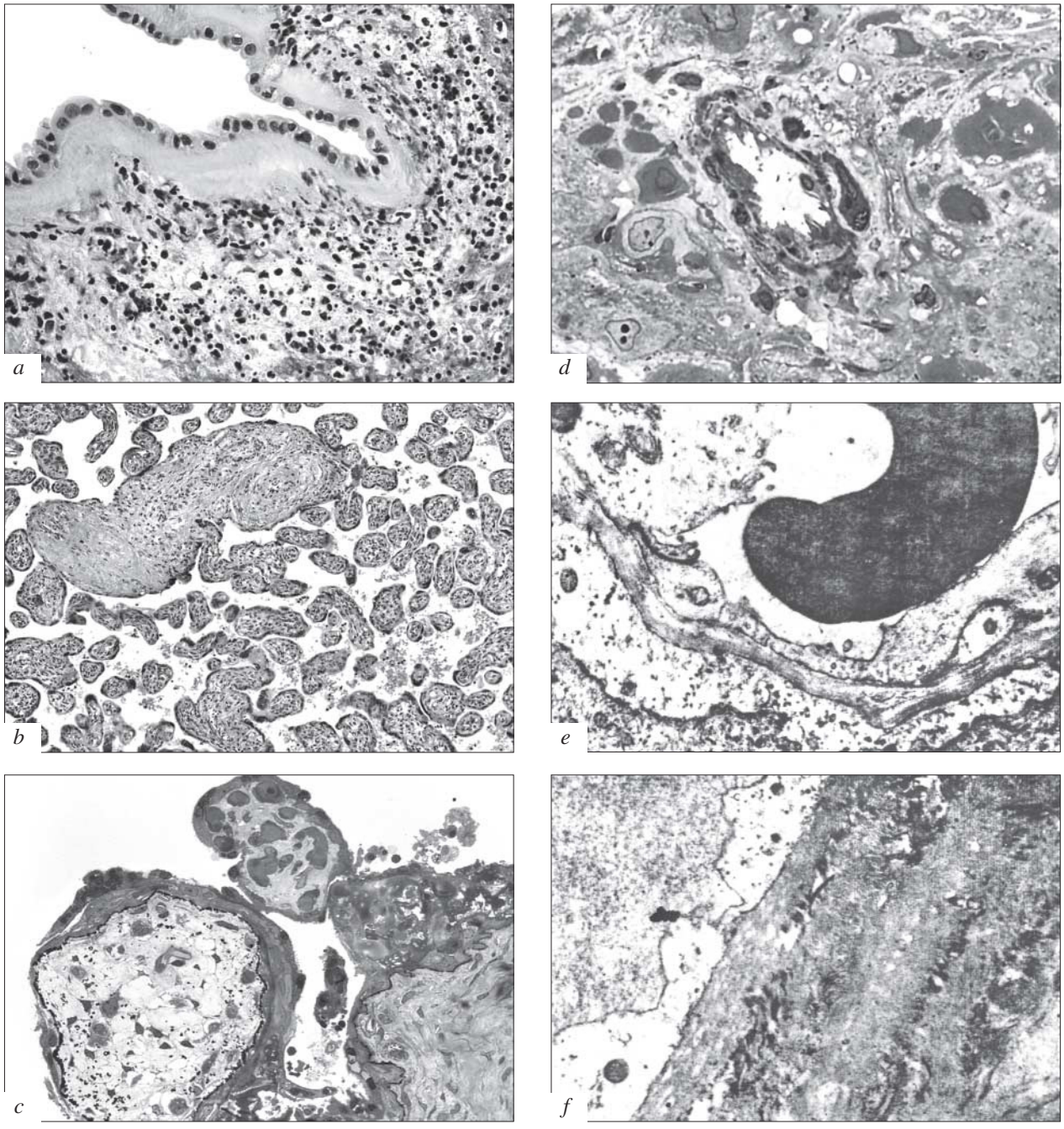

Fig. 1. Structural changes in the placenta in suppurative chorioamnionitis of infectious (viral) origin. *a*) amniochorionic plate with pronounced diffuse inflammatory cell infiltration, ×500; *b*) stem and terminal villi with inflammatory cell infiltration, ×150; *c*) microcirculatory disorders and perivascular edema in the subchorionic villous stroma, ×600; *d*) degeneration and destruction of capillary endotheliocytes in decidual membrane, ×800; *e*) edema of an endotheliocyte and chorionic capillary basal membrane, ×10,000; *f*) endotheliocyte necrobiosis, fibrinoid imbibition of vascular wall, ×8000. *a*, *b*: hematoxylin and eosin staining; *c*, *d*: semithin sections, staining with Schiff reagent and azur II; *e*, *f*: electronograms.

The index of labeling with RNA synthesis precursor decreased significantly in glomerular endotheliocytes at the expense of atrophic cells, which, together with their ultrastructural changes, indicated severe (presumably primary) involvement of the glomerular capillary endothelium caused by inhibition of the endotheliocyte biosynthetic function.

Vessels with walls thickened because of edema, plasma imbibition, paralleled by degeneration and compensatory hyperplasia of endotheliocytes, often with obstruction of the lumen, predominated in the derma and subcutaneous fat in APS. Capillary endotheliocytes were characterized by ultrastructural polymorphism caused by degenerative changes of

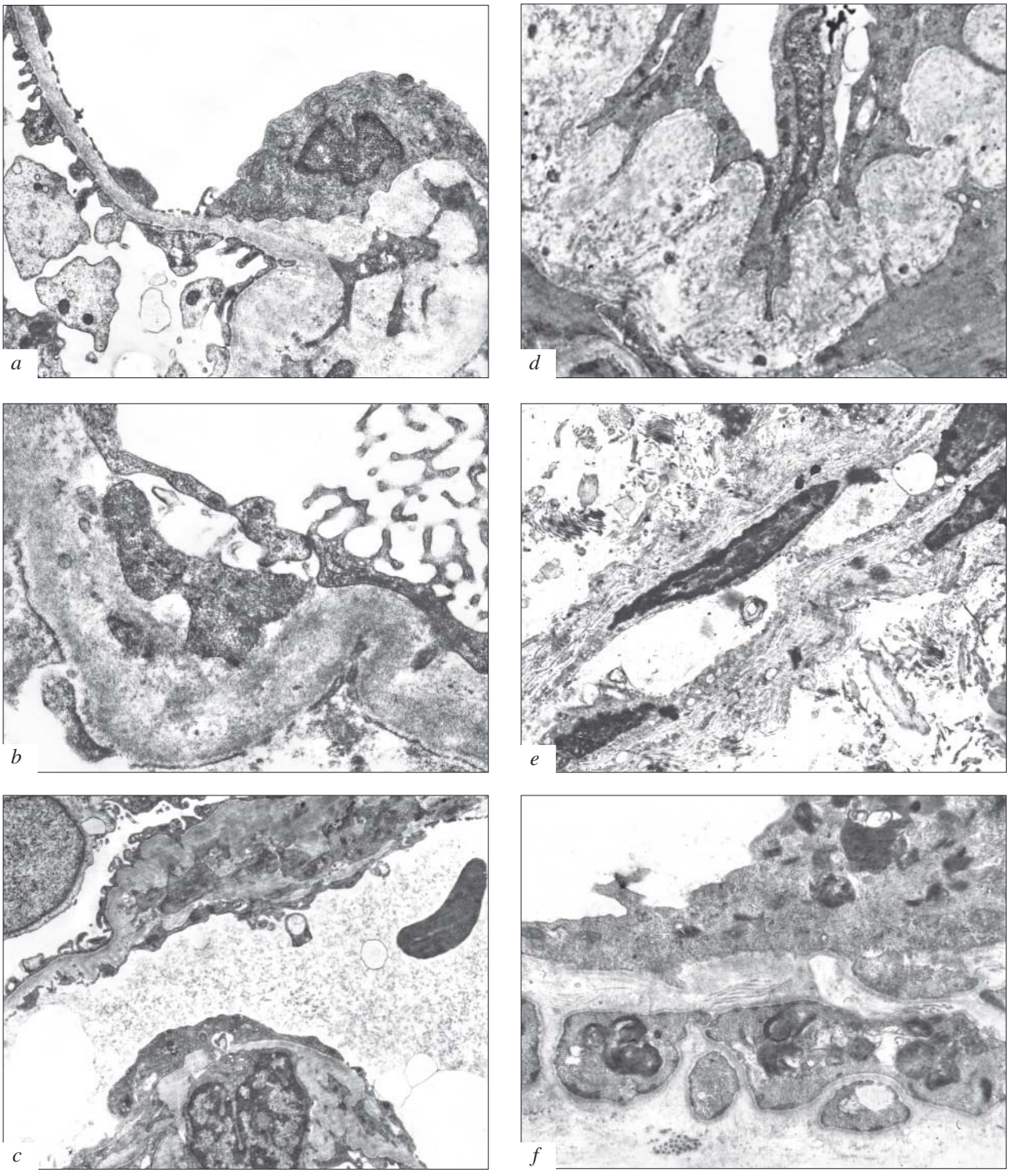

Fig. 2. Ultrastructural characteristics of endotheliocytes in microangiopathies of different origin. *a*, *b*) nephrobiopsy specimens in glomerular pathologies; *c*, *d*) nephrobiopsy specimens in APS concomitant with SLE; *e*) biopsy specimen from the fundal compartment of the stomach in vibration gastropathy; *f*) skin biopsy specimen in diffuse angiokeratoma. *a*) endotheliocyte degeneration, ×400; *b*) endotheliocyte hypertrophy with formation of arcades by the luminal plasmalemma, ×15,000; *c*) degenerative changes in endotheliocyte cytoplasm, ×600; *d*) periglomerular arteriolar endothelial degeneration, ×400; *e*) significant degenerative changes in lamina propria capillary endothelium of the gastric mucosa, ×3000; *f*) osmiophilic granules in skin capillary endotheliocyte and pericytes, ×8000.

different severity and different levels of pinocytic activity modulating the luminal surface relief. The nuclei of the majority of endotheliocytes in the papillary capillaries and arterioles were euchromatic, with numerous microfilamentous structures, solitary profiles of the granular cytoplasmic reticu-

lum, and disorganization of mitochondrial cristae. Signs of alteration of cytoplasmic membrane organelles were seen (large polymorphic osmiophilic residual bodies). The endothelial basal membrane was multiplied in the majority of capillaries, which indicated repeated desquamation and proliferation of endotheliocytes.

Vibration exposure led to the development of a complex of structural and functional changes in the stomach and urinary bladder (vibration gastro- and cystopathies). Their major structural signs were degenerative and atrophic changes in the endothelial (Fig. 2, *e*) and epithelial compartments of the mucosa, reduction and aneurysm-like transformation of the microcirculatory network, diffuse fibrosis and absence of inflammatory cell infiltration, and drastic reduction of biosynthetic reactions of epitheliocytes and endotheliocytes shown by *in vitro* radioautography. Systemic microangiopathy and the relevant primary degenerative process in the absence of compensatory reactions of capillaries paralleled by subsequent atrophy of all layers of the organ walls underlie the pathogenesis of vibration gastro- and cystopathies.

In diffuse angiokeratoma, endotheliocytes of the surface capillary network of the skin were hypertrophic with signs of macro- and microclasmatosis caused by accumulation of large polymorphic osmiophilic granules of lamellar structure (Fig. 2, *f*). Some granules were transformed into large residual bodies. The endothelial basal membrane was edematous and loosened, had multilamellar structure, pericyte processes and collagen fibrils, indicating repeated desquamation of endotheliocytes. Pericytes are transformed into depocytes with very large granular conglomerations looking as giant osmiophilic residual bodies. Accumulations of poorly differentiated pericytes located near degenerative vessels, in which all cells were transformed into depocytes, formed perivascularly.

Systemic and stereotypical structural reorganization of the endothelium in different pathological processes associated with endothelial dysfunction syndrome is noteworthy. Despite structural and functional stereotypical reactions of the endothelial associations, specific features of cell damage and compensatory reactions were noted. Placental failure of infectious (viral) origin was characterized by pronounced endothelial damage and hemodynamic disorders, paralleled by significant changes in the stromal component (massive cell infiltration and necroses). Glomerular disease was characterized by predominant degeneration of endotheliocytes associated with compensatory proliferation of mesangiocytes, hyperproduction of the mesangial matrix, and podocyte metaplasia. Vibration microangiopathies presented as a systemic pathology and were characterized by the most severe atrophy of endotheliocytes against the background of capillary reduction and diffuse stromal fibrosis. In diffuse angiokeratoma, capillary endotheliocyte death was paralleled by compensatory activation of pericytes.

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