ULTRASTRUCTURAL CHANGES IN THE SARCOLEMMA IN THE EARLY STAGES OF IMMUNE HEART DAMAGE

L. F. Popovich and A. A. Moibenko

UDC 616.12-056.43-036.4-07:
616.12-018.63:576.314

KEY WORDS: immune damage; phospholipid degradation; sarcolemma; multilaminar structures

Recent investigations have shown that damage to the sarcolemma plays a decisive role in the development of pathological changes in the cardiomyocytes in various heart diseases [7]. One of the most important factors in this case is disturbance of the permeability and structural integrity of the glycocalyx and plasma membrane, leading to disturbance of the mechanisms of trans-sarcolemmal ion transport and to the entry of an excess of calcium into the cell [7, 9, 13]. An important role in the development of disturbances of the barrier function of the cardiomyocyte membranes is attributed to structural changes in and degradation of the phospholipid bilayer of the plasma membrane [10, 11, 14]. The writers showed previously that immune heart damage is characterized by the rapid development of disturbances of the structure and function of the cardiomyocyte sarcolemma [3, 6] and evidence was obtained by support of the view that activation of phospholipases may take place, resulting in the formation of biologically active products of phospholipid catabolism, namely prostaglandins and leucotrienes [4].

It was accordingly decided to undertake special investigations of structural changes in the cardiomyocyte sarcolemma in order to study morphological features of phospholipid degradation of the plasmalemma in immune heart damage, and the results are described below.

EXPERIMENTAL METHOD

Experiments were carried out on 15 mongrel dogs weighing 15-20 kg under general anesthesia. Acute heart damage was produced by injecting anticardiac cytotoxic serum (ACS) in a volume of 1-2 ml, with a titer of 1:800 in the complement fixation test, obtained from rabbits after immunization with the supernatant fraction of dog heart homogenate, directly into the blood stream in the circumflex or descending branch of the left coronary artery. Details of the technique of production of local immune heart damage were described previously [1]. In two series of experiments, undertaken 5-7 min and 1 h respectively after the procedure, the hearts were removed from the thorax of anesthetized dogs, left to stand in ice-cold KCl solution, after which pieces of myocardium were excised, fixed in glutaraldehyde solution in 0.1 M phosphate buffer, or in parallel experiments in glutaraldehyde with ruthenium red [15] and with colloidal lanthanum [18] in cacodylate buffer, postfixed in 1% osmic acid, and embedded in Epon-Araldite. Ultrathin sections were stained in uranyl acetate and examined in the IEM-100CX electron microscope.

EXPERIMENTAL RESULTS

In all the experiments the development of an antigen-antibody reaction in the territory supplied by the circumflex or descending branch of the left coronary artery was accompanied by focal damage to the coronary vascular bed and myocardium, depending on the site of injection of the anticardiac antibodies and the development of typical immunogenic shock, with a fall of pressure in the aorta on average by 45% and reduction of the cardiac output by 43% compared with its initial values. Morphological changes in the myocardium consisted essentially of disturbance of permeability of the sarcolemma, the development of overcontraction of myofibrils, myocytolysis, and various changes in the mitochondria, leading ultimately to the formation of foci of myocardial necrosis with marked calcification. Structural changes

Department of Experimental Cardiology, A. A. Bogomolets Institute of Physiology, Academy of Sciences of the Ukrainian SSR, Kiev. (Presented by Academician of the Academy of Medical Sciences of the USSR, N. N. Gorev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 103, No. 5, pp. 621-625, May, 1987. Original article submitted July 9, 1986.

 0007-4888/87/0005- 0708$12.50

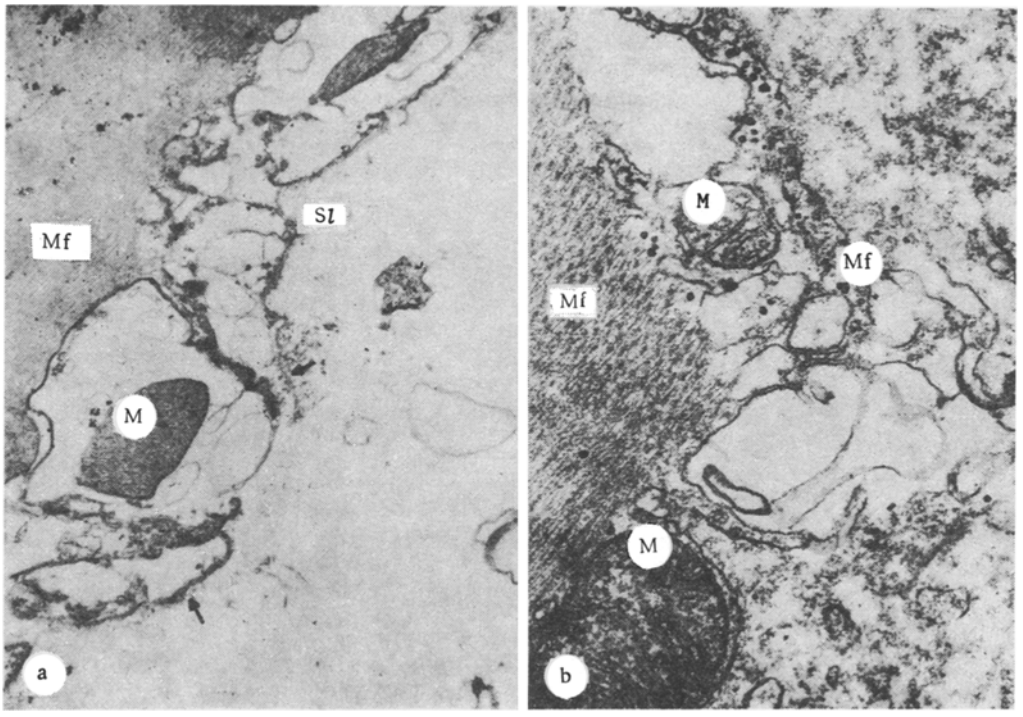

Fig. 1. Ultrastructure of myocardium of dog's left ventricle 7 min after injection of anticardiac serum. Formation of numerous vacuoles of different sizes in the subsarcolemmal zone, stripping of the plasmalemma, glycocalyx raised above the plasma membrane (arrow), integrity of the plasma membrane is disturbed. SL) Sarcolemma; M) mitochondrion; MF) myofibrils. Fixation with glutaraldehyde and osmium. Magnification: a) 12,000; b) 48,000.

in the cardiomyocytes were observed in the experiments of series I as early as 5-7 min after injection of ACS (Fig. 1a, b). However, they were exhibited most completely and were extensive in character at later times of observation. Ultrastructural changes in the sarcolemma 7 min after injection of ACS are illustrated in Fig. 1. A mosaic pattern of stripping of the glycocalyx is observed, with numerous vacuoles of different sizes, bounded by a membrane, in the subsarcolemmal region, appearing in the section as ring-like structures. The formation of these large vacuoles was accompanied by disturbance of the integrity of the sarcolemma and, in particular, of the plasma membrane, and direct contact was observed between the vacuoles and the plasma membrane, which had the appearance of a "simmering surface," on which liposomes are formed.

After fixation of the myocardial tissue with colloidal lanthanum, an electron-microscopic tracer revealing microdefects in the sarcolemma [7, 12], the permeability of the membrane was found to be disturbed early in the zone of injection of ACS, and these disturbances progress with the course of time. The ultrastructure of a typical modified cardiomyocyte 1 h after injection of ACS is shown in Fig. 2. The plasma membrane formed layers, and areas of aggregation of membranous material, marked with lanthanum granules, and areas with indistinct outlines of the plasmalemma could be seen. In the subsarcolemmal zone laminated structures were visible, and were evidently of membrane origin. Significant disturbance of the barrier function of the sarcolemma was shown by penetration of quite large particles of colloidal lanthanum inside the cardiomyocytes. At the same time, areas of overcontraction of the muscle fibers and of their lysis were clearly visible. Thus immune heart damage was characterized by the formation of numerous vacuoles of various sizes in the juxtamembranous layers of the cardiomyocytes, were revealed after fixation of the tissue even by ordinary methods.

After fixation of pieces of myocardium with ruthenium red osmiophilic multilaminar structures consisting of tightly packed bilayers could be seen in the cardiomyocytes (Fig. 3). The multilaminar structures were located mainly in the subsarcolemmal zone, sometimes in the immedicate vicinity of the plasma membrane which, as a rule, lost its clarity of outline in these situations (Fig. 2) and also on the outer mitochondrial membrane (Fig. 3) and in the

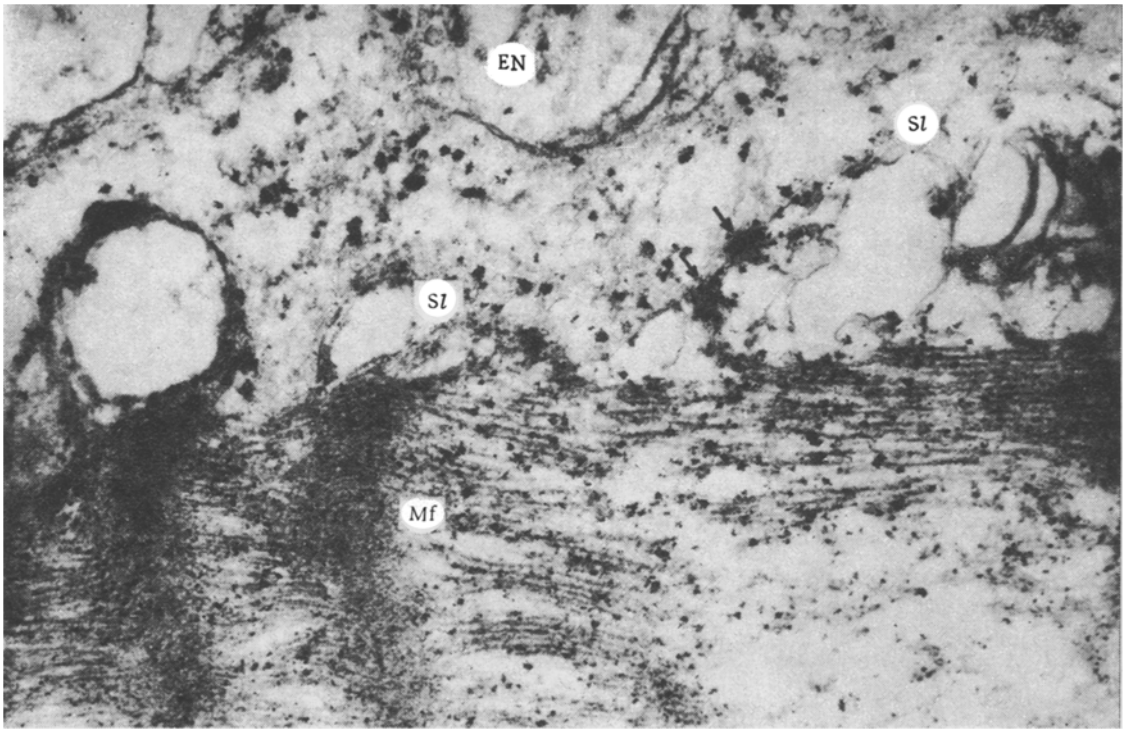

Fig. 2. Ultrastructure of left ventricular myocardium of a dog 1 h after injection of anticardiac serum. Aggregation of membranous material (arrow) of sarcolemma, mosaic pattern of indistinctness of plasma membrane, laminated structures beneath the sarcolemma, penetration of colloidal lanthanum inside the cardiomyocyte, overcontraction and focal lysis of mylfibrils. EN) Capillary endothelium. Fixation with colloidal lanthanum, 14,000 ×.

sarcoplasmic reticulum. Staining with ruthenium red, which reacts with substances possessing a negative charge, is used to strain extracellular polyanions in tissue fragments, for this dye does not pass through the intact cell membrane [15, 20], and it is used to stain the glycocalyx of cell membranes. The writers showed previously that the glycocalyx layer in an area of myocardium subjected to the action of anticardiac antibodies is thinner and it stains less strongly than in the control. Simultaneous reduction of binding of the dye in the outer layers of the sarcolemma and its penetration into the myoplasm, where it evidently binds with and oxidizes negatively charged phospholipids (amphiphilic lipids), also are evidence of increased permeability of the plasmalemma and of its structural modification. There is every reason to suppose that the osmiophilic multilaminar structures are derivatives of degraded membrane phospholipids, forming liposomes consisting of densely packed bilayers in the cytosol. Multilaminar structures are formed *in vitro* during swelling of phospholipids or phosphoglycerides in an aqueous medium, and it has been shown that vesicles composing these structures are formed by a lipid bilayer in which polar groups of phospholipids face outward [5].

The formation of analogous ultrastructures in the cardiomyocytes has been demonstrated by injecting lipid drops into the myocardium under conditions of activation of lipolysis [20]. The formation of multilaminar structures of phospholipid origin from plasma membranes of the sarcolemma and outer membranes of the mitochondria has been described in experimental ischemia and reperfusion of the myocardium, and also in the "calcium paradox" [11, 15, 16]. Degradation of phospholipids of cardiomyocyte membranes, recorded by biochemical methods, was accompanied by the formation of circular lipid structures [19]. It must be considered that the destabilization developing under conditions of heart disease, with liquefaction of the phospholipid bilayers of the cell membranes, led to leakage of lipids into the juxtamembranous zone, where they are organized into bilayers, with the formation of multiple vesicles. A bilayered structure is known to be optimal for amphiphilic lipids [5]. To judge from our own data and those in the literature, differences in liposomal ultrastructure are largely dependent on the method of fixation of the myocardial tissue. Workers who have described the formation of multilaminer structures used methods of fixation in glutaraldehyde with tannic acid, or the freeze-etching method [11, 17, 21]. During routine fixation in glutaraldehyde lipo-

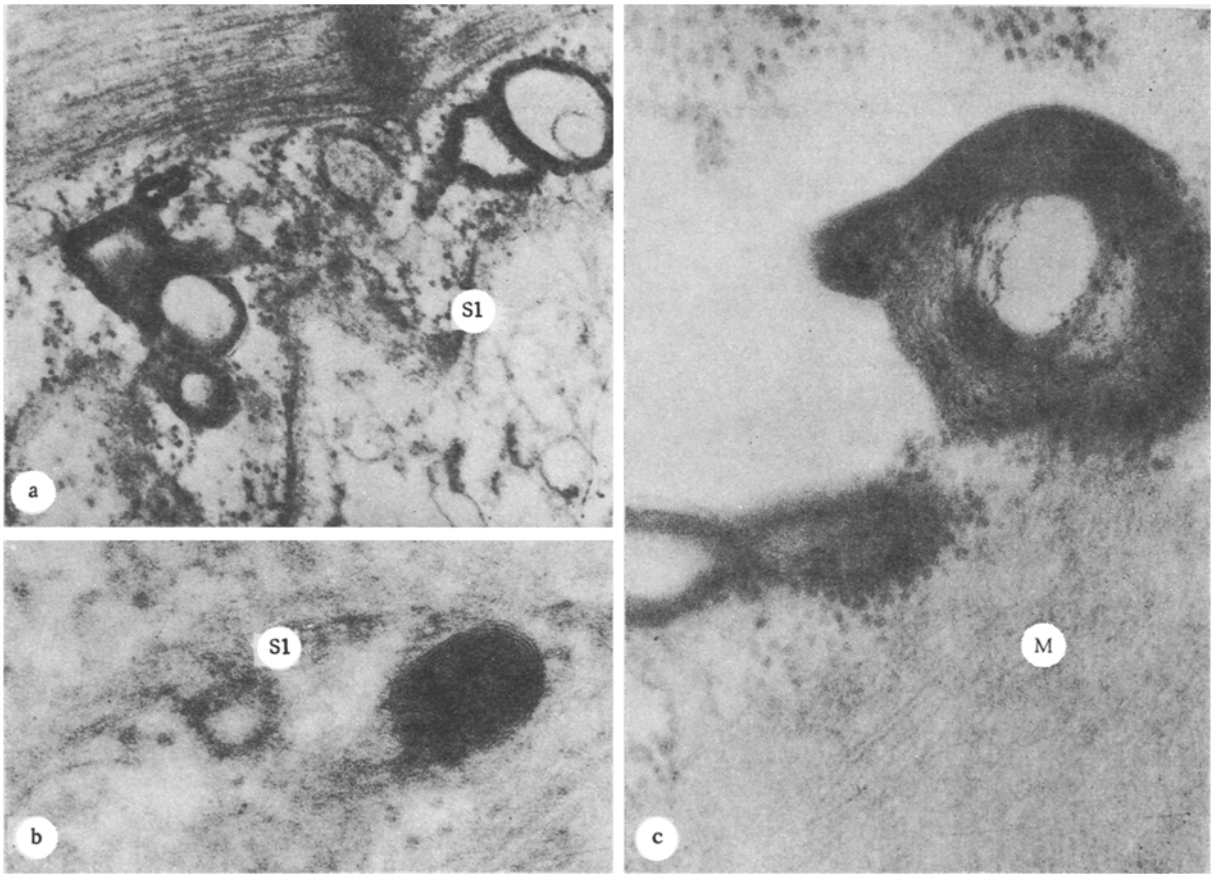

Fig. 3. Ultrastructure of left ventricular myocardium of a dog 1 h after injection of anticardiac serum. a, b) Multilaminar structures in subsarcolemmal region; mosaic pattern of indistinctness of outline of plasma membrane; c) multilaminar structure on mitochondrial membranes. Fixation with ruthenium red. Magnification: a) 2400 ×; b, c) 48,000.

somes have the appearance of circular structures [19]. It must be emphasized that in the case of immune heart damage structural changes in the sarcolemma, resulting in the appearance of microdefects and disturbances of permeability, take place much earlier (after 5-7 min) than in ischemic heart damage (90-150 min) and subsequent reperfusion [16]. The increase in phospholipase activity in immune heart damage and degradation of phospholipids of the plasmalemma may be connected with activation of the complement system [8], an increase in the calcium concentration in the cytosol, together with possible pH changes arising as the result of the development of myocardial hypoxia in the zone of immune heart damage [2].

LITERATURE CITED

1. A. A. Moibenko, M. M. Povzhitkov, and G. M. Butenko, Cytotoxic Heart Lesions and Cardiogenic Shock [in Russian], N. N. Gorev, ed., Kiev (1977).
2. A. A. Moibenko and L. F. Popovich, Vest. Akad. Med. Nauk SSSR, No. 7, 58 (1982).
3. A. White, P. Handler, E. L. Smith, et al., Principles of Biochemistry, New York (1978).
4. L. F. Popovich, V. F. Sagach, and V. G. Sharov, Byull. Éksp. Biol. Med., No. 11, 101 (1982).
5. V. F. Sagach, Byull. Éksp. Biol. Med., No. 2, 151 (1986).
6. G. A. Cherednichenko, Z. D. Vorobets, M. D. Kurskii, et al., Fiziol. Zh. (Kiev), No. 1, 43 (1986).
7. V. G. Sharov, Byull. Vses. Kardiol. Nauch. Tsent., No. 1, 48 (1984).
8. M. Betz and G. M. Hansch, Immunobiology, 166, 473 (1984).
9. L. M. Buja, K. R. Chien, K. P. Burton, et al., Myocardial Injury, New York (1983), pp. 421-431.
10. K. R. Chien, J. Willerson, and L. M. Buja, Adv. Myocardiol., 5, 347 (1985).
11. J. S. Frank, T. L. Rich, S. Beydler, and M. Kreman, Circulat. Res., 51, 117 (1982).

12. S. Hoffstein, D. E. Gennaro, A. C. Rox, et al., Am. J. Path., 79, 207 (1975).
13. R. B. Jennings and K. A. Reimer, Am. J. Path., 102, 214 (1981).
14. A. A. Katz and F. S. Messineo, Circulat. Res., 48, No. 1, 1 (1981).
15. J. H. Luft, Fed. Proc., 25, 1773 (1966).
16. J. A. Post, J. Leunissen-Bijvelt, T. J. Ruigrok, and A. J. Verkleij, Biochim. Biophys. Acta, 845, 119 (1985).
17. J. Post. P. Nievels, J. Leunissen-Bijvelt, et al., J. Mol. Cell. Cardiol., 17, 265 (1985).
18. J. P. Revel and M. J. Karnovsky, J. Cell Biol., 33, 7 (1967).
19. C. Steenberg and R. Jennings, J. Mol. Cell. Cardiol., 16, 605 (1984).
20. E. Tani and T. Ametani, J. Cell Biol., 46, 159 (1970).
21. M. G. Wetzel and R. O. Scow, Am. J. Physiol., 246, C467 (1984).