MYOSIN DISTRIBUTION IN PRIMARY CULTURES OF HUMAN AORTIC INTIMAL CELLS

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According to several workers [4, 11, 13] a key role in the pathogenesis of atherosclerosis is played by smooth-muscle cells (SMC). Accordingly, there is great interest in the study of SMC and the description of their functional and morphological features. Wide prospects are afforded in this respect by the use of SMC cultures, whereby single cells can be investigated and the conditions of their culture varied. It has been shown, for instance, that vascular SMC change their phenotype during culture and this affects the character of distribution of myosin in them [5-7]. However, these results were obtained in experiments on animals, whereas we know that SMC in the intima of the human aorta differ considerably from one another in their ultrastructure [9].

In the present investigation the character of the distribution of myosin in cells of a primary culture of human aortic intima was studied, using antiserum against smooth muscle myosin.

EXPERIMENTAL METHOD

Under aseptic conditions a segment of the thoracic portion of the aorta was excised within 1.5-3 h after death from three men who had died suddenly at the ages of 31, 54, and 70 years. The outer connective-tissue membrane was detached from the media and the vessel was cut longitudinally and washed free from blood. Areas of intima containing atherosclerotic plaques were removed. The endothelium was removed by treatment with 0.1% collagenase solution (type I, from Sigma, USA) in the course of 30 min at 37°C, after which the vessel was incubated under the same conditions with 0.2% collagenase solution for 2 h. During this time about one-third of the intima adjacent to the endothelium was dispersed, as verified by stained frozen sections of the vessels. The cell suspension was washed off and seeded on coverslips measuring 22 × 22 mm with a density of $2.5 \cdot 10^4$ to $3.0 \cdot 10^4$ cells/cm^2. The cells were cultured in medium 199 containing 2 mM glutamine, 25 mM HEPES, 1 mM sodium pyruvate, 100 U/ml penicillin, 100 μg/ml streptomycin, and 10% calf serum (all reagents from Gibco, USA). After 24 h the nonadherent cells were washed off, and the medium was changed thereafter every 3 days. Effectiveness of adhesion and rate of growth of the cells were determined as described previously [1].

The cells were fixed between 1 and 14 days of culture with absolute acetone for 10 min at −10°C and dried in air. Rabbit antiserum against human uterine myosin, the characteristics of which were described previously [2], was used. The antiserum was passed additionally through a sorbent of platelet actomyosin complex, prepared by means of glutaraldehyde [3]. Some cells were incubated with antiserum against uterine myosin, others with rabbit antiserum against antihemophilic factor VIII (Behring Diagnostic Corp.), or with normal rabbit serum in dilutions of 1:5 and 1:3, respectively. The cells were then incubated with goat antibodies against rabbit IgG, labeled with fluorescein by the standard method [12]. The coverslips were washed and examined under an "Opton" microscope. The ratio between cells differing in their myosin distribution was counted in preparations fixed and stained on the 5th, 7th, and

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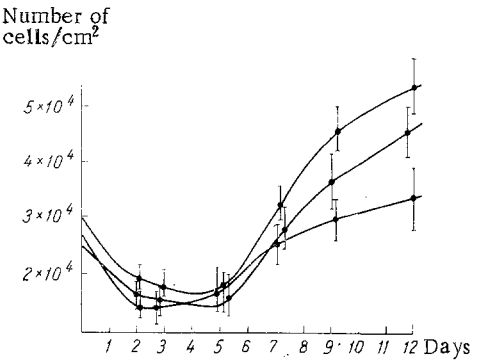

Fig. 1. Curves showing increase in number of human aortic intimal cells in primary culture. Abscissa, time of culture (in days); ordinate, number of cells/cm^2.

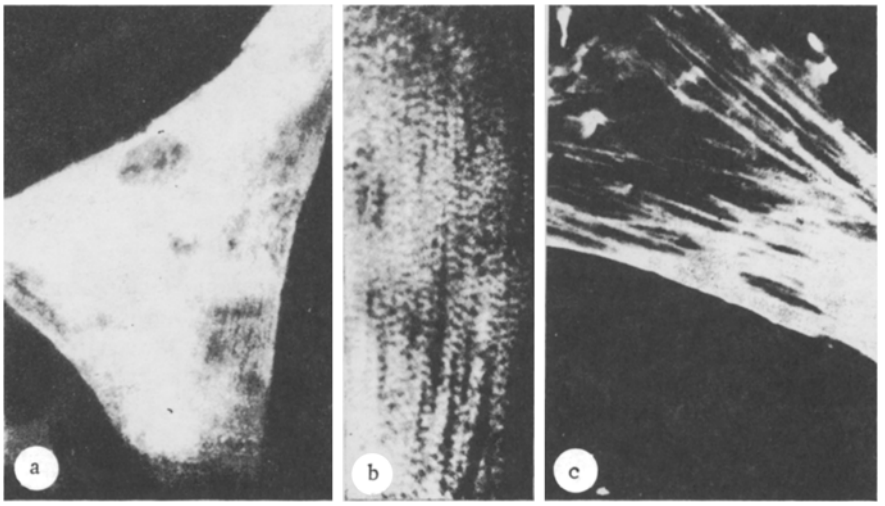

Fig. 2. Variants of myosin distribution in cells of primary culture of human aortic intima. a) Homogeneous type; b) myosin aggregates in interrupted fibrils; c) myosin in the form of continuous fibrils, with unstained cell alongside. Coons' indirect method, treatment with antiserum against smooth muscle myosin. Magnification: a, c) 80; b) 160.

14th days of culture. The results were subjected to statistical analysis. Some cells were stained with Oil Red.

EXPERIMENTAL RESULTS

After treatment with collagenase a suspension was obtained in which more than 90% of the cells were viable. In the course of 24 h 67-84% of the cells adhered to the substrate, and during the next 2 days they spread out into a monolayer. Analysis of the growth curve of the cells in culture showed that during the first 5 days of culture the number of cells remained constant, but after the 7th day it increased (Fig. 1). The time taken for the number of cells to double in the logarithmic phase of growth was 48-96 h.

The culture of human aortic intimal cells was heterogeneous and in every case it varied mainly on account of foam cells and, to a lesser degree, of endothelium. Foam cells were small and oval in shape. Their cytoplasm was filled with round inclusions, which stained bright red with Oil Red. Some inclusions gave intense yellow fluorescence in UV light. Endothelial cells also were small and oval in shape, and they had one or several nuclei. These cells contained factor VIII, which aided their identification. Foam and endothelial cells were not stained by antiserum against smooth muscle myosin and they were not counted when the number of cells with different distributions of myosin was analyzed.

TABLE 1. Distribution of Myosin (in %) in Aortic Intimal Cells from Cadavers of Three Men, at Different Stages of Culture

Age of man at death	Time of culture, days	Character of myosin distribution		
		homogeneous	in the form of continuous fibrils	absence of staining
31 years	5	73,88±3,80	1,49±1,05	24,62±3,73
	7	54,26±4,40	7,75±2,36	37,98±4,28
	14	5,00±1,54	30,50±3,26	64,50±3,39
54 years	5	69,00±4,64	1,00±1,00	30,00±4,60
	7	59,00±4,94	16,00±3,68	25,00±4,35
	14	3,50±1,30	50,00±3,54	46,50±3,53
70 years	5	78,23±3,17	0	21,76±3,17
	7	63,37±3,68	7,56±2,02	29,06±3,47
	14	9,00±2,02	26,50±3,12	64,50±3,39
Arithmetic mean	5	74,50±2,17	0,74±0,42	24,74±2,14
	7	59,34±2,45	9,72±1,48	30,91±2,31
	14	5,83±0,95	35,66±1,95	58,50±2,01

Most of the cells were comparatively large in size, elongated, polygonal, or stellate in shape, and they stained variously with antiserum against smooth muscle myosin. In some cells, for instance, the myosin was distributed homogeneously, filling all the cytoplasm except a small zone around the nucleus (Fig. 2a, b). Under high power interrupted fibrils in which myosin was distributed as aggregates, could be observed in these cells (Fig. 2b). These aggregates gave the cells a cross-striated appearance. As well as this type of myosin distribution, as early as during the first day of culture, cells were found in which myosin was distributed as continuous fibrils, arranged in parallel bundles along the long axis of the cells, mainly at their periphery, with a free zone around the nucleus (Fig. 2c).

Furthermore, during the first day in culture, cells which did not stain with antiserum against smooth muscle myosin could be found. These cells were indistinguishable in shape and size from the cells containing myosin. They had a central oblong nucleus and had no lipid inclusions.

The ratio between the above-mentioned types of cells with different myosin distributions varied during culture. Their number remained constant only for the first 5 days and the largest number of cells in culture had a homogeneous distribution of myosin (Table 1). Starting with the 7th day of the experiment the number of cells with myosin distributed in the form of continuous fibrils increased ($P < 0.001$). In the later stages of the experiment the number of these cells continued to increase, and on the 14th day of culture they were 66 times more numerous than when the experiment began. The proportion of cells not stained with antiserum against smooth muscle myosin was increased under these circumstances ($P < 0.001$). All these events took place on account of a more than 12-fold decrease in the number of cells with a homogeneous myosin distribution.

It will be noted that on the 6th and 7th days of culture continuous fibrils appeared in single cells with homogeneous staining for myosin, at the periphery of the cytoplasm.

In human aortic intimal cells during culture changes thus take place in the character of myosin distribution. Some cells lose their ability to stain with antiserum against smooth muscle myosin. A similar picture also was observed during culture of vascular SMC of experimental animals, which had a homogeneous type of myosin distribution during the first 5 days, whereas later their myosin distribution changed, and this was accompanied by changes in structural and functional properties of these cells [5-7, 10]. This process may be called modulation instead of the term "transformation" commonly used previously [7]. As some workers have suggested [8, 14], as a result of modulation, SMC change from their usual contractile state into a synthetic state, and this is followed by unrestrained collagen synthesis which, strictly speaking, is characteristic of atherosclerosis also.

A distinguishing feature of cultures of human aortic intimal cells compared with vascular SMC cultures from experimental animals is that during the first few days of the experiment, before any changes characteristic of culture have taken place, cells with different distributions of myosin are found, including some which do not stain with antiserum against smooth muscle myosin. Evidently this fraction of cells is in different stages of the modula-

tion process from the beginning and, consequently, it differs from the other cells in its structural and functional properties. An explanation of these functional differences is essential for the understanding of the role of SMC in the pathogenesis of atherosclerosis.

LITERATURE CITED

1. A. S. Antonov, A. S. Krushinskii, M. A. Nikolaeva, et al., Tsitologiya, No. 10, 1154 (1981).
2. B. V. Shekhonin, A. S. Alkadarskii, V. S. Rukosuev, et al., Byull. Éksp. Biol. Med., No. 5, 618 (1981).
3. S. Avrameas and T. Ternynck, Immunochemistry, 6, 53 (1969).
4. E. P. Benditt, Ann. N. Y. Acad. Sci., 275, 96 (1976).
5. B. Burki, C. Mahlmeister, U. Gröschel-Stewart, et al., Histochemistry, 60, 135 (1979).
6. J. H. Chamley, G. R. Campbell, J. D. McConnell, et al., Cell Tissue Res., 177, 503 (1977).
7. J. H. Chamley-Campbell, G. R. Campbell, and B. Ross, Physiol. Rev., 59, 1 (1979).
8. G. R. Campbell and J. H. Chamley-Campbell, Pathology, 13, 423 (1981).
9. J. C. Geer and M. D. Haus, Smooth Muscle Cells in Atherosclerosis, Vol. 2, Basel (1972).
10. U. Gröschel-Stewart, J. H. Chamley, G. R. Campbell, et al., Cell Tissue Res., 165, 13 (1975).
11. G. M. Martin and C. A. Sprague, Exp. Mol. Pathol., 18, 125 (1973).
12. R. C. Nairn, Fluorescent Protein Tracing, Edinburgh (1976), p. 648.
13. R. Ross and J. Glomset, New Engl. J. Med., 295, 869 and 420 (1976).
14. R. Ross, Atherosclerosis, 1, 293 (1981).

TRANSMISSION OF "REGENERATION" INFORMATION BY LYMPHOCYTES OF RATS AFTER WIDE INTESTINAL RESECTION

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The ability of lymphocytes to transmit "regeneration" information has been demonstrated in relation to regeneration of various organs both after surgical operations [1, 2, 6, 7] and after a pathologic process [3, 4]. It is not yet clear whether this ability is expressed after surgical trauma on organs with an initially high level of spontaneous proliferative activity.

To examine this problem it was decided to study how wide resection of the small intestine is reflected in information transmission by lymphocytes.

EXPERIMENTAL METHOD

Experiments were carried out on 38 male (August × Wistar Black)F_1 rats. Half of the intestine at a distance of 10 cm from the stomach was removed from rats which later acted as donors of lymphocytes. The animals were killed 17 h after the operation, a suspension of their spleen cells was made in medium 199 and centrifuged, viability of the lymphocytes was determined by the method in [1], after which they were transplanted into the femoral vein of intact syngeneic recipients in a dose of $4 \cdot 10^8$ cells. Rats receiving lymphocytes from animals undergoing mock operations (control recipients) and intact rats of the same age (intact control) acted as the control. All the animals were killed with chloroform vapor (at 9-10 a.m.) 48 h after transfer of the spleen cells. The small intestine (at a distance of 10 cm from

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