MORPHOGENESIS OF THICKENINGS OF THE INTIMA OBSERVED IN NONSPECIFIC AORTOARTERITIS

D. S. Sarkisov, E. G. Kolokol'chikova,
B. N. Varava, O. Yu. Printseva, and A. V. Tyurin

UDC 616.132-002-06:616.13-018.74-007.61-091

KEY WORDS: aortoarteritis, intima, morphogenesis.

The problem of thickenings in the intima of large blood vessels has been studied experimentally by many workers. Most of them have concluded that thickening of the intima, especially in atherosclerosis, is formed on account of smooth-muscle cells (SMC) which migrate into it from the media [7, 8, 10, 12-14]. These investigations have been conducted mainly by electron microscopy. The method of light autoradiography has been used in only three investigations [9, 11, 15], and their results have led to questioning of the view that the only source of formation of the thickened intima is SMC from the media. For instance, it has been suggested on the basis of experimental data that thickening of the intima takes place on account of capillaries invading it from the vasa vasorum [9]. This view is in harmony with the fact established previously that the basis of proliferative processes in different types of connective tissue consists of very small vessels and, in particular, the pericytes surrounding them. This has been shown by electron-autoradiographic study of the histogenesis of benign tumors [2, 4], osteogenesis after fractures [5], and hyperplasia of the intima of blood vessels in certain pathological processes [1].

The aim of this investigation was a further study of the nature of foci of proliferation of the intima characteristic of nonspecific aortoarteritis.

EXPERIMENTAL METHOD

Segments of the aorta, and also of the carotid, femoral, and renal arteries measuring from 0.5 to 2 cm, obtained during operations for nonspecific aortoarteritis on nine patients (eight women aged from 21 to 47 years and one boy aged 11 years) were investigated. Material for histologic examination was embedded in paraffin wax and sections were stained with hematoxylin and eosin, by Van Gieson's method, with toluidine blue, and for elastic fibers.

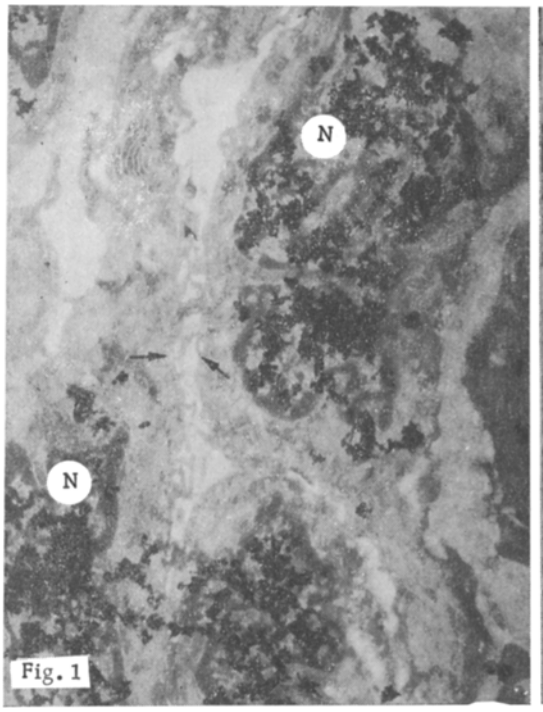
The shape of the cells in the vessel wall was studied by alkaline tissue dissociation. For this purpose the aorta was fixed, layer by layer (intima, media, adventitia) with 2.5% glutaraldehyde solution and then subjected to dissociation in a mixture of 33% KOH solution and 96% ethanol (1:1) for 2-3 h at 25°C. The single cells thus isolated were stained with methylene blue and the percentage of cells of different shapes determined (by counting 1000 cells). The results were subjected to statistical analysis.

Pieces measuring about 1 mm were excised for electron-autoradiographic investigation and incubated for 1.5 h at 37-38°C in medium 199 containing radioactive precursors of nucleic acids: ^{3}H-tymidine (specific radioactivity 21.6 Ci/mmole) in a dose of 20 μCi/ml or ^{3}H-uridine (26 Ci/mmole) in a dose of 100 μCi/ml. After incubation the fragments were washed with cold phosphate buffer, pH 7.4. The material was fixed in 2.5% glutaraldehyde solution and 1% OsO_4 solution and embedded in Epon. First, semithin sections were studied by light-microscopic autoradiography. Depending on the results of this analysis, regions for cutting of ultrathin sections were chosen in semithin sections. Electron-microscopic autoradiographs were prepared by the method described perviously [3] and studied in the JEM-100B electron microscope.

The distribution of the different types of collagen in the aortic wall in nonspecific aortoarteritis was studied by the indirect immunofluorescence method in freshly frozen sections (5-10 μ). Monospecific antibodies against collagens of types I and III were used as the first antibodies and antirabbit sheep IgG antibodies, labeled with fluorescein isothio-

A. V. Vishnevskii Institute of Surgery, Academy of Medical Sciences of the USSR. Institute of Experimental Cardiology, All-Union Cardiologic Science Center, Academy of Medical Sciences of the USSR, Moscow. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 102, No. 8, pp. 233-235, August, 1986. Original article submitted March 10, 1986.

 0007-4888/86/0008-1136$12.50



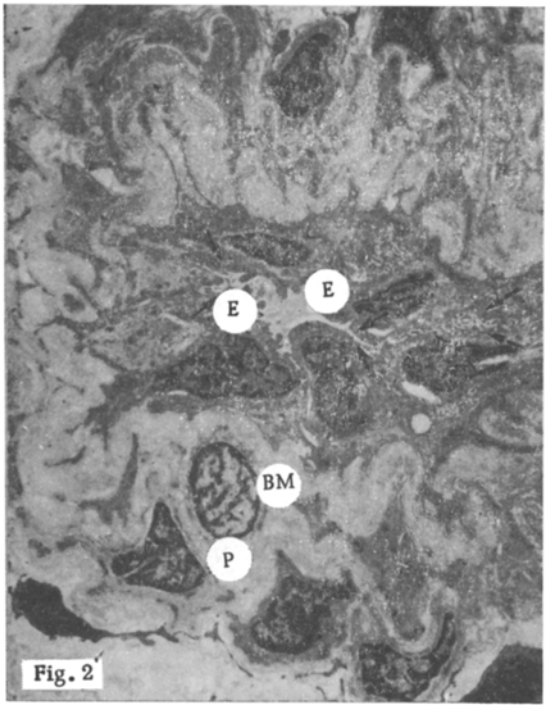


Fig. 1. Capillary in thickening of intima of carotid artery. Endotheliocyte nuclei (N) intensely stained with ^{3}H-uridine (black grains of silver). Lumen of vessel (arrows) constricted. 12,000 ×. Here and in Figs. 2 and 3: electron-microscopic autoradiograph.

Fig. 2. Vessel in thickening of aortic intima. Endotheliocytes (E) and pericytes (P) labeled with ^{3}H-uridine (black grains of silver). Well-marked basement membrane (BM). Lumen of vessel (arrows) greatly twisted and narrowed. 2000 ×.

cyanate (FITC) as the second antibodies. Sections were examined in the JCM-405 microscope (Opton, West Germany) with luminescence attachment.

EXPERIMENTAL RESULTS

Macroscopic examination of the arterial segments revealed narrowing of their lumen. As a rule, this was due to cushion-like growth of the intima. Histologic investigation of these growths revealed loose connective tissue, the cells of which were haphazardly arranged among amorphous ground substance, containing a few fibrous structures. Electron-autoradiography showed that the cellular composition of the cushion-like thickenings of the intima was heterogeneous. It included fibroblast-like cells, cells ultrastructurally resembling SMC, and forms transitional between the first two. Light-optical quantitative analysis showed that the majority of cells here were fibroblast-like (69.9 ± 4.5% of the total number), whereas in the media 73.1 ± 7.1% of the cells were SMC.

The space between the cells was filled with bundles of collagen fibers, surrounded by amorphous substance. Depending on the results of the immunomorphological study of the distribution of collagens of types I and III in the aortic wall in nonspecific aortoarteritis, mainly type I collagen was present in the intima and type III in the media.

Fibroblast-like cells are the most interesting, because it is in them that intensive RNA synthesis was found. Consequently, these functionally active cells are the source of formation of the intercellular substance, an excess of which leads to the development of thickening of the intima.

As was stated above, the view is widely held at present that SMC migrates from the media into the intima, where it can be converted into a "modified" SMC, which differs from the differentiated SMC in its well-developed synthetic apparatus. These cells can produce large quantities of intercellular substance, on account of which the intimal thickenings are formed.

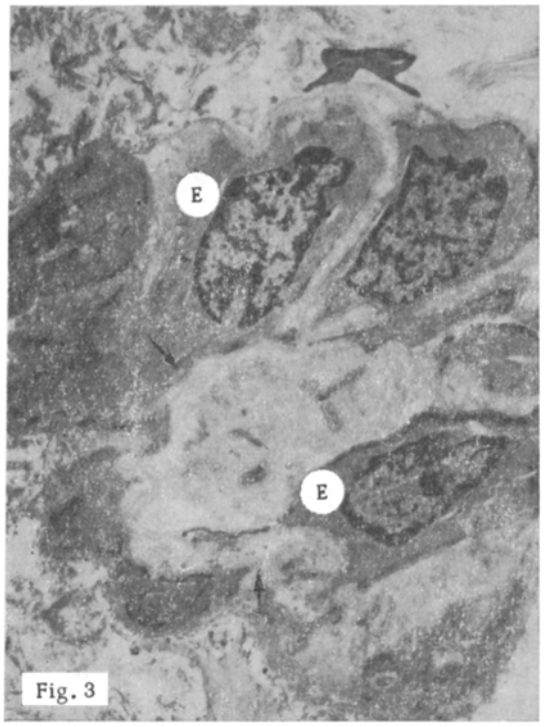


Fig. 3. Vessel in thickening of aortic intima. Lumen of vessel (arrows) dilated, endotheliocytes (E) shifted apart, their nuclei labeled with ^{3}H-uridine (small black grains of silver). 4000×.

There is no direct proof of this view, since migration of the cells cannot be observed directly. Moreover, we do not know whether SMC and fibroblasts in general are capable of migrating.

The writers have shown for the first time that among the cells and ground substance mentioned above there are many small vessels of capillary and precapillary type (Fig. 1). This suggested that cells of the intimal thickening appear there not on account of migration of SMC, but as a result of ingrowths of small vessels of capillary type, with their surrounding pericytes, which are converted into fibroblast-like cells that produce the intercellular substance. The presence of vessels in the thickened intima cannot be explained by migration of cells there from the media, for it is difficult to imagine migration of whole groups of cells, forming even the smallest vessel.

Larger vessels, with blood cells present in their lumen, also are found in the thickenings of the intima. Both endotheliocytes and pericytes of these vessels were well labeled with ^{3}H-uridine. Sometimes the vessel was the center around which many cells were concentrated (Fig. 2). The lumen of some of the vessels described above was filled with fibrillary material, and the cells forming the vessel were some distance apart and no longer formed a closed ring on transverse section, as is the case normally (Fig. 3). This suggests that the initial stage in the formation of intimal thickenings is ingrowth of very small vessels from the media into the intima. Cells forming the walls of these vessels produce interstitial fibrillary substances, as a result of which the structure of the vessel is disturbed and the cells forming it lie freely among bundles of delicate collagen fibers. It is this which makes migration of single cells into the intima here visible. The process described above can be traced only by electron microscopy, for in the light microscope the lumen of the very small vessels is indistinguishable, and the general impression is obtained that single cells and small groups of cells, unconnected with vessels, are present. This hypothesis is in harmony with the modern view that the various hyperplastic processes taking place in connective tissue are based on proliferation of cells which form the blood vessel wall, and in particular, pericytes [6].

LITERATURE CITED

1. B. N. Varava, O. Yu. Printseva, A. V. Tyurmin, et al., Arkh. Patol. (1986).
2. M. I. Kuzin, A. A. Pal'tsyn, A. A. Adamyan, and D. S. Sarkisov, Arkh. Patol., No. 6, 40 (1980).
3. D. S. Sarkisov, A. A. Pal'tsyn, and B. V. Vtyurin, Electron-Microscopic Autoradiography of the Cell [in Russian], Moscow (1980).
4. D. S. Sarkisov, A. A. Pal'tsyn, A. A. Adamyan, and E. G. Kolokol'chikova, Byull. Éksp. Biol. Med., No. 12, 100 (1983).

5. D. S. Sarkisov, B. M. Kostyuchenko, Yu. A. Amiraslanov, et al., Arkh. Patol., No. 2, 17 (1985).
6. A. V. Smol'yannikov, D. S. Sarkisov, and A. A. Pal'tsyn, Arkh. Patol., No. 1, 3 (1984).
7. J. Bhawan, I. Joris, U. Degirolami, and G. Majno, Amer. J. Path., 88, 355 (1977).
8. A. W. Clowes, R. E. Collazzo, and M. J. Karnovsky, Lab. Invest., 39, 141 (1978).
9. L. Diaz-Flores and C. Dominguez, Arch. Path. Anat. Abt. A, 406, 165 (1985).
10. J. R. Guyton and M. J. Karnovsky, Amer. J. Path., 94, 585 (1979).
11. O. Hassler, Lab. Invest., 22, 286 (1970).
12. P. R. Potvliege and R. H. Bourgain, Br. J. Exp. Path., 60, 382 (1979).
13. R. Ross and J. A. Glomset, New Engl. J. Med., 295, 369 (1976).
14. R. G. Schaub, C. A. Rawlings, and J. C. Keith, Am. J. Pathol., 104, 13 (1981).
15. W. S. Webster, S. P. Bishop, and J. C. Geer, Lab. Invest., 39, 370 (1974).

AUTORADIOGRAPHIC STUDY OF DNA SYNTHESIS IN BRONCHOALVEOLAR-LAVAGE MACROPHAGES IN CHRONIC INFLAMMATORY LUNG DISEASE

G. I. Nepomnyashchikh, V. N. Efremov, L. M. Nepomnyashchikh, and V. P. Tumanov

UDC 612.24-002.2-07:616.233].24-008.953.3-008.939.633.2

KEY WORDS: chronic inflammation of the lungs; bronchoalveolar-lavage; proliferation of alveolar macrophages; autoradiography.

Alveolar macrophages account for about 90% of the total number of inflammatory and immunoeffector cells of respiratory tissue and they play an important role in the development of many pathological situations, a fact that largely determines interest in the study of their structural and metabolic characteristics [1-3, 7, 13, 15]. The results of a study of material obtained by bronchoalveolar-lavage has shown the great mobility of these cells, on the basis of which a number of diagnostic and prognostic criteria have been developed. The dynamic character of the macrophages is expressed not only by the ease with which they penetrate into the lumen of the alveoli and from it into the air passages, but also by the considerable and rapid increase in the number of cells of this population in various experimental situations and pathological processes, and as is now well known, in smokers [13].

Experiments on animals have shown that the pulmonary macrophage population can be replenished quantitatively through proliferation of these cells in situ [8, 10, 11, 14, 16]. Attempts have been made to determine whether these cells can proliferate in human respiratory tissue [9, 12].

The aim of this investigation was to study the proliferative activity of human alveolar macrophages in chronic inflammatory diseases of the lungs by the use of a radioisotope method [4, 6].

EXPERIMENTAL METHOD

Bronchial washings were obtained from 23 patients (14 men and nine women) between the ages of 26 and 61 years. Fifteen patients had chronic bronchitis, four had a chronic lung abscess, and four had fibrocavernous tuberculosis of the lungs. Bronchoalveolar lavage was carried out during bronchoscopy, when the patients were anesthetized and artificially ventilated. In the presence of destructive inflammatory changes segments of lung tissue collateral relative to the focus of destruction were investigated, whereas in patients with chronic bronchitis, various segments were studied. A No. 7 cardiac catheter was passed through a Friedel's bronchoscope as far as the origin of the subsegmental bronchus and sterile physiological saline, heated to 37°C, was injected in two portions, each of 50 ml. The fluid was aspirated 5-10

Laboratory of Ultrastructural Bases of Pathology, Department of Pathomorphology and Morphometry, Institute of Clinical and Experimental Medicine, Siberian Branch, Academy of Medical Sciences of the USSR, Novosibirsk. Laboratory of Histochemistry and Autoradiography, Department of Pathological Anatomy, A. V. Vishnevskii Institute of Surgery, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR D. S. Sarkisov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 102, No. 8, pp. 236-240, August, 1986. Original article submitted February 4, 1986.

0007-4888/86/0008- 1139$12.50