INTERACTION BETWEEN BLOOD MONOCYTES AND ARTERIAL INTIMA DAMAGED BY CATECHOLAMINES IN SWINE

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The initial stages of atherosclerosis are characterized by increased permeability of the vascular wall for components of the blood plasma and cells, including monocytes [1, 9]. With its ability to take part in phagocytosis, with its broad spectrum of hydrolytic enzymes [2, 14], and containing a powerful mitogen for fibroblasts and smooth-muscle cells [8] and a developed receptor apparatus [5, 14, 11], the monocyte, penetrating into the intima of arteries, becomes actively involved in a complex chain of pathological mechanisms of atherogenesis. In experiments using cell cultures and Boyden's chambers, certain plasma [10, 13] and tissue [6, 7, 15] factors were shown to be capable of acting as the stimulus for adhesion and migration of monocytes. In long-term experiments on the whole animal, an increase in colonization of the intima of arteries by mononuclear cells was the result of the integral effect of both plasma and tissue factors, and for that reason it was impossible to determine which of them plays the leading role, individually, in mechanisms of infiltration of the vascular wall.

The aim of this investigation was to study the effect of tissue factors of the vascular wall on interaction between monocytes and the intima of the carotid arteries of domestic pigs, before and after damage induced by catecholamines in organ culture.

EXPERIMENTAL METHOD

The common carotid arteries and blood of 15 mature pigs of the same sex and age were used. The arteries were transported in a sterile container containing Hanks' medium with the addition of 0.3% papaverine solution and antibiotics (300 mg/ml). Autologous blood in a volume of 50 ml was collected in plastic tubes containing EDTA (1:9). Under aseptic conditions the carotid arteries were cut into equal segments each 45 mm long, freed from adventitia, and decontaminated in Hanks' solution with the addition of antibiotics: penicillin 125 U/ml, streptomycin 150 U/ml, and nystatin 250 U/ml, for 1 h at 21°C. The segments of the arteries were then cannulated and four of them connected to a perfusion system. The vessels were perfused under open conditions for 6 h at 37°C, under the hydrostatic pressure of 10 mm Hg, at the rate of 5 ml/h. The perfusion medium consisted of medium 199 with the addition of glutamine (0.57 g/liter), sodium bicarbonate (0.8 mg/ml), zwitterion buffer (HEPES, 20 mM), and antibiotics (100 U/ml). No serum was added to the perfusion medium, to avoid any effect of plasma factors on the course of the experiments. The perfusion solution was saturated with carbogen through a bacterial air filter (Millipore, France), by means of a bubble oxygenator. A suspension of monocytes was obtained from the pigs' blood by the method in [12].

There were two series of experiments in which a suspension of autologous monocytes was added to the perfusion system at the rate of $20 \cdot 10^6$ cells/ml for each vessel. In the control series of experiments (seven arterial segments) intact vessels were perfused with the monocytes. In the experimental series (eight segments) before injection of the monocytes, the vessels were damaged with adrenalin in a concentration of 10^{-6} M for 1 h, after which they were rinsed to remove catecholamines by perfusion medium for 1 h. After perfusion of the monocyte suspension for 3 h, the arterial segments were investigated morphologically.

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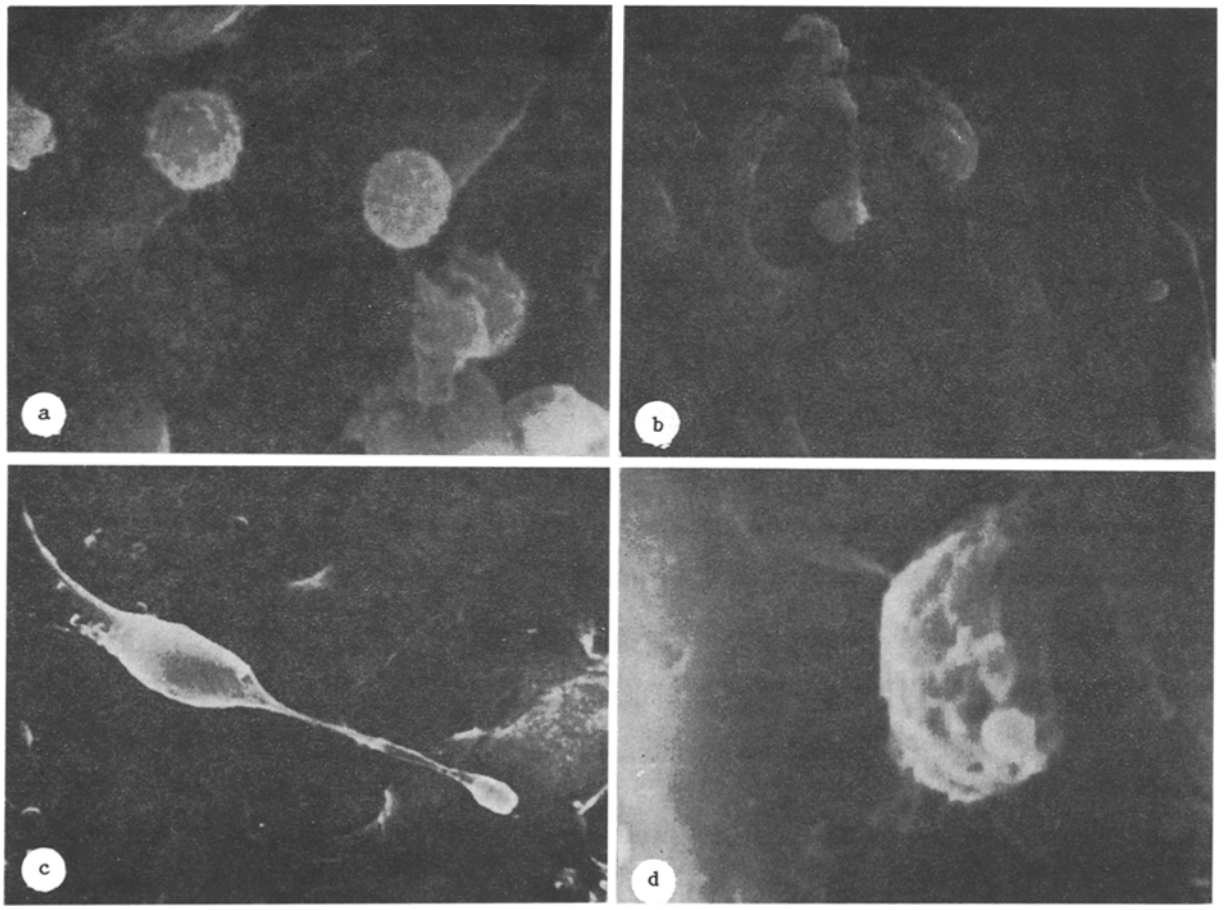

Fig. 1. SEM of luminal surface of pig carotid arteries, critical point drying. a) Adhesion of blood monocytes to E of pig carotid artery. Control. 5000×; b) Bipolar monocytes on surface of E close to a crater. Damage induced by adrenalin (10^{-6} M). 3500×; c) Bipolar monocyte on surface of damaged E. Damage by adrenalin (10^{-6} M). 8000×; d) Migration of monocytes into subendothelial space through defect in interendothelial junction. Guided pole of penetrating monocyte is covered with large vesicles. Damage induced by adrenalin (10^{-6} M). 10,000×.

Pieces of vascular tissue for scanning electron microscopy (SEM) were fixed in 2.5% glutaraldehyde solution (pH 7.2) cut open longitudinally, straightened out on a cork slab with needles, dehydrated in a series of alcohols and acetone, and dried by the "critical point" method. Specimens of arteries prepared in this way were sprayed with gold and palladium and examined in the PSEM-500 scanning electron microscope. Pieces of the arteries for light and transmission electron microscopy (TEM) were postfixed in 1% OsO_4 solution, dehydrated in a series of alcohols and acetone, and embedded in Epon-Araldite ("Fluka"). Semithin sections were stained by the method in [3] and examined in the light microscope. Ultrathin sections were studied and photographed in the JEOL-100CX transmission electron microscope.

The injured epithelium (E) was subjected to quantitative analysis by SEM. No fewer than 30-40 randomly chosen fields with an area of 0.11 mm^2 were analyzed. Changes in E were evaluated by the number of craters and stomata per unit area of blood vessel. The number of adherent monocytes per unit area of vessel was counted on the luminal surface of the arteries. In semithin sections under a magnification of 100, the number of monocytes, histiocytes, and macrophages per 1000 μ length of section was determined in the subendothelial space.

EXPERIMENTAL RESULTS

According to the SEM data, the endothelial layer of the control segments of the carotid arteries remained intact for 5 h of isolated perfusion. The number of desquamated cells did

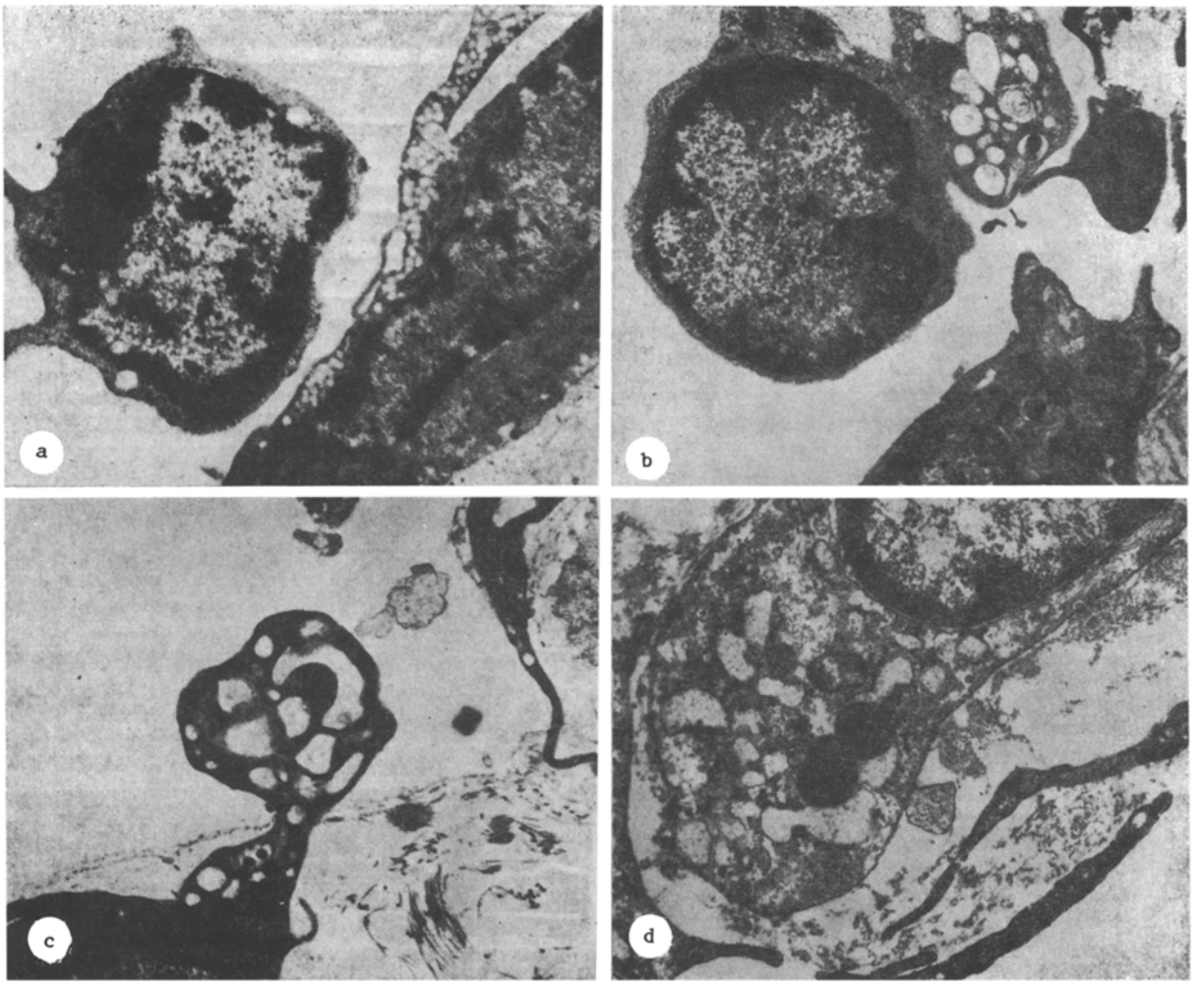

Fig. 2. TEM of different phases of adhesion and transendothelial migration of pig blood monocytes. a) Adhesion of monocytes to luminal surface of E. Control. 35,000×; b) Initial phase of migration of monocytes into subendothelial space through widened interendothelial channel. Damage induced by adrenalin (10^{-6} M). 35,000×; c) Migration of monocytes into subendothelial space in region of crater. Many monocytes present in subendothelial space, vacuolated guided pole of cytoplasm of monocytes remains above surface of basement membrane of E. Damage induced by adrenalin (10^{-6} M). 40,000×; d) Final phase of migration of monocytes into subendothelial space. Monocyte located directly below tapering E in region of dilated interendothelial channel. Cytoplasm of monocyte vacuolated and contains large lysosomes. Damage induced by adrenalin (10^{-6} M). 40,000×.

not exceed 3.2 ± 0.5/mm² of vessel studied (Table 1). The number of E with modified intercellular boundaries of the crater and stoma type was 74 ± 15/mm². After addition of the monocyte suspension to the perfusion system, adherent cells were discovered on the surface of the arteries; the density of their distribution along the vessel was 511 ± 30/mm² (Table 1). The population of monocytes adherent to E was heterogeneous, and morphologically they consisted of round and bipolar monocytes and mononuclear cells migrating into the arterial intima.

Round monocytes were almost indistinguishable in their morphology from blood monocytes. Their surface was covered by numerous short cytoplasmic processes and curiously shaped crests (Fig. 1a). Monocytes of this type, numbering not more than 34.5% of the total number of adherent monocytes (Table 1) were located on the surface of the plasmalemma of outwardly unchanged E. Bipolar monocytes were located mainly on the surface of E with minimal damage, close to craters and stomata (Fig. 1b). These were fusiform cells with a smooth surface, and with long cytoplasmic processes located at their poles (Fig. 1c). They accounted for 61.6% of the population of adherent monocytes. Monocytes migrating into the arterial intima were located at the edge or in the lumen of the craters and stomata, and on SEM examination

TABLE 1. Frequency and Distribution of Injuries to E in Carotid Arteries of Pigs and Number of Blood Monocytes Interacting with Them before and after Damage Induced by Adrenalin (10^{-6} M)

Parameters	Conditions of culture	
	before treatment with adrenalin (control)	after damage by adrenalin
Number of experiments	7	8
Number of desquamated endotheliocytes/mm^2	3.2 ± 0.5	15.2 ± 1.2
Number of craters and stomata/mm^2	74 ± 15	154 ± 25
Total number of monocytes adherent to luminal surface of vessel/mm^2	511 ± 30	637 ± 50
Number of round monocytes, %	34.5	6.1
Number of bipolar monocytes, %	61.6	34.4
Number of migrating monocytes, %	3.9	59.5
Number of monocytes colonizing arterial intima per 0.001 μ of semithin section	2.3 ± 0.6	11.2 ± 0.9

they had the appearance of nodular, cigar-shaped formation projecting from the interendothelial defects (Fig. 1d), and accounted for not more than 3.9% of the total number of adherent monocytes (Table 1). It must be emphasized that penetration of mononuclear cells into the marginal zones of the cytoplasm of E was never observed.

Perfusion of the carotid artery segments with medium with the addition of adrenalin (10^{-6} M) led to a significant increase in the frequency of lesions of E. According to the SEM data the number of endothelial cells (EC) with modified intercellular boundaries in the form of craters and stomata was more than doubled compared with the control (Table 1). During the TEM study, destruction of the basement membrane, vacuolation of the cytoplasm, and edema and disintegrative swelling of the nucleus, accompanied by increase in the thickness of the marginal zones of the cytoplasm of EC with dilatation of the interendothelial channels were observed in these cells. In the intima and the inner zones of the media, marked edema, fragmentation of collagen fibers, and dystrophy and necrosis of smooth-muscle cells were observed.

Perfusion of the damaged arteries with a monocyte suspension was accompanied by some increase in the total number of monocytes adherent to the luminal surface of E (Table 1). The population of these monocytes consisted mainly of cells migrating into the intima of the vessel (59.5% compared with 3.9% in the control). The relative decrease in the number of round and bipolar cells observed in this case is evidently an indication that damage to the vessel not only triggers the mechanism of adhesion, but also stimulates migration of monocytes into the arterial intima. The sequence of changes in ultrastructure of the monocytes during adhesion and their transendothelial migration is illustrated in Fig. 2: a, b, c. So far as monocytes migrating into the intima are concerned, they were located immediately below E and preserved their usual morphological features (Fig. 2d). According to the results of the morphometric investigation, colonization of the intima of the pig carotid artery by blood monocytes was significantly increased after adrenalin-induced damage, up to 11.2 ± 0.9 cells/1000 μ of semithin section compared with 2.3 ± 0.6 in the control (Table 1).

According to data in the literature, the stimulus for adhesion and migration of monocytes into the vascular wall is provided by several humoral factors: turbulence of the blood flow, blood platelet factor [4], and also fraction C of complement [13]. In the present experiments these inducers were almost completely excluded. Nevertheless, adhesion of monocytes took place both in the control and after catecholamine-induced damage. The correlation found between the degree of damage to E and the number of monocytes adherent to it indicates that besides humoral factors, an important role in this process is played by local tissue chemoattractants of the vascular wall. It has been shown that a smooth-muscle-cell factor [8], decomposition products of elastin and collagen [6], necrohormones [15], etc., may possess chemoattractant activity for blood monocytes. Damage to the vascular wall evidently increases the concentration of tissue chemoattractants in it, and this stimulates migration of monocytes into these regions.

Several stages can be distinguished in the mechanism of infiltration of the arterial intima by mononuclear cells: adhesion, transformation into bipolar cells and transendothelial migration of monocytes into the intima. The quantitative parameters of the ratio of adherent, bipolar, and migrating monocytes on the luminal surface of the vessels can be used as an objective criterion for assessing the severity of the pathological process in the vascular wall.

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