MORPHOLOGICAL SIGNS OF REPARATIVE REGENERATION OF THE AORTIC ENDOTHELIUM AFTER REPEATED INJURY

M. D. Rekhter and A. A. Mironov

UDC 616.132-018.74-001-003.93-091.8

KEY WORDS: regeneration; aorta; endothelium; repeated injuries

Besides changes in the lipoprotein spectrum of the blood plasma, the state of the endothelium of the arteries plays an important role in the pathogenesis of atherosclerosis [11]. A key role is ascribed to it in the "response to injury" hypothesis. Particular attention has been paid to chronic, repeated exposure to the injuring agent [10].

The aim of this investigation was to study the mechanisms of reparative regeneration of the aortic endothelium after repeated injuries.

EXPERIMENTAL METHOD

Experiments were carried out on 30 male KWR albino rats aged 30-50 weeks, kept on a standard diet with a normal content of lipids. The abdominal aorta was de-endothelized by the method in [8], under pentobarbital anesthesia and sterile conditions. A copper rod (diameter of the base 3 mm), cooled with liquid nitrogen, was applied for 30 sec to the aortic wall. The same area of the vessel was injured 5 times at intervals of 14 days. Material for investigation was taken 1, 3, and 14 days after the 5th operation. Rats exposed to cold once, and rats undergoing mock operations served as the controls. Specimens for scanning electron microscopy were prepared by retrograde perfusion through the iliac artery, under average arterial pressure, initially with medium 199 with the addition of heparin (10) U/ml) for 30 sec, then with a 2.5% solution of glutaldehyde in medium 199 for 5 min, followed by immersion postfixation for at least 24 h. The cell boundaries were visualized by the silver impregnation method, for which purpose the following solutions were injected successively after perfusion fixation: 5.5% glucose solution (30 sec), 0.15% $AgNO_3$ solution (60 sec), 5.5% glucose solution (30 sec), 3% NH_4Br and 1% $CoBr_2$ solution (60 sec). Subsequent treatment of the specimens included dehydration in acetone, drying by passage through the critical point in CO_2, ionic spraying with gold [1], and examination in the Hitachi S-405A scanning electron microscope. The specimens were prepared for scanning electron microscopy after perfusion fixation by the usual method [4].

EXPERIMENTAL RESULTS

In the control group 24 h after injury the de-endothelized surface was covered by platelets and monocytes, which spread out to form a monolayer (Fig. 1a). Concentrations of platelets, arranged in a mosaic, were found 24 h after the 5th de-endothelization. Stratified platelet aggregates were frequently seen, with monocytes and erhthrocytes adherent to them (Fig. 1b). Some platelets had undergone degranulation. By this time, the zones of activation, formed by spreading and migration of endothelial cells (EC) could be seen by this time in areas of endothelium adjacent to the "wound," in animals which had undergone a single cryodestruction. In size, these zones consisted of one or two rows of cells proximally and distally to the defect. In rats which had undergone five deendothelizations the size of the zone was increased to 5 or 6 rows. The cells were aligned in the direction of the blood flow, and the zone containing their nucleus was elevated. On the surface of the migrating EC adherent erythrocytes and platelets were often found, which was never observed in the control. Single mitotically dividing EC, covered with vesicles and microvilli were seen.

Central Research Laboratory, A. S. Bubnov Ivanovo Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR N. N. Trapeznikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 103, No. 1, pp. 98-101, January, 1987. Original article submitted January 7, 1986.

0007-4888/87/0103-0115$12.50

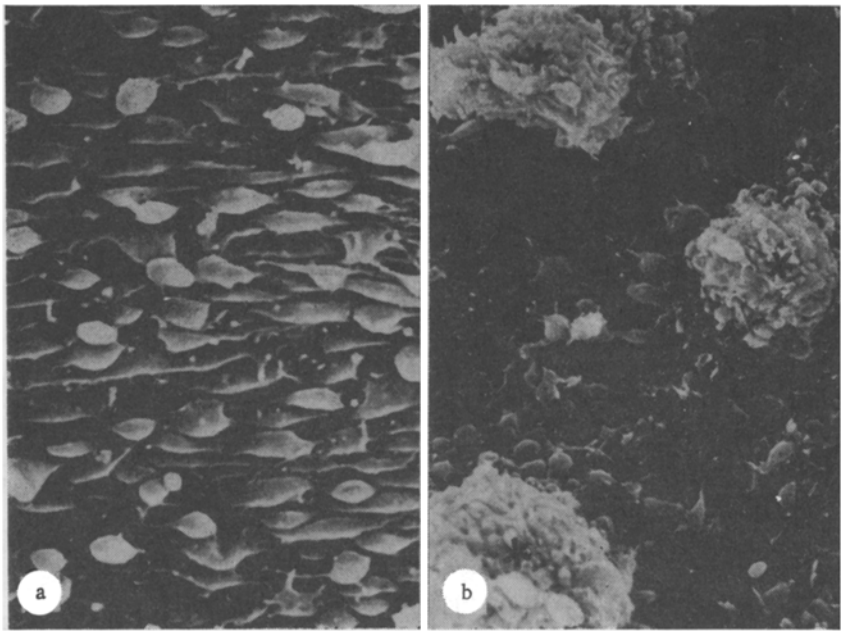

Fig. 1. De-endothelized area of rat abdominal aorta 24 h after injury: a) single de-endothelization: platelets form a monolayer. 3,500 ×; b) 5 de-endothelizations. Asterisks indicate platelet aggregates arranged in a mosaic. 2,500 ×. Here and in Fig. 2: scanning electron microscopy.

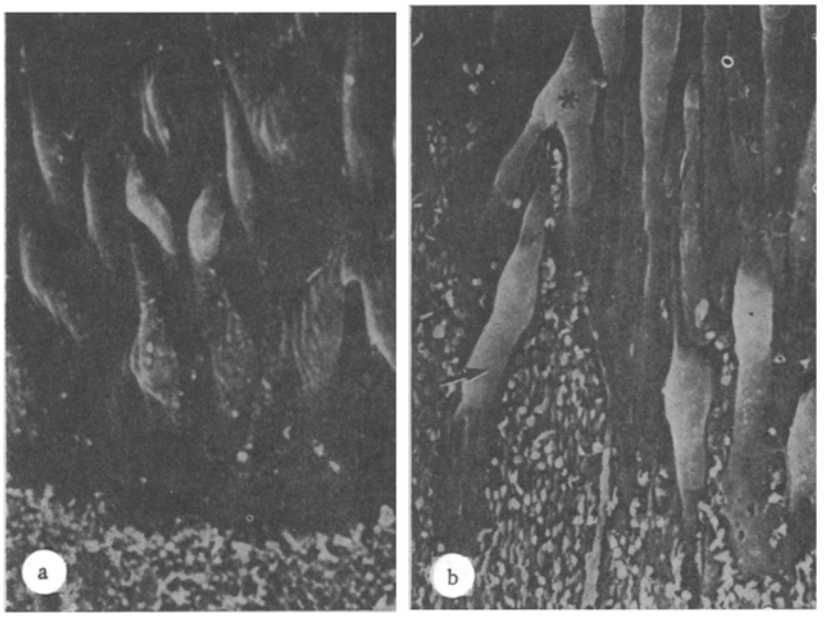

Fig. 2. Partially re-endothelized area of rat abdominal aorta 3 days after injury: a) single de-endothelization; lamelloplasms of peripheral EC form a smooth front. E) Endothelium; DE) zone of de-endothelization; 1,200 ×; b) 5 de-endothelizations: peripheral EC have uneven outlines. Y-shaped (asterisk) and leading EC (arrows) can be seen. 1,400 ×.

After 3 days the process of re-endothelization developed intensively, and in the repeatedly injured animals it followed a more rapid course. The area of the defect of the monolayer not yet covered by EC was 1.2-1.3 times smaller in them than in the control. After a single injury the eodothelium migrated in the form of a continuous sheet, the edge of which formed a smooth front (Fig. 2a). In vessels after 5 de-endothelizations the general character of migration remained the same, but the broad leading lamelloplasm was not yet formed in all the peripheral EC. Many of them were elongated, often spindle-shaped, on account of

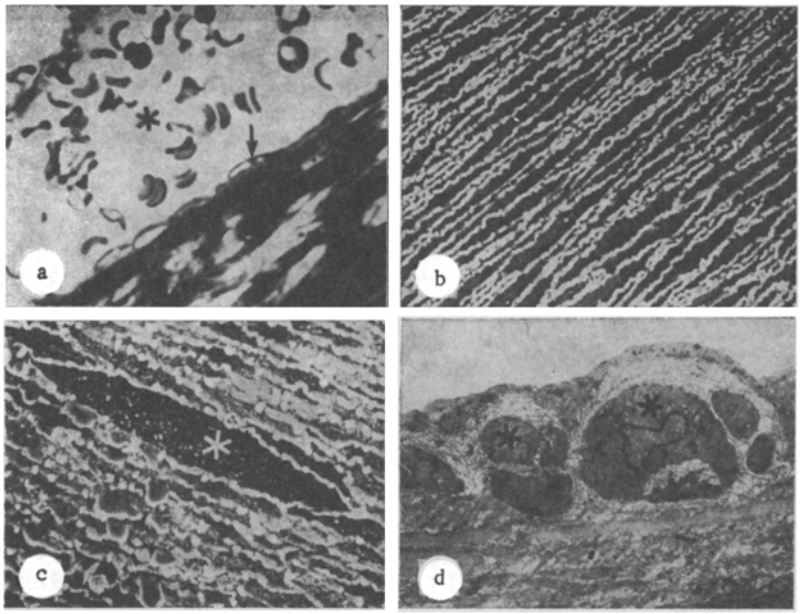

Fig. 3. Re-endothelized region 14 days after injury. a) 5 de-endothelizations: formation of juxtamural thrombus (asterisk) above endothelium (arrow). Semithin section. 350 x; b) single de-endothelization: regular packing of EC in layer is preserved. Scanning electron microscopy. $AgNO_3$. 400 ×; c) 5 de-endothelizations; appearance of giant EC (asterisk). Scanning electron microscopy. $AgNO_3$. 400 ×; d) 5 de-endothelizations: thickening of subendothelial layer. Asterisk indicates smooth muscle cells; arrow EC. Transmission electron microscopy. 2,000 ×.

which the edge of the sheet appeared not as a continuous front, but dentate in character. Many autonomous leader cells, preceding the main sheet of EC and frequently unconnected with it, appeared. The endothelial monolayer became polymorphic: Y-shaped cells, cells with many branches, and giant cells were seen in it (Fig. 2b). Besides axial (in the direction of the blood flow) regeneration, lateral migration of EC was more conspicuous than after a single injury. Whereas in the control group migrating EC were closely connected with each other, after repeated injuries small defects ("breaches"), covered with platelets and leukocytes, could be seen in the composition of the sheet, more frequently in the region of interendothelial junctions. Groups of leukocytes were adherent to the newly formed endothelium in the region of the intercellular junctions. The junctions showed greater affinity for silver salts than after a single injury, and they appeared widened in ultrathin sections. Single argentophilic EC were observed. Weibel-Palade bodies were more frequently found in the cytoplasm of EC.

On the 14th day no defect was found in either experimental or control groups, but in animals with repeated trauma de-endothelized microregions, not exceeding 4-5 μ in diameter, were still present. In the control newly formed EC remained athrombogenic, whereas in vessels subjected to repeated trauma, in some cases juxtamural thrombi formed above the layer of young endothelium, with deposition of fibrin threads (Fig. 3a). In both groups marked hyperplasia of EC was observed in the zone of re-endothelization. In vessels denuded of their endothelium only once the arrangement of the cells continued to be oriented in character (Fig. 3b), whereas after repeated trauma the regular arrangement of the cellular mosaic of the endothelium in impregnated preparations was distinctly disturbed (Fig. 3c). The subendothelial layer (SEL) was considerably thickened after repeated trauma, and its thickness was directly proportional to the duration of de-endothelization (greatest in the center of the former defect, least at the periphery). The structure of SEL consisted of loose alternating layers of granular and fibrillary material, the number of which corresponded to the number of denudations. Beneath the endothelium smooth muscle cells (SMC) with lipid inclusions in their cytoplasm were immured in the ground substance (Fig. 3d).

Comparison of the results with data obtained after a single cryodestruction shows that the fundamental cellular mechanisms and stages of regeneration (migration and proliferation

of EC) were preserved, but after repeated trauma there were significant differences: modification of SEL, a change in the character of interaction between platelets and the injured and repaired aortic wall, increased cellular polymorphism and autonomy, and also rapid restoration.

A distinguishing feature of the model used is injury by cold to all layers of the vascular wall [8]. For that reason the typical myointimal thickening, frequently described previously [10, 11], did not form at the site of the "wound." However, the presence of single SMC beneath the young endothelium is evidence of their migration from intact regions of the media of the aorta. The view is held that growth factors of platelets and macrophages are the main stimuli for migration of SMC [10]. This evidently explains the appearance of the many SMC in the center of the zone of injury, i.e., at the site of longest contact of the de-endothelized vascular wall with blood cells. The ability of SMC to synthesize noncellular connective-tissue elements of SEL [10] accounts for its uneven thickness.

Changes in the platelet response are also probably connected with the processes described above. We know that stratified platelet aggregates are formed only on collagen of types I and III [2]. Since only collagen of types IV and V is present in SEL of the intact aorta [3], after single de-endothelization the adherent platelets form a monolayer. However, after only the second injury, the appearance of stratified microthrombi has been described [9]. It can be tentatively suggested that this is due to expression of collagen of types I and II here. The mosaic arrangement of the aggregates discovered in the present experiments may be the result of the corresponding arrangement of collagen-synthesizing SMC in the intima of the vessel.

In our opinion the variability of the structural and functional properties of SEL appearing after repeated injuries is due to the corresponding topography of SMC and it can induce a change in the adhesiveness and mobility of EC adherent to it, leading to an increase in cellular polymorphism in the zone of reendothelization. It has been shown *in vitro* that the character of migration and the shape of EC depend on the type of collagen of the subendothelial matrix [7]. It is also known that giant cells adhere better to the underlying substrate [6]. Their appearance can therefore be interpreted as a response to thickening and a change in the properties of SEL. At the same time, it has been shown that changes in the morphological phenotype of EC in culture correlate with changes in collagen synthesis by them [5]. Consequently, the appearance of discrete loci of SEL with new properties after repeated trauma can be partly explained by an increase in polymorphism of the EC themselves.

Substrate-dependent changes in mobility and proliferative activity of EC may also, perhaps, be a cause of the more rapid re-endothelization.

The character of regeneration of the aortic endothelium after repeated trauma thus has distinguishing features, in the development of which a key factor, in our opinion, is a change in the structure and properties of the subendothelial layer.

LITERATURE CITED

1. A. A. Mirnoov, V. A. Mironov, and M. D. Rekhter, Arkh. Anat., No. 11, 85 (1983).
2. F. Missel'vitts, V. L. Leitin, S. P. Domogatskii, et al., Byull. Éksp. Biol. Med., No. 9, 359 (1984).
3. B. S. Weakley, Beginner's Handbook in Biological Electron Microscopy, Churchill (1972).
4. B. V. Shekhomin, S. P. Domogatskii, A. V. Rudin, and V. S. Rukosuev, Arkh. Patol., No. 3, 18 (1984).
5. C. I. Levene, C. Bartlet, and G. Heale, Atherosclerosis, 52, 59 (1984).
6. L. A. Lyass, A. D. Bershadsky, V. I. Gelfand, et al., Proc. Natl. Acad. Sci. USA: Biol. Sci., 81, 3098 (1984).
7. J. A. Madri and K. S. Stenn, Am. J. Pathol., 106, 180 (1982).
8. H. T. Malczak and R. C. Buck, Am. J. Pathol., 86, 133 (1977).
9. M. Richardson, R. L. Kinlough-Rathbone, H. M. Groves, et al., Br. J. Exp. Pathol., 65, 597 (1984).
10. R. Ross, A. Faggiotto, D. Bowen-Pope, and E. Raines, Circulation, 70, 77 (1984).
11. S. M. Schwartz and R. Ross, Prog. Cardiovasc. Dis., 26, 355 (1984).