Lymphology 39 (2006) 76-83

THE ABSENCE OF LYMPHATICS IN NORMAL AND ATHEROSCLEROTIC CORONARY ARTERIES IN MAN: A MORPHOLOGIC STUDY

O. Eliska, M. Eliskova, A.J. Miller

Department of Anatomy (OE,ME), 1st Medical Faculty, Charles University, Prague, Czech Republic, and Cardiology Division (AJM), Feinberg School of Medicine, Northwestern University, Chicago, Illinois, USA

ABSTRACT

It has been suggested by various investigators that the impairment of lymphatic drainage from the coronary arteries may play a role in predisposition to coronary atherosclerosis, the pathogenesis of which is certainly multifactorial. In our study, no lymphatic vessels were found in the walls of the coronary arteries (adventitia, media and intima) in 51 human hearts from patients ranging in ages from 3 months to 83 years with normal coronary arteries, coronary atherosclerosis, and cardiomyopathy. Visualized lymphatics were located solely in the periadventitial area, and these lymphatics were more irregular in hearts from older persons. With injection, histology, and electronmicroscopy methods we could not detect penetration of lymphatics into the wall of coronary trunks in normal as well atherosclerotic arteries. In all coronary arteries studied, and particularly in the atherosclerotic lesions, blood vasa vasorum could be visualized. In the atherosclerotic areas, vasa vasorum (angiogenesis) could be seen penetrating into the media and intima. Many of the thin-walled vasa vasorum could easily be mistaken for lymphatics. The absence of lymphatics draining the epicardial coronary arteries may be a predisposing factor to coronary atherosclerosis.

Keywords: lymphatics of heart; coronary lymphatics; coronary artery lymphatics; coronary artery anatomy; coronary atherosclerosis; coronary artery histology

A number of investigators have suggested that the integrity of the lymphatic system draining the coronary arterial wall may have an important role in the lipid efflux mechanism and the occurrence of atherosclerosis in man (1-6). In man and other mammals, numerous reports have described grossly visible epicardial lymphatic collectors adjacent to epicardial coronary vessels (4,7-10). However, in histologic studies in the dog we found only scarce lymphatics in the outer adventitial layer of the coronary arteries (11), and data on the lymph drainage of the coronary arterial wall in man are scant. In the studies in man previously reported, lymphatics have been described as penetrating the adventitia and reaching the outer zone of the media (12-14). Johnson (12) emphasized that he saw no lymphatics penetrating the media.

Studies producing acute interference with the principal cardiac lymphatics in the dog have reported subsequent changes in the coronary arterial wall, including edema and infiltration with plasma proteins (3,5). Such studies have indirectly suggested the possibility of a significant lymphatic drainage in coronary arteries.

We wished to clarify the important question of lymphatic drainage of coronary arteries in man, the extent of such drainage possibly representing a major anatomical factor relating to the occurrence of coronary atherosclerosis. Coronary atherosclerosis is a remarkable skip disease with areas of atherosclerosis and plaque separated by essentially normal tissue, and we also wished to ascertain whether this characteristic might be related to the nature of possible lymph drainage from the arterial wall.

MATERIALS AND METHODS

We divided our study of human hearts into two groups.

Group 1

There were 32 human hearts in this group, from 20 males and 12 females. Eleven hearts from individuals 3 months to 32 years of age (3 hearts age 3 months to 4 years – the causes of death were sarcoma and lymphoma; 8 hearts age 5 to 32 years – traumatic accidents and suicides). Twenty one hearts were from individuals 40 to 83 years of age [6 hearts (traffic trauma), 6 hearts (suicides), 4 hearts (abdominal tumors), 5 hearts (acute infarcts)]. In all hearts, the lymphatics in the epicardium were injected with a mixture of 2 percent gelatin and India ink. The major trunks of the coronary arteries and veins were injected with 5 percent gelatin and colored dyes in half of the hearts studied. In four of the hearts (from individuals aged 40 to 70 years of age), the epicardial coronary artery was incised longitudinally and 0.1 ml of Gerota mass (Prussian blue and Iron ferrocyanide in oil mixed with terpentine) was injected to beneath the intima at the site of atheroma or fatty streaks. In another four hearts (from males aged 28 to 67 years of age), 0.1 ml of Gerota mass was injected into the adventitia and media. Lymphatics visualized in this way were then injected with India ink using a micropipette under the dissecting microscope. The specimens were studied under the dissecting microscope after fixation and clearing of the heart and by histology (see below).

Group 2

Eleven hearts, 8 males and 3 females, were obtained from patients ages 28 to 60 years undergoing cardiac transplantation. Eight patients 48 to 60 years of age had coronary atherosclerosis; three patients aged 28 to 36 years had a diagnosis of cardiomyopathy. Specimens were taken at various sites (see below) from the left and right coronary arteries in each of the hearts, all of which were studied by light and electron microscopy.

Handling of Specimens

Method of clearing

The heart was initially fixed in 10 percent formaldehyde and thereafter was dehydrated with increasing concentrations of alcohol. The injected epicardial lymphatics, arteries and veins and adjacent muscle were then cleared with methylsalicylate.

Light microscopy

Specimens from Group 1 were fixed in 10 percent formol saline. Transverse sections were stained with hematoxylin eosin blue. Masson trichrome, resorcin fuchsin (modification of Weigert method for elastic tissue staining) and azur fuchsin stains were utilized for elastic tissue staining.

Electron microscopy

The right and left coronary arteries from all hearts in Group 2 were perfused immediately with Karnovsky fixative solution (a mixture of formaldehyde and glutaraldehyde) (15) under pressure of 100-120 mm Hg after removing the heart from the thorax.

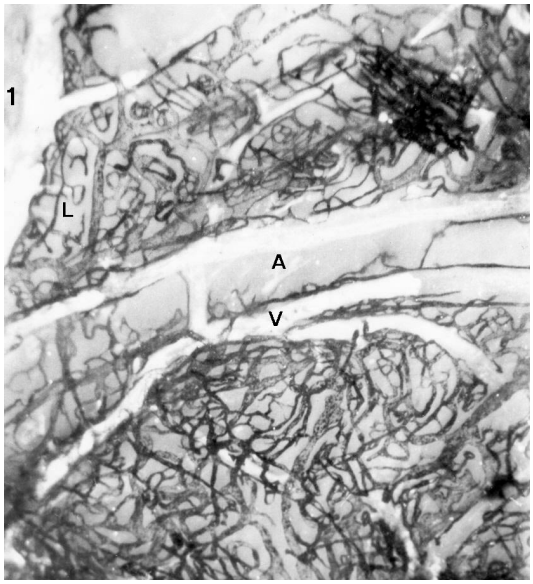

Fig. 1. Injected lymphatic vessels (L) accompanying the proximal part of the anterior interventricular artery (A) and veins (V). Female age 18 years. Cleared specimen. Artery and vein injected with distinguishing markers. Original magnification x 4.

Fig. 2. Injected lymphatic vessels (L) of irregular shape accompanying the proximal half of the anterior interventricular artery (A). Cleared specimen. Male aged 80 years. Original magnification x 4.

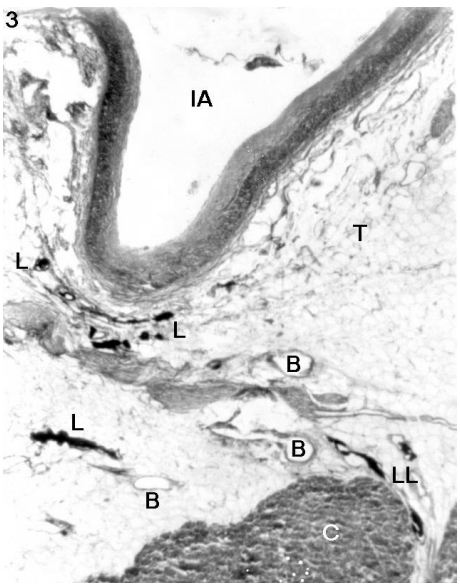

Fig. 3. Injected lymphatics (L) in the periadventitial tissue (T) of the interventricular artery (IA), without atherosclerotic changes. LL = lymphatics between bands of cardiac muscle (C). B = blood vessels. Female aged 64 years. Masson stain. Original magnification x 10.

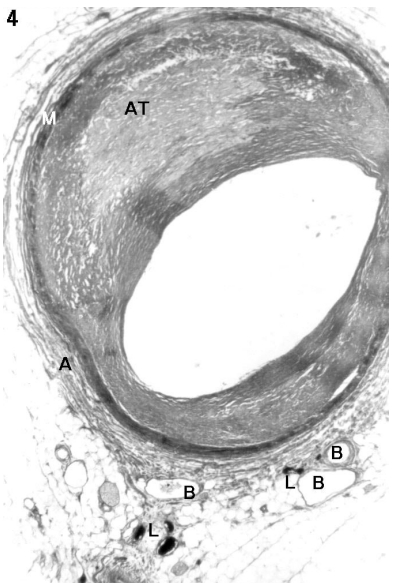

Fig. 4. Section of the interventricular artery, with atheroma (AT). M = media. A = adventitia. L = injected lymphatics in the periadventitial tissue. B = blood vessels. Female 64 years of age. Masson stain. Original magnification x 10.

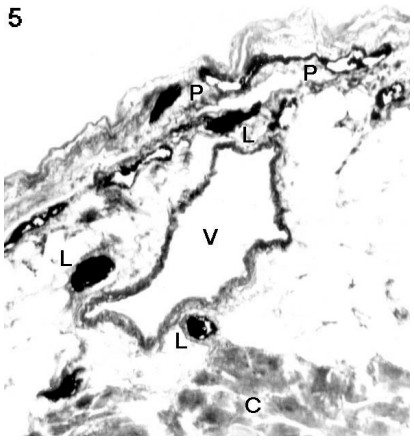

Fig. 5. Injected lymphatics (L) around the interventricular vein (V). P = Pericardial lymphatics. C = cardiac muscle. Female 50 years of age. Masson stain. Original magnification x 10.

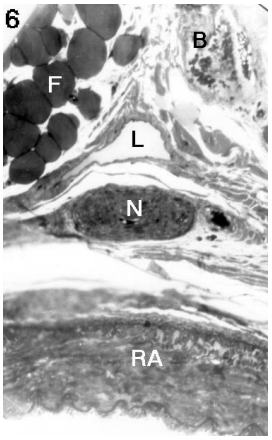

Fig. 6. Lymphatic vessel (L) in periadventitial tissue of right coronary artery (RA). N = nerve. B = blood vessel. F = fat cells. Male aged 49 years. Azur-fuchsin stain. Original magnification x 32.

Specimens were obtained from the proximal, middle and distal areas of the anterior interventricular, left circumflex and right coronary arteries. The sections for electron microscopy (Philips electron microscope) were fixed in Karnovsky fixative, post-fixed in 2 percent osmium tetroxide and embedded in araldite. Semithin sections were stained with toluidine blue and azur fuchsin. Thin sections were contrasted with uranyl acetate and lead citrate.

RESULTS

Group 1

As noted above, these specimens were studied with the dissecting microscope. As has been observed repeatedly by many investigators in the past, epicardial and subepicardial collecting lymphatics paralleled the right and left coronary arteries. However, smaller drainage lymphatics were seen only periadventially in loose connective tissue (*Figs. 1-5*). No lymphatics could be discerned in the adventitia itself. Lymphatics were absent on both the outer (ventral) and myocardial (dorsal) surfaces of the coronary arteries. A rich network of subepicardial lymphatics was identified adjacent to the outer adventitia of both the coronary arteries and veins, being more prominent near the veins. It was noted that the lymphatics were more irregular in hearts from older persons. No lymphatics were found in the coronary wall or periadventially in specimens obtained from sites (subintimal and adventitial) where the Gerota mass was injected adjacent to areas of coronary atherosclerosis. The injected material did fill the blood capillaries and the vasa vasorum visualized.

Group 2

These coronary arteries were studied from areas of varying severity of atherosclerosis (16) and from where the arteries appeared normal. The coronary arteries from the patients with cardiomyopathy showed minimal localized atherosclerosis, with rare localized macrophages and foam cells or fatty streaks. The coronary arteries from the patients with severe disease showed extensive atherosclerosis that varied from minimal to far advanced lesions, with essentially normal tissue in neighboring areas. With neither light microscopy or electron microscopy did we find any lymphatics in any layers of the

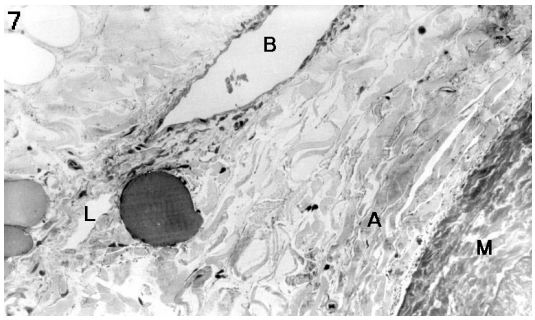

Fig. 7. Lymphatic vessel (L) in periadventitial tissue of circumflex artery, without atherosclerotic changes. B = blood vessel. M = media. A = adventitia of artery. Female aged 29 years. Semithin section. Original magnification x 64.

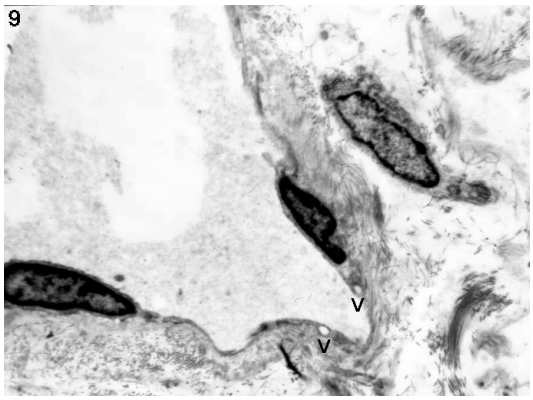

Fig. 9. Section of periadventitial lymph capillary of anterior interventricular artery with atherosclerotic (grade 4 and 5). V = vacuoles in the wall of the lymph capillary. Male, aged 55 years. Electron-micrograph. Original magnification x 2000.

coronary arteries at the normal or atherosclerotic sites. The lymphatics that were visualized were solely localized periadventially in loose connective tissue and never entered the adventitia of the artery itself (*Figs. 4,6-9*). In these latter lymphatics, the lumen was patent and no pathological changes were observed. Smaller lymphatics at 3 to 100 µm distance from the outer adventitial area measured 8 to 50 µm; larger lymphatics in the 100 to 1000 µm distance measured 60 to 400 µm.

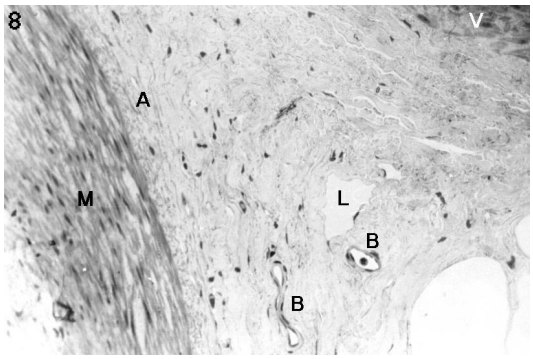

Fig. 8. Lymph capillary (L) and blood capillary (B) in periadventitial tissue of the atherosclerotic anterior interventricular coronary artery. M = media. A = adventitia of artery. V = vein. Male aged 57 years. Semithin section. Original magni-fication x 64.

In contrast to the absence of lymphatics, blood vessels were readily identified in the adventitia and in the outer zone of the media in the coronary arteries. In the areas of atherosclerosis, blood vessels could be found in the altered media and subintima. Vasa vasorum were frequently discerned in the more advanced atherosclerotic lesions. In the atheroma and fibroatheroma they entered the media and subintimal spaces as well as the margin of the fibrous cap.

DISCUSSION

It is generally accepted that coronary atherosclerosis is a multifactorial disease. The various factors that have been implicated in its pathogenesis include the mechanisms of extracellular lipid accumulation and leukocyte recruitment (17-19), inflammation and infection (20-23), wound healing reactions (24), cell apoptosis (25,26), collagen and fibrin deposition (27-29), hypoxia and vasa vasorum (20,30), high transmural pressure (31), and expression of tissue factors and genes (32). Two mechanical factors appear to be important in the development of atherosclerosis: (a) increased permeability of the endothelium to macromolecules, and (b)

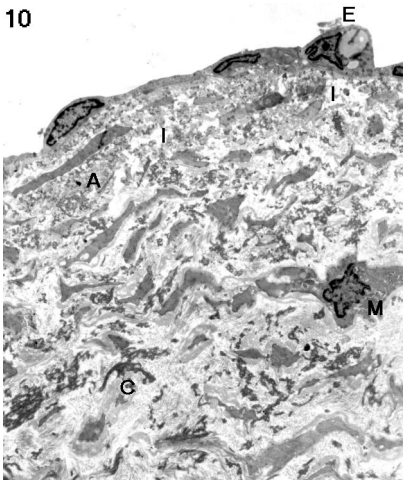

Fig. 10. Showing first two stages of atherosclerosis. Subintimal space is filled with modified muscle cells (M), collagen fibers (C) and amorphous substances (A). Interstitial edema is present (I). Endothelial cells (E) are flat and some of them are lipid laden. No lymphatics are present. Male, aged 51 years. Electronmicrograph. Original magnification x 2000.

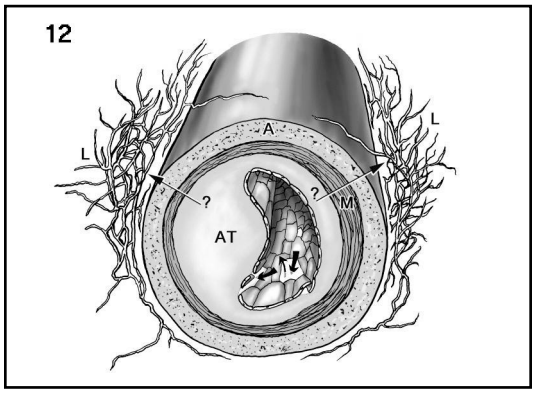

Fig. 12. Diagram of the coronary artery and periadventitial lymphatics. Lymphatics are located only in the periadventitial tissue. A = adventitia. M = media. AT = atheroma. Thick arrows show the possible morphological route of plasma proteins and lipids through interendothelial gaps from the lumen of the coronary artery to the subendothelial space. The thin arrow shows a possible morphological route of interstitial fluid from the subendothelial space through interendothelial channels to the lumen of the coronary artery. The long thin arrows show possible, but unlikely, drainage of interstitial fluid through the wall of the coronary artery to the periadventitial lymphatics.

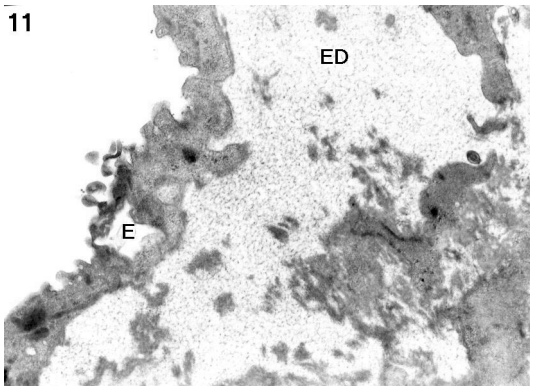

Fig. 11. Large subendothelial edema (ED) of anterior interventricular artery. No lymphatics are seen in the wall of the artery. E = endothelial cells with interendothelial channel. Male, aged 54 years. Electronmicrograph. Original magnification x 6800.

decreased filtration of these macromolecules through the thicker intimal and medial layers of the arterial wall. In exploring the multifactorial nature of coronary atherosclerosis, some investigators have stressed the influx-efflux aspects of lipid metabolism in the arterial wall (33). Noteworthy has been the relatively minimal attention paid the anatomical characteristics of the coronary arteries, though some important attention has been given the histologic anatomy (34).

Our studies showed no evidence of lymphatic vessels draining coronary arteries from normal segments or from atherosclerotic lesions. As has been described by other workers (35,36), we found microvasculature in the subintima and media of the atherosclerotic plaques. Such microvasculature of neovascularization typically extended from the adventitia, through the media, and into the thickened intima. It is very easy to confuse the presence of vasa vasorum with small lymphatics because many of the former are very thin-walled, and it is possible that this led previous investigators to describe these thin-walled vasa vasorum as lymphatics.

We consistently found myocardial lymphatics to be present only in the

periadventitial area, but never in the coronary adventitia itself. Our studies provide no evidence for the concept of lymph stasis leading to coronary artery wall edema or fibrosis. Drainage of significant protein and fluid amounts from the coronary wall to the periadventitial lymphatics appears anatomically unlikely, though myocardial edema could indirectly affect coronary arteries. We consider the absence of lymphatics draining the epicardial coronary arteries to be a factor predisposing to coronary atherosclerosis by virtue of the absence of a potential system for removing protein, fluid and lipids from the arterial wall. The epicardial coronary arteries are in a rather unique metabolic situation as compared to the intramural coronary arteries, with the latter surrounded by an extensive plexus of myocardial lymphatics. This may be one of the reasons why the intramural coronary arteries are so remarkably spared from coronary atherosclerosis except in the transplanted heart, where the cardiac lymphatic drainage is impaired (37,38).

ACKNOWLEDGMENTS

This research was supported by Research Project of Ministry of Education Youth and Sport 0021620807, Grant Agenture Czech Republic no. 0607, and by the Lymphatic Research Fund No. 785, Northwestern Memorial Hospital, Chicago, IL, USA.

REFERENCES

1. Lemole, GM: The role of lymphostasis in atherogenesis. Ann. Thorac. Surg. 31 (1981), 290-293.
2. Sims, FH: The arterial wall in malignant disease. Atherosclerosis 32 (1979), 445.
3. Jellinek, H: The role of transmural fluxes and vascular permeability in atherogenesis. In: *Atherosclerosis Biology and Clinical Science*. Ollson, AG (Ed.), Churchill Livingstone, Edinburgh, 1987.
4. Miller, AJ: *The Lymphatics of the Heart*. Raven Press, New York City, 1982.
5. Solti, F, H Jellinek; *Cardiac Lymph Circulation and Cardiac Disorders*. Akadedemiai Kaido, Budapest, 1989.
6. Miller, AJ, R Pick, LN Katz: The importance of the lymphatics of the mammalian heart: Experimental observation and some speculations. Circulation 29 (1964), 485-487.
7. Patek, PR: The morphology of the lymphatics of the mammalian heart. Am. J. Anat. 64 (1939), 203-250.
8. Aagaard, OC: *Les Vaisseux Lymphatique du Coeur Chez L'Homme et Chez Quelques Mammiferes*. Levin and Munksgaard, Copenhagen, 1924.
9. Feola, M, R Merklin, S Cho, et al: The terminal pathway of the lymphatic system of the human heart. Ann. Thorac. Surg. 24 (1977), 531-536.
10. Eliskova, M, O Eliska: Lymphatic vessels of the dog heart. Cor Vasa 16 (1974), 301-310.
11. Eliska, O, M Eliskova, AJ Miller: The morphology of the lymphatics of the coronary arteries in the dog. Lymphology 12 (1999), 45-57.
12. Johnson, RA: Lymphatics of blood vessels. Lymphology 2 (1969), 44-56.
13. Pappadia, F, GC Setti: The lymphatic drainage system of the great blood vessels in normal and pathologic and experimental conditions. Arteno Parmese 43 (1972), 133-135. cit after Berry, CL: Organogenesis of the arterial wall. In: *Diseases of the Arterial Wall*. Camilleri, JP, CL Berry, JA Fiessinger, et al (Eds.), J Springer Verlag, London, Berlin, 1989, p. 71-55-70.
14. Hansson, GK, J Nilsson: Pathogenesis of atherosclerosis. In: *Cardiology*. Crawford, MH, JP DiMarco, WJ Paulus (Eds.), Mosby, Edinburgh, London, 2004, pp 3-14.
15. Karnovsky, MJ: A formaldehyde-glutaraldehyde fixative of high osmolarity for use in electron microscopy. J. Cell Biol. 27 (1965), 137a.
16. Stary, HC: Natural history and histological classification of atherosclerotic lesions: An update. Arterioscler. Thromb. Vasc. Biol. 20 (2000), 1177-1178.
17. Libby, P: The vascular biology of atherosclerosis. In: *Heart Disease*, 6th edition. Braunwald, E, DP Zipes, P Libby (Eds.), WB Saunders, 2001, pp 995-1009.
18. Rainger, G, GB Nash: Cellular pathology of atherosclerosis. Smooth muscle cells prime cultured endothelial cells for enhanced leukocyte adhesion. Circ. Res. 88 (2001), 615-622.
19. Stary, HC, AB Chandler, RE Dinsmore, et al: A definition of advanced types of atherosclerotic lesions and a histological classification of atherosclerosis. A report from the Committee on Vascular Lesions of the Council on Atherosclerosis, American Heart

Association. Arterioscler. Thromb. Vasc. Biol. 15 (1995), 1512-1531.
20. Watanabe, T, S Haraoka, T Shimokama: Inflammatory and immunological nature of atherosclerosis. Internat. J. Cardiol. 54 (1996) (suppl): S25-S34.
21. Ross, R: Atherosclerosis – an inflammatory disease. N. Eng J. Med. 340 (1999), 115-126.
22. Vink, A, G Pasterkamp, M Poppen, et al: The adventitia of atherosclerotic coronary arteries frequently contains Chlamydia pneumoniae. Atherosclerosis 157 (2001), 117-122.
23. Singh, RJ, SA Mengi, YJ Xu, et al: Pathogenesis of atherosclerosis: A multifactorial process. Exp. Clin. Cardiol. 7 (2002), 40-53.
24. Beranek, JT: Atherosclerosis: A personal view. Cardiac Chronicle 5 (1991), 1-6.
25. Bauriedel, G, R Hutter, U Welsch, et al: Role of smooth muscle cell death in advanced coronary primary lesions implications for plaque instability. Cardiovasc. Res. 41 (1999), 480-488.
26. Kockx, NM, NWM Knaapen: The role of apoptosis in vascular disease. J. Pathol. 190 (2000), 267-280.
27. Gordon, D, MD Rekhter: The growth of human atherosclerosis: Cell proliferation and collagen synthesis. Cardiovasc. Pathol. 6 (1997), 103-106.
28. Rekhter, MD: Collagen synthesis in atherosclerosis: too much and not enough. Cardiovasc. Res. 41 (1999), 376-384.
29. Smith, EB: Fibrin deposition and fibrin degradation products in atherosclerotic plaques. Thromb. Res. 75 (1994). 329-335.
30. Gossl, M, M Rosol, NM Malyar, et al: Functional anatomy and hemodynamic characteristics of vasa vasorum in the walls of porcine coronary arteries. Anat. Rec. (part A) 272A (2003), 526-537.
31. Saul, GD, HM Gerard: Physical fitness, dynamic extra-arterial pressures, and the pathogenesis and distribution of atherosclerosis. Med. Hypotheses 36 (1991), 228-237.
32. Schwartz, SM, DDE Blois, ERM O'Brien: The intima: Soil for atherosclerosis and restenosis. Circ. Res. 77 (1995), 445-465.
33. Wolinsky, H: A proposal linking clearance of circulating lipoproteins to tissue metabolic activity as a basis for understanding atherogenesis. Circ. Res. 47 (1980), 301-311.
34. Sims, FH: A comparison of coronary and internal mammary arteries and implication of the results in the etiology of arteriosclerosis. Am. Heart J. 105 (1983), 560-566.
35. Zamir, M, MD Silver: Vasculature in the walls of human coronary arteries. Arch. Path. Lab. Med. 109 (1985), 659-662.
36. Desinan, L, M Rocco, S Pizzolitto, et al: Parietal centripetal and centrifugal thickening neovasculrization in the descending anterior coronary artery. Virchows Arch. A Pathol. Anat. 415 (1989), 211-217.
37. Miller, AJ, A DeBoer, A Palmer: The role of the lymphatic system in coronary atherosclerosis. Med. Hypotheses 37 (1992), 31-36.
38. Miller, AJ, AS Palmer, O Eliska, et al: Failure of the canine principal ascending epicardial lymphatic to regenerate after transection. Lymphology 32 (1999). 29-35.

Prof. Oldrich Eliska M.D., DrSc
Department of Anatomy
1st Medical Faculty
Charles University
Prague 2, U nemocnice 3
Czech Rep.
oldrich.eliska@Lf1.cuni.cz