IMMUNOHISTOCHEMICAL AND ENZYME-HISTOCHEMICAL CHARACTERISTICS OF INTIMAL CELLS OF ARTERIES IN NONSPECIFIC AORTO-ARTERITIS

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KEY WORDS: nonspecific aorto-arteritis; intima of the aorta

Nonspecific aorto-arteritis (NAA) is characterized by a lesion of the aorta at various levels, and also of its main branches. The morphology of the affected vessels differs depending on the stage and course of the disease. Infiltration of the adventitia and media by plasma cells and lymphocytes, destruction of the elastic carcase of the media, and hyperplasia of the intima are typical of the acute stage of NAA. In the chronic stage of NAA sclerotic changes predominate, with marked hyperplasia of the intima, and this is the main case of stenosis and occlusion of the lumen of the vessel [2, 5].

It has now been shown that focal hyperplasia of the intima of the great vessels in the NAA is due to the loose connective tissue, large numbers of cells, chiefly fibroblast-like, and numerous small vessels of the capillary and precapillary type [1]. However, the nature of the cells responsible for the formation of the intimal thickenings of NAA and for production of the extracellular matrix (ECM) is not sufficiently clear. The study of the enzyme activity in addition to antigenic cell markers and components of ECM could provide sufficiently complete information about the function of these cells and the degree of their differentiation.

The aim of the present investigation was accordingly to conduct an enzyme-histochemical and immunohistochemical study of the thickened intima of the aorta and its branches in NAA.

EXPERIMENTAL METHOD

Segments of the aorta and carotid artery, removed during operations for NAA (eight cases) were investigated. Sections were stained with hematoxylin and eosin and by Van Gieson's and Verhoeff's methods. Thiamine pyrophosphatase (TPP) activity was determined histochemically by the lead method of Novikoff and Goldfischer, and myosin ATPase activity was determined by the calcium-cobalt method of Padykula and Herman. Smooth muscle myosin, fibronectin, and collagen of types I, III, IV, and V were detected by indirect immunofluorescence, using specific polyclonal antibodies. The characteristics of the antibodies and the procedure of the immunohistochemical investigation were described previously [8. 9].

EXPERIMENTAL RESULTS

During histochemical investigation the main mass of the cells of the subendothelial layer of the thickened intima of the vessels with NAA showed high TPP activity (Fig. 1a). The latter also was observed in the endothelium and smooth muscle cells (SMC) of the media of vessels located in the thickened subendothelial layer. A characteristic feature of the subendothelial cells in NAA, besides the above, was a positive reaction for myosin ATPase (Fig. 1b).

The immunomorphological investigation showed that nearly all cells of the subendothelial layer of the intima of arteries with NAA, except cells of inflammatory infiltrates, contained smooth-muscle myosin (Fig. 1c). The shape, orientation, and number of subendothelial cells varied from case to case. Sometimes quite loosely and chaotically arranged long and branching cells were discovered in transverse sections through the intima. In some cases there was a definite orientation of these cells parallel to the lumen of the artery, with the formation of powerful bundles of cells. Another characteristic feature of these cells was that

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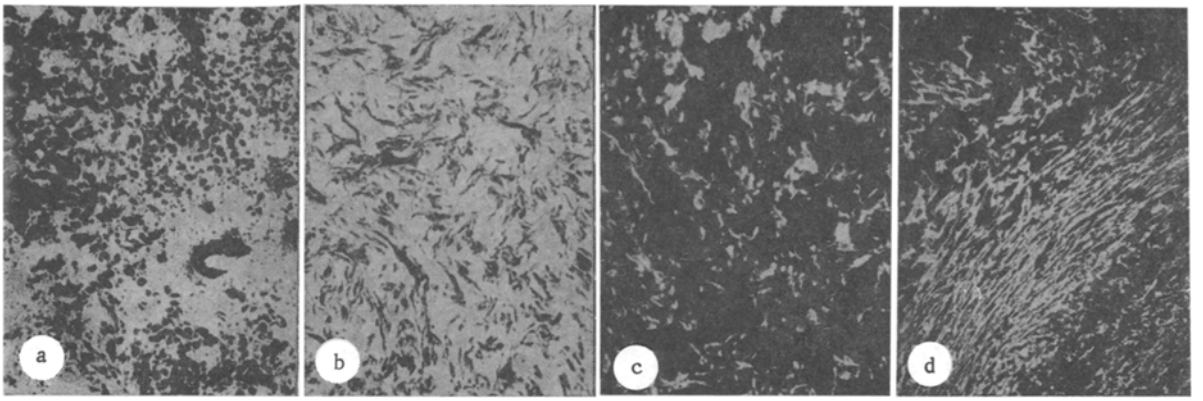

Fig. 1. Enzyme histochemical and immunomorphological characteristics of cells of subendothelial layer of thickened intima of carotid artery in NAA. a) High TTPase activity in cells of hyperplastic intima of carotid artery in NAA. Lead method of Novikoff and Goldfischer. 100 ×; b) Myosin ATPase in cells of thickened intima of carotid artery in NAA. Calcium cobalt method of Padykula and Herman. 150 ; c) smooth muscle myosin in chaotically arranged cells. 150 ; d) fibronectin around subendothelial cells arranged in bundles. 80 . a, b) Enzyme-histochemical investigation; c, d) indirect immunofluorescence method.

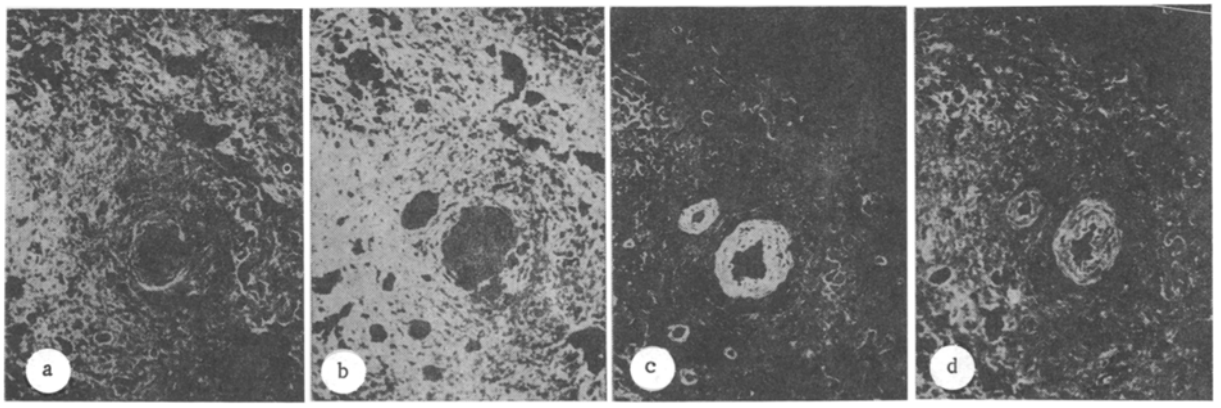

Fig. 2. Components of ECM in thickened intima of carotid artery in NAA. a) Very small quantity of type I collagen in ECM; b) large quantity of type III collagen in ECM; c) localization of type IV collagen around subendothelial cells and in walls of vasa vasorum; d) distribution of fibronectin in thickened intima. Indirect immunofluorescence method. Here and in Fig. 3: serial sections. 80 ×.

they were surrounded by fibronectin and collagen of type IV and, to a lesser degree, of type V (Fig. 1d).

Despite the differences in each concrete case, some general rules also were observed in the structure of ECM of the subendothelium of the thickened intima. A predominance of type III collagen was found in ECM and, as a rule, it contained less interstitial collagen of type I (Fig. 2a, b). The content of type V collagen varied: sometimes it was well represented in the thickened subendothelial layer of the intima. Fibronectin and type IV collagen could not be found actually in ECM, but they were localized only around the cells and in the walls of the numerous blood vessels located in the thickened intima (Fig. 2c, d). Depending on the presence of fibronectin and type IV collagen in the region of the basement membrane of the endothelium and around SMC in the subendothelial layer, vessels of varied caliber were clearly visible: they ranged from small vessels such as capillaries and arterioles to well-formed arteries of muscular type. Sometimes vessels could be seen to invade the intima from the adventitia through the injured media. Vessels of this kind had the appearance of slits lined with endothelium, in the region of the basement membrane of which fibronectin and collagen of types IV and V could be seen (Fig. 3a, b).

We know that cells in the subendothelial layer of the intima of arteries consist mainly of myofibroblasts or, as they are still called, modified smooth muscle cells [12]. These cells have lost part of their contractile apparatus and show signs of high synthetic activity, characteristic of fibroblasts. This was confirmed by the high TPP activity discovered

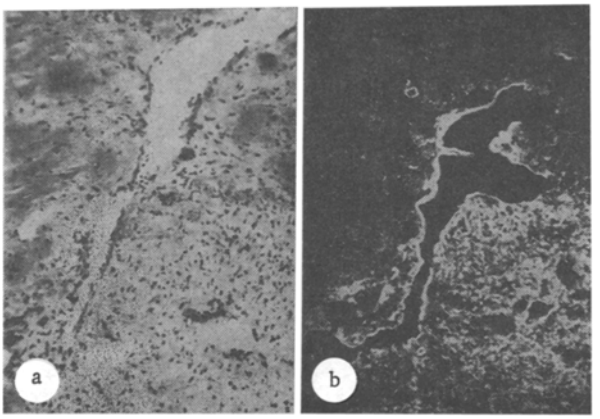

Fig. 3. Invasion of intima by blood vessel from adventitia of the carotid artery. a) General staining with hexatoxylin and eosin; b) type IV collagen in region of basement membrane of endothelium.

in cells of the subendothelial layer of the intima of arteries in NAA. Histochemical investigation revealed TPP in the cells mainly in the Golgi complex, and it is therefore regarded as a marker for that structure [14]. The presence of myosin of smooth muscle cells and of active myosin ATPase in the subendothelial cells indicates their genetic kinship with SMC. This is supported indirectly by the localization of collagen of types IV and V and of fibronectin around these cells, which is typical of SMC [8, 9]. The predominance of type III collagen in ECM of the subendothelial layer of the thickened intima in NAA also is more characteristic of SMC which, unlike fibroblasts, synthesize predominantly interstitial collagen of type III [10, 13].

The opinion is now widely held that SMC migrate from the media into the intima in response to its injury. This conclusion has arisen mainly on the basis of model experiments with diffuse intima thickening after denudation of the artery. Besides migration from the media, proliferation of undifferentiated cells, evidently precursors of modified SMC, preexisting in the subendothelial layer of the intima cannot be ruled out [3, 15].

It has also been shown that under certain experimental conditions thickening of the intima can be induced with active invasion of it by capillaries from the media [11]. Under these circumstances proliferation of pericytes surrounding the capillaries and their differentiation into modified SMC are observed. In the cases of NAA which we investigated, invasion of the intima by vessels from the adventitia and marked vascularization of the thickened subendothelial layer of the intima also were observed. The possibility therefore cannot be ruled out that pericytes accompanying capillaries and small vessels may act as precursor cells for the population of modified SMC of the injured intima in NAA. This is in agreement also with the results of electron-autoradiographic investigations [4, 6, 7], which indicate that pericytes surrounding very small newly formed vessels are the basis of proliferative processes in different kinds of connective tissue.

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QUANTITATIVE HISTOAUTORADIOGRAPHIC ANALYSIS OF CELL PROLIFERATION IN THE AORTIC WALL OF RABBITS WITH EXPERIMENTAL ATHEROSCLEROSIS

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Excessive deposition of apo-B-containing lipoproteins in the intima of arteries is regarded as a key phemomenon at the basis of atherogenesis [1, 2, 6]. Data obtained in recent years show that smooth muscle cells (SMC) and macrophages are quickly transformed into foam cells only after contact with aggregated or modified lipoproteins (LP), i.e., with modified particles [5]. Under these circumstances, an important place in the chain of events to be investigated is occupied by the problem of proliferation of the intimal cells that participate in assimilation of LP or in desmoplastic reactions. This process has not hitherto been studied, although a number of factors causing proliferation of cells of the vascular wall have been isolated [7]. Since the most convincing data, characterizing proliferation of cells of the vascular wall in atherogenesis can be obtained by the use of specific tritium labeling of the precursor of DNA synthesis, investigations of this kind are possible only by experiments using ^{3}H-thymidine.

The aim of this investigation was to study the character of proliferation of cells of the aortic wall in rabbits during the formation of atherosclerotic lesions against the background of hypercholesterolemia, and to determine the types of cells which participate in this process.

EXPERIMENTAL METHOD

Experiments were carried out on 49 male rabbits weighing 2.5-3 kg. Of this total number 32 animals were kept on an atherogenic diet (0.2 g cholesterol per kilogram body weight five times a week, in 5 ml of sunflower oil, given by gastric tube) for 1, 2, 3, 4, 6, 8, 12, and 16 weeks, five intact animals served as the control, and 12 rabbits were used in experiments with delayed labeling. For each of the times of the experiment studied, animals with approximately equal blood cholesterol levels were chosen. ^{3}H-thymidine (specific activity 19.8 Ci/mole) was injected intravenously in a dose of 0.3 μCi/g body weight into all the animals five times in the course of 24 h (at the end of the period on an atherogenic diet) at intervals of 6 h. The animals were killed 1 h after the last injection of the isotope. The aorta, cut longitudinally, was fixed in toto in Carnoy's fluid, and the material was prepared so that the whole aorta was accommodated on one section 3-4 μ thick. To determine whether the dividing cells could be transformed into foam cells experiments were carried out with delayed labeling. The animals were kept for 4 weeks on an atherogenic diet, after which they were given seven injections of ^{3}H-thymidine in the course of 36 h, at intervals of 6 h. Some of the rabbits were then killed, to serve as the control, whereas the remaining animals continued on the atherogenic diet for a further 4 weeks. Histoautoradiographs were prepared by the standard method, using Ilford K-5 emulsion, exposed for 18 days at 4°C, and then stained with hematoxylin and eosin. The number of labeled nuclei was counted separately for each type of lesion and for each zone of the atherosclerotic plaque, after which the index of labeled nuclei (ILN) was calculated. Low-density lipoproteins, labeled with ^{125}I (^{125}I-LDL), were in-

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