

Cellular Mechanisms of Human Atherosclerosis: Subendothelial Intimal Cells

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INTRODUCTION

Pathogenetically relevant manifestations of atherosclerosis are linked to the development of plaques in the arterial wall. Many serious conditions, such as ischemic heart disease, stroke, renal hypertension, occlusion of blood vessels of lower limbs and other disorders associated with atherosclerosis develop as a result of dramatic decrease of the blood flow in strategically significant blood vessels [1-3].

Arterial wall is the site of atherosclerotic lesion development. The blood vessel wall structure is schematically presented on Figure 1. Arterial wall consists of 3 distinct layers. The innermost layer, which is located immediately below the endothelium, is called intima. Adjacent to intima is the layer called media. The outer layer of the arterial wall is called adventitia.

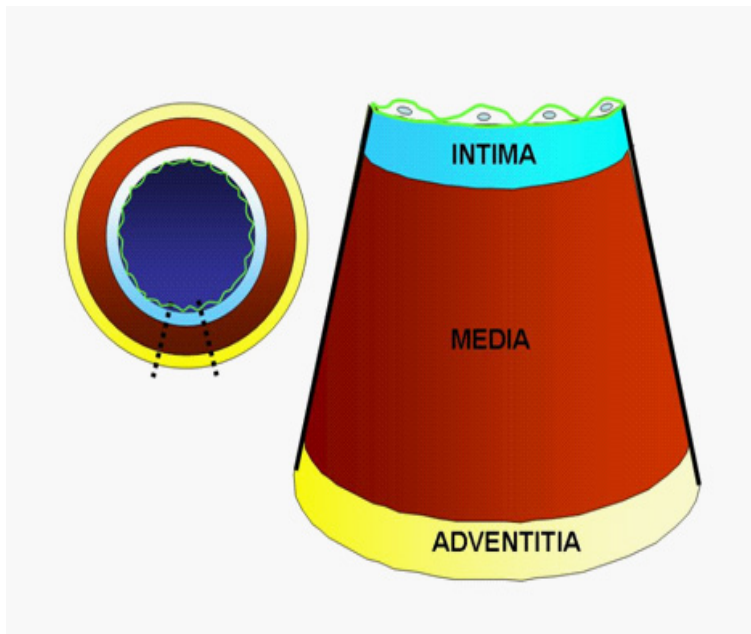


Figure 1: Schematic structure of the arterial wall.

Intima is the principal site of atherosclerotic lesion development. This layer is separated from the blood vessel lumen by a layer of endothelial cells, and from the media – by the internal elastic membrane (Figure 2).

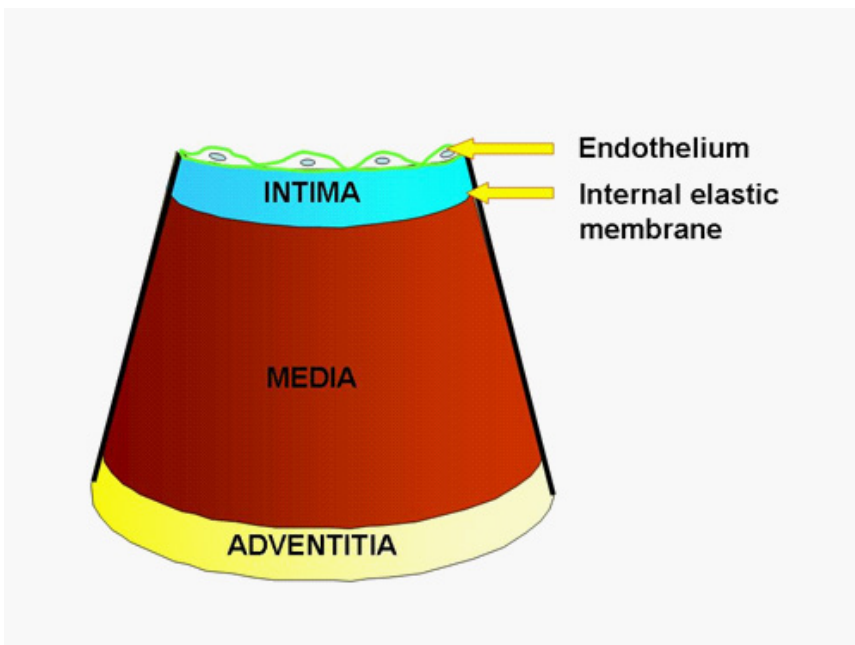


Figure 2: Morphological borders of the intima.

Atherosclerotic plaque is a local thickening of the intimal layer, protruding into the blood vessel lumen (Figure 3). It is the major cause of the blood supply decrease in vital organs associated with atherosclerosis. Atherosclerotic plaque has a lipid (atheronecrotic) core covered by the so-called fibrous cap [2,4]. The plaque contains mesenchymal cells surrounded by the extracellular matrix components, such as collagen and elastic fibres, fibronectin, proteoglycans, microfibrils and others [5-7].

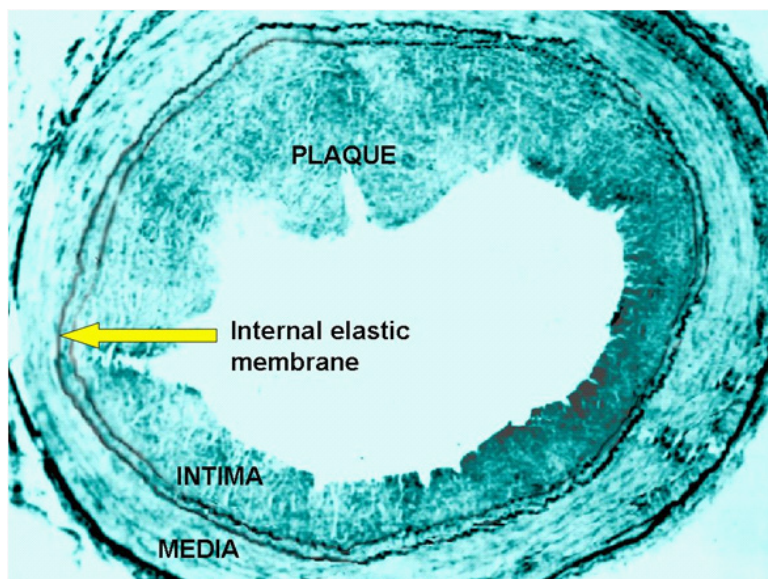


Figure 3: Section of aorta with an atherosclerotic plaque.

Despite the significant progress in studying the mechanisms of atherogenesis, many components of this complex process remain poorly understood. First of all, cellular mechanisms of initiation and progression of atherosclerotic lesion are not sufficiently studied. The following questions still have to be answered: 1) what is the cellular population of normal arterial wall and atherosclerotic plaque; 2) what changes take place in the arterial wall cells upon the development of atherosclerotic lesion; 3) what are the causes of these changes; 4) what is their role in atherogenesis. Detailed information on the role of mesenchymal cells of arterial intima in atherogenesis is missing. Furthermore, mechanisms of local lipid accumulation in the intimal layer of the arterial wall, which is currently considered as the primary cause of atherosclerosis, requires a more detailed study.

MODERN CLASSIFICATION OF ATHEROSCLEROTIC LESIONS

Main types of atherosclerotic lesions in human arteries are schematically presented on Figure 4. Hypothetical transition from one lesion type to another are indicated with arrows. Although such transitions cannot be proven by real-time observation, there exist currently no contradictory data that disproves this hypothesis.

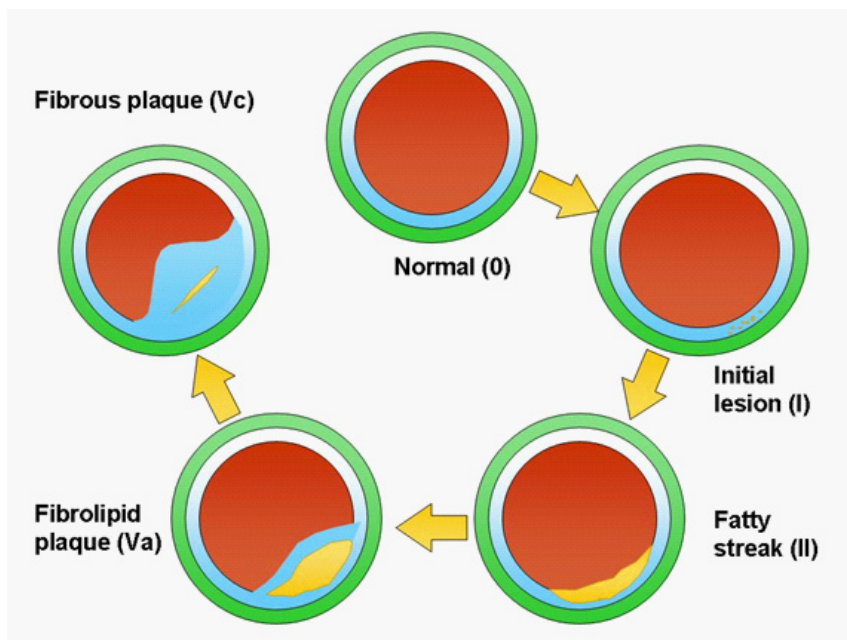


Figure 4: Main types of atherosclerotic lesions in human arteries.

Atherosclerotic lesion sites and uninvolved areas of the arterial wall can be distinguished macroscopically and then studied microscopically according to the classification of the American Heart Association [8-11]. Uninvolved areas of aorta have smooth luminal surface. Cross sections reveal two clearly definable layers of the intima: proteoglycan-rich layer next to the lumen and muscular-elastic layer adjacent to the media. Macroscopically, initial atherosclerotic lesions (type I lesions) are detected as areas with smooth yellowish surface, sometimes containing tiny yellow spots. Microscopic changes are minimal. Small lipid droplet deposits can be seen in the connective tissue matrix. Apart from the resident cells, initial lesion sites contain migrated mononuclear cells. Their amounts are increased in the lesions in comparison to macroscopically uninvolved intima. Occasional foam cells loaded with lipids can be seen. No disturbance of the tissue structure can be detected.

Fatty streaks (type II lesions) can be distinguished macroscopically as yellow streaks and dots slightly projecting above the surface of the blood vessel wall. Singular fatty streaks often form bigger clusters. Microscopic analysis of the cross-sections reveals mostly intracellular accumulation of lipids both in smooth muscular cells and macrophages. Extracellular lipids can also be seen in the connective tissue matrix. In some cases, hypertrophy of the extracellular matrix can be detected. Foam cells form layers and aggregations.

Fibrolipid plaques (type Va lesions) are detected macroscopically as yellow or opalescent, significantly protruding rounded structures on the luminal surface. Microscopic study reveals all the features observed in fatty streaks, including intracellular lipid accumulation and extracellular

matrix hypertrophy. At the same time, fibrolipid plaques can contain a massive necrotic core covered by a connective tissue cap. Shoulder areas of fibrolipid plaques morphologically resemble fatty streak lesions.

Fibrous plaques (type Vc lesions) are significantly protruding opalescent rounded structures made mostly of connective tissue matrix with embedded cells. Lipid component can be minimal or even absent.

Lesion types VI, VII and VIII are complicated atherosclerotic plaques with signs of rupture, thrombosis, haemorrhage or calcinosis. These types of plaques have an important clinical value to evaluate the patient's prognosis and represent final stages of atherosclerotic lesion development that can lead to fatal consequences. However, these types of lesions are currently less interesting for improvement of atherosclerosis prevention and therapy, as they provide little or no opportunities for therapeutic intervention [10]. Modern pathomorphological classification of atherosclerotic lesions adequately reflects the process of atherosclerosis development and allows distinguishing key stages macro- and microscopically. Importantly, this allows defining the therapeutic possibilities even at the subclinical stages of atherosclerosis, where no symptoms are present.

SUBENDOTHELIAL INTIMAL CELLS

Despite the fact that subendothelial intimal cells are being studied already for 120 years, their identification still remains challenging.

Currently, only the cell type from the muscular layer of arterial intima is fully characterized and identified. These are the smooth muscular cells, as has been suggested already by early researchers that first described and named this layer [12]. The development of morphological techniques led to further consolidation of opinion on the nature of these cells. Especially informative were the electron microscopy studies conducted in the 1960s. Cells forming the muscular layer of the intima have all the ultrastructural features of typical differentiated smooth muscular cells: limiting basal membrane, numerous myofilaments with dense bodies that fill the cytoplasm almost completely [13-15]. Cells of the muscular layer have elongated shape and are oriented along the blood vessel axis in close contact with each other forming therefore dense bundles (Figure 5).

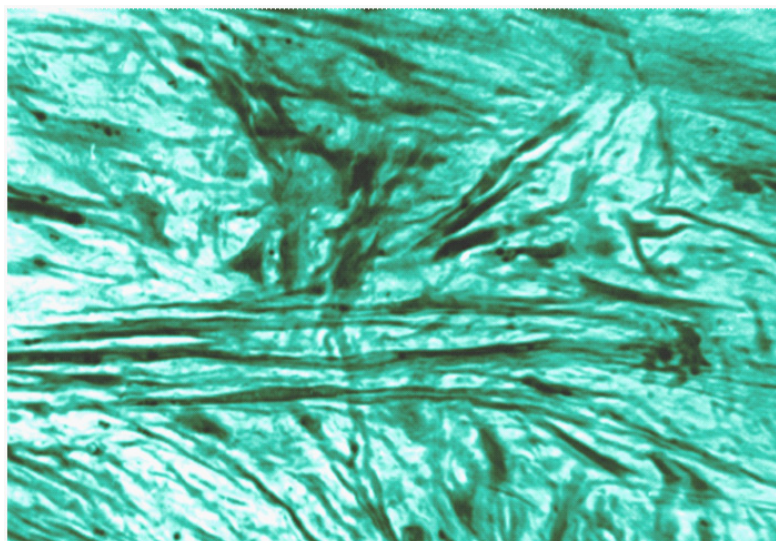


Figure 5: Muscular layer of human artery intima.

Using the method of alkaline dissociation of fixed aortic tissue, we could determine that cells populating this layer are morphologically homogeneous and closely resemble the smooth muscular cells of the media that have elongated bipolar shape [16]. Brought into suspension, dissociated cells from the media and the muscular layer of intima are barely distinguishable (Figure 6).

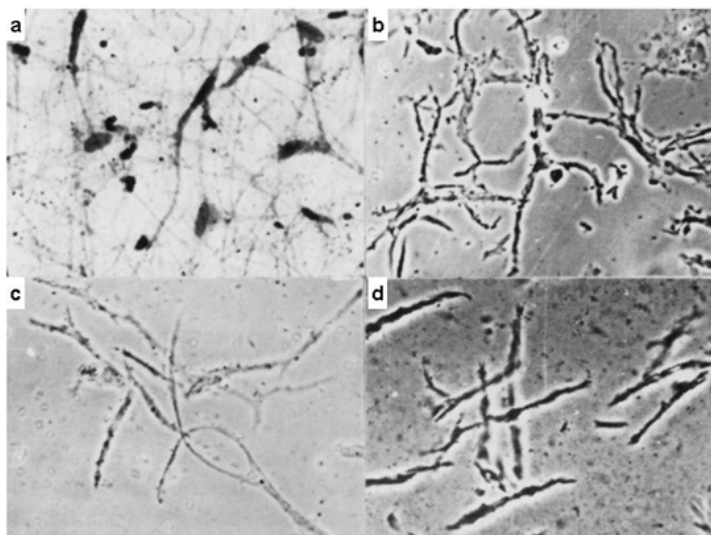


Figure 6: Polymorphism of human aortic cells.

- (a) Juxtaluminal layer. Thin section.
- (b) Suspension of cells from the juxtaluminal layer.
- (c) Suspension of cells from the muscular layer.
- (d) Suspension of cells from the media.

(a)-Haematoxylin, x 200.

(b-d)-Phase contrast. x 200.

Unlike the cellular population of the muscular layer, cells of the proteoglycan-rich subendothelial layer are morphologically heterogeneous [16]. Polymorphism of the subendothelial cells can be observed on thin en face preparations parallel to the endothelial lining (Figure 7).

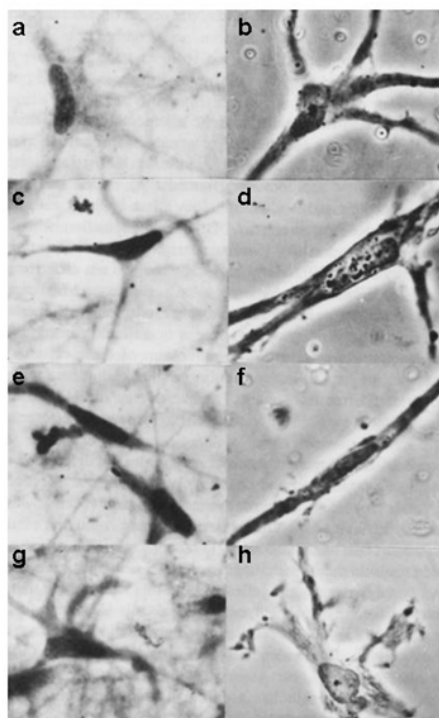


Figure 7: Morphological types of human aortic intimal cells.

- (a, b) – stellate cells;
- (c, d) – elongated cells with processes;
- (e, f) – bipolar elongated cells;
- (g, h) – irregular shape cells.
- (a, c, e, g) – thin sections, haematoxylin, x 500.
- (b, d, f, h) – suspension, phase contrast, x 500.

Such preparations allow distinguishing cells of various shapes: elongated cells with long and thin branches, stellate cells, with relatively small bodies and 3 to 12 and more long branching radial processes, irregular shape cells and ovoid cells (Figure 7). Branched and irregularly shaped cells are flattened, their processes being parallel to the endothelium. For this reason, conventional vertical sections are not suitable for studying the complexity of morphological forms of the subendothelial cells. On such sections, most cells have similar, more or less elongated shape.

The proteoglycan-rich layer is characterized by a less dense cell population than the muscular-elastic layer or the media. As can be observed on en face preparations, branches of subendothelial cells interconnect forming a cellular network in the intima. In such network, cells represent connecting «hubs». Areas surrounded by cellular processes can be observed as well. These areas penetrate the arterial wall towards the media like channels [17]. Channels formed by loosely distributed cells can be detected using scanning electron microscopy after complete removal of the extracellular matrix (Figure 8).

Morphological heterogeneity of the subendothelial cell population has been described as early as in 1866 by Langhans [18], whose name bear the cells populating the proteoglycan-rich layer [17,19,20] and sometimes the layer itself [21,22]. This observation was later confirmed by many other researchers [23-25]. However, the origin of the subendothelial cells still remains to be fully clarified.

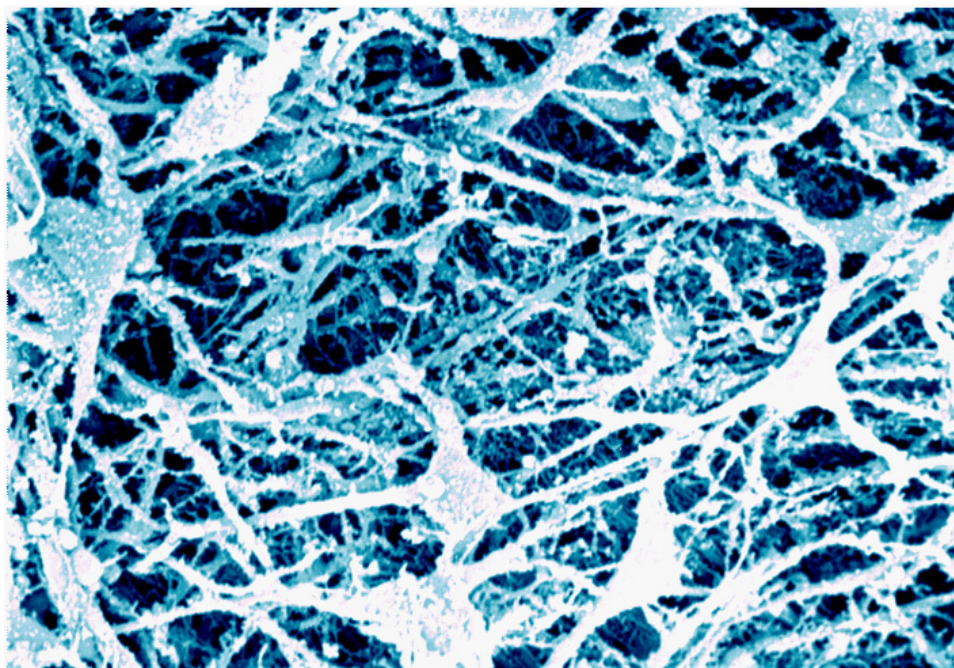


Figure 8: Cellular network of the proteoglycan-rich layer of human aortic intima. Vertical channels formed by cell branches can be seen. Scanning electron microscopy.

ORIGIN OF THE CELLS POPULATING THE JUXTALUMINAL LAYER

Identification of some of the cell types populating the subendothelial layer is straightforward. Round and ovoid cells are most probably inflammatory cells that penetrate into the intima from the circulating blood. Most often, they are localized immediately below the endothelial layer. These cells, for the large part, resemble blood lymphocytes by their size and morphological features. Another, less numerous population is comprised of mononuclear cells of various size and morphology, mostly of ovoid shape [20]. Morphologically, they are not different from blood monocytes [25]. These cells have no basal membrane. Few micropinocytic vesicles can be seen in the proximity of plasma membrane. The cells have numerous short finger-like cytoplasmic processes that reach into the surrounding connective tissue in all directions. The cytoplasm contains few organells, mostly smooth endoplasmic reticulum, mitochondria and free ribosomes. The nucleus is usually kidney-shaped, has a single nucleole and is filled with heterochromatin [25].

Inflammatory cells represent a small part of subendothelial cell population, never exceeding 5% in healthy intima [26]. The principal part of the subendothelial population is comprised of heterogeneous cells that have different shape and organell content. Since the first description of these cells by Langhans [18] these cells received various names: myointimal cells [27,28], multipotent and multifunctional mesenchymal cells [29], intermediate smooth muscular cells [30], intermediate cells [31], myoendothelial cells [32], modified smooth muscular cells [25,33], cambial subendothelial cells [34], intimacytes [35], Landhans cells [17,20], unidentified cells [36], primitive cells [30], poorly differentiated cells [37] and others.

The possible origin of these cells has long been disputed. Langhans, who was the first to describe the subendothelial cells, suggested that they could be fibroblasts or fibrocytes [18]. For a long time, researchers, making use of light microscopy technique, held the same opinion. Even 100 years later, cells from the subendothelial layer were classified as fibroblasts [38], fibrocytes, or histocytes and monocytoïd cells [39]. At the same time, other authors suggested that these cells originate from the transformed endothelial cells that took place below the endothelial lining and perform functions, unusual for the typical endothelial cells [23,32,40]. There have been also suggestions that subendothelial cells represent recently divided cells that subsequently differentiate either into smooth muscular or endothelial cells [30,36]. Some researchers described these cells as mesenchymal, intermediate between the fibroblasts and smooth muscular cells [20]. Another theory was that these cells derive from pluripotent fibroblasts or are pluripotent themselves and can transform into fibroblasts, endothelial and smooth muscular cells depending on the surrounding stimuli [23]. Ham and Cormack [41] described these cells not as resting fibroblasts, but as cells that can be transformed, when needed, into stem cells of cellular defence, endothelial cells, fibroblasts and macrophages. Shchelkunov classified them as undifferentiated elements of the organism's mesenchymal reserve [42].

Intruduction of the electron microscopy technique for morphological studies led to an opinion shift regarding the nature of the subendothelial cells. Geer [24] and Geer, Haust [25] described the ultrastructural features of these cells as follows.

Subendothelial cells have heterogeneous, usually stellate, shape. Cytoplasmic processes penetrate into the surrounding connective tissue, sometimes for long distances from the cellular body. Plasma membrane contains variable numbers of micropinocytic vesicles. Some cells have a partial limiting basal membrane. Cytoplasm contains scattered mitochondria, granular endoplasmic reticulum and free ribosomes. Granular endoplasmic reticulum cisterns contain thin filamentous material. Cytoplasm can contain dispersed small filaments resembling myofilaments of smooth muscular cells. Sometimes, they contain areas of increased electron density similar to dense bodies that can be found in myofilament bundles. Filaments are usually located within the narrow border zone in the proximity of the plasma membrane. Unlike typical smooth muscular cells, these cells contain more cytoplasmic organells and significantly less cytoplasmic filaments. Some subendothelial cells, usually of stellate or irregular shape, have no signs of smooth muscular cells and resemble fibroblasts and fibrocytes [24]. They have no limiting basal membrane. Few pinocytic vesicles can be seen in the proximity of the plasma membrane. The cytoplasm contains mostly membranes of smooth and granular endoplasmic reticulum.

Some authors, based on the observations of myofilament-resembling filaments and basal membrane adjoining plasma membrane in some subendothelial cells, argued in favour of smooth muscular nature of these cells [43]. Since ultrastructural elements of smooth muscular cells have been observed in these cells[13,14,15], the term “modified smooth muscular cells” became common for their identification. The significant differences in the morphology of these cell populations were explained as different stages of differentiation of the common cell type [25]. Currently, the opinion that the subendothelial cells have smooth muscular nature prevails [25,44]. Smooth muscular cells of the media are supposed to be precursors for these cells, and morphological changes are believed to occur upon migration of smooth muscular cells into the intima [45,46] .

It has been noticed previously that many subendothelial cells had a very different ultrastructure than typical smooth muscular cells. The prominence of the observed difference led some authors to the conclusion that these cells represent a distinct cell type in the intima [24]. Such possibility is currently not discussed. However, there is no evidence that would allow rejecting this theory. The presence of certain smooth muscular cell features in subendothelial cells does not contradict to a previously suggested model, according to which, these cells are pluripotent mesenchymal cells that can differentiate into fibroblasts, macrophages, endothelial or smooth muscular cells when needed [23,21]. This suggestion is grounded on the fact that, among the cells of the adult organism, there are examples of progenitor multipotent cells such as pericytes.

Pericytes can be found along the capillaries and small precalipary arterioles and postcapillaryvenules [47]. These cells have long and thin branching processes and often have

stellate shape. They wrap around the endothelial tubule of the small vessels, forming contacts with endothelial cells. In larger blood vessels, pericytes are substituted by smooth muscle cells. In contrast to smooth muscle cells, pericytes have a more diffused distribution of organelles and have no visible filaments and dense bodies in the cytoplasm. However, similar to smooth muscle cells, each pericyte is limited by a basal membrane [41]. It is known currently that smooth muscular cells are most probably formed from pericytes following the blood vessel enlargement [41]. It has been proposed that smooth muscle cell type is not the only possible direction of pericyte differentiation. Spontaneous bone formation in the connective tissue can be explained by the presence of pericytes that can differentiate into osteoblasts. The abovementioned authors argue that many of the dividing cells present at the regeneration sites in normal connective tissue (such as tendon) derive from pericytes. Likewise, adult adipose tissue contains precursors of adipocytes, and pericytes are the likely candidates for being such precursors. Moreover, it has been suggested that mast cells derive from cells that retain to a significant degree their mesenchymal potential. Here again, pericytes are the likely candidates for being such cells [41]. In certain pathologies, such as haemangioma, pericytes acquire some ultrastructural features of endothelial cells, smooth muscular cells or fibroblasts. As a result, lesion sites contain fibroblast- endothelial- and smooth muscle-like pericytes [48]. Ultrastructural characteristics, typical stellate shape and juxtaendothelial location are characteristic features of subendothelial cells of human aorta, which leads to the possibility of a link between these cells and pericytes.

The hypothesis suggesting the presence of multipotentmesenchymal cells similar to pericytes in the vascular wall of adult humans has been formulated long ago. Shchelkunov [22] distinguished subendothelial cells that form the cambial layer of the intima, which produces all the tissue elements both in the direction of the outer surface of the vessel (the media) and towards the inner surface and endothelium. He postulated that this layer can be regarded as the remainings of the subendothelialmesenchymal layer, which is present during the embryonic development. The outer part of this layer differentiated into smooth muscular and connective tissue elements of the vessel. According to Shchelkunov, endothelial cells lining the blood vessel walls have limited lifespan and are substituted by subendothelial cells at younger developmental stages. He linked these cells to all the hyperplastic processes in the intima. These cells were regarded as the source of rapid tissue neogenesis in endarteritis and various hypertrophies including atherosclerosis. They actively participate in blood vessel remodelling from the early until the old age.

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