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Intracellular transports and atherogenesis

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1. ABSTRACT

There is a great progress in understanding the cellular and molecular aspects of atherosclerosis, which is one of the leading causes of death. Yet, there are questions regarding the cellular and metabolic mechanisms that lead to atherogenesis. Among the many factors that influence this process, food plays a significant role. Among other factors that play a paramount role in atherogensis are alterations of the transport of food in enterocytes, oxysterols, development of an atherogenic serum, endothelial damage, accumulation of foam cells within the vessel wall, lysosome-ER transport, and hypertension. Here, we discuss the contribution of secretion, transcytosis, endocytosis of chylomicrons, low-density lipoproteins (LDL), very LDL, and high-density lipoproteins to atherogenesis.

2. INTRODUCTION

In 1904, Marshand described the accumulation of lipids in atherosclerotic plaques and proposed the name "atherosclerosis". In 1913, Anichkov (Anitschkow) and Chalatov discovered the main role of cholesterol in the development of atherosclerosis (1-4). Later on, atherosclerosis was studied in the following areas:

1. Animal experiments on the basis of animal experimentation;
2. Studies of different type of family hypercholesterolemia;
3. Analysis of newly created genetic models of atherosclerosis in animals;
4. Epidemiological studies of the relationship between human diet and atherosclerosis.

5. Study of the effect of drugs with a known molecular mechanism of action on a large number of treated people;
6. Study of cells obtained from people with the earliest manifestations of atherogenesis in test-tube (Orekhov's and Smirnov's groups from the Russian Cardiology Centre in Moscow).

There are many excellent reviews on this topic including the role of inflammation and mitochondria damage (5-7). On the other hand, this disease is not of purely of genetic origin (8-10). Therefore, here we will focus our attention only on a few aspects of atherogenesis related to intracellular transport and present here only a very brief analysis.

Secretion, transcytosis and endocytosis of low density lipoproteins (LDL), very LDL (VLDL), high-density lipoprotein (HDL), and chylomicrons (ChMs) are important for atherogenesis because most tissue cells (cells of nervous system and hepatocytes are exclusions) do not synthesize cholesterol and need to acquire this lipid from the blood apolipoproteins. In hepatocytes, cholesterol and free fatty acids (FFAs) are synthesized in the cytosol and then on the cytosolic leaflet of the membrane of the smooth endoplasmic reticulum ER (SER). VLDLs are formed in the lumen of the SER. Apolipoprotein (Apo) B is synthesized in the granular ER (GER), diffuses to the SER and extracts cholesterol and FFAs from the SER membrane generating VLDL. These particles are formed inside the lumen of the SER near the border between the SER and GER. Assembly of VLDLs at SER is regulated by a chaperone called microsomal triglyceride transfer protein (11).

In intestine, bile acids emulsify fats and form micelles. The micelles pass through the glycocalyx of the apical PM and come into contact with the apical plasma membrane (APM). There, the FFAs and cholesterol from the micelles move inside the APM and diffuse towards the tight junctions where their diffusion is stopped by these junctions. Transfer proteins able to deliver FFAs and cholesterol from one bilayer to another act only in the cytosol. Therefore, cholesterol and FFAs should jump from the outer monolayer of the APM to the inner (cytosolic) leaflet of this membrane. Then they reach the place where the membrane of the cisternae of the SER cisternae is tightly attached to the basolateral plasma membrane (BLPM) of the enterocyte and form two-dimensional network. This attachment is observed just below the belt of adhesive junctions (12).

After the ApoB-dependent formation ChMs or VLDL inside the lumen of SER of enterocytes or hepatocytes these particles are transported towards the Golgi complex (GC; 13). The role of COPII in this process remains enigmatic. At least COPII-coated buds and typical ER exit sites were found in neither enterocytes nor hepatocytes. In the GC, ApoB acquired glycosylation pattern. During this transport Ca2+ is released from the ER and the GC (14). The particles passed through the GC and reach the BLPM. Nascent VLDL released from the liver contains ApoB100, ApoC1, ApoE, cholesterol, its esters, and triglycerides. VLDLs are secreted into the Disse's space and through the pores in endothelial cells (ECs) of hepatic sinusoids VLDLs reach the blood. In enterocytes, after glycosylation of pre-ChMs, these particle become ChMs. ChMs are delivered to the BLPM, appeared between enterocytes and then are formed to go to the basement membrane (BM) and through its pores to interstitium. Then, ChMs are absorbed by lymphatic capillaries (15) and after ChM passage through the lymphatic system are delivered to the blood.

In blood, ChMs and VLDL come in contact with lipoprotein lipase presumably localized on the APM of ECs. This lipase removes triglycerides from VLDL. As more and more triglycerides are removed from VLDL and ChM, VLDL becomes intermediate-density lipoprotein and then LDL whereas ChM is transformed into ChM remnants. VLDL now meets back up with HDL where ApoC-II is transferred back to HDL (but keeps ApoE). HDL also transfers cholesterol esters to the VLDL in exchange for phospholipids and triglycerides via cholesterol-ester transfer protein. As more and more triglycerides are

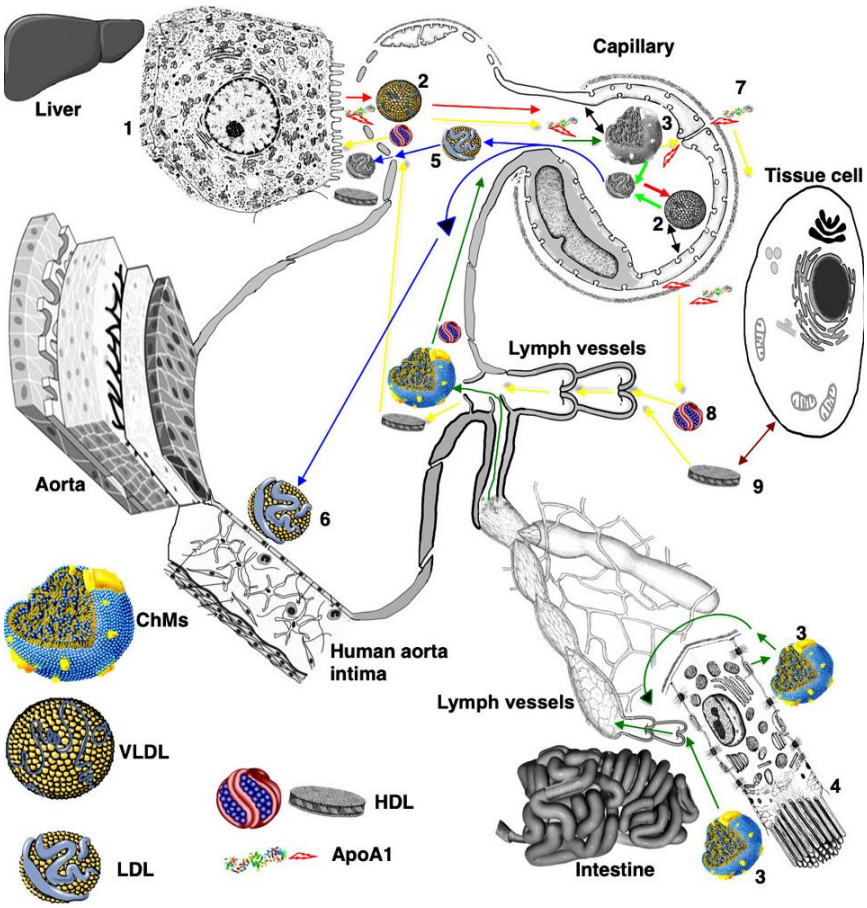

Figure 1. Scheme of lipid circulation in the human body. Hepatocyte (1) synthesizes VLDL (2), which moves into the blood through pore in endothelial cells of liver sinusoid (red arrows). Next, VLDL arrives to the blood capillary (Capillary) with continuous endothelium and gives cholesterol to the APM of ECs. Unfolded ApoA1 is synthesized by hepatocytes and also arrived to this capillary (yellow arrows). It passes endothelium through intercellular contacts and appears in interstitium. There, it contacts with the BLPM of ECs and takes cholesterol there forming HDL. ChM (3) is formed by enterocyte (4). It is transported to interstitium and then to the lumen of lymph capillary and delivered to the blood capillary lined with the continuous endothelium dark green arrows). In the capillary, VLDL and ChM contact with the APM (black double-sided arrows) and insert cholesterol and fatty acids into it. After this, ChM and VLDL are transformed into LDL (5), which are delivered to hepatocytes passing through pores in sinusoidal endothelium (blue arrow). Finally, LDLs are taken by LDL receptors on the PM of hepatocytes. Part of LDL (6) contacts with ECs, lining aorta in the areas of altered flow pattern and can pass into intima there through leaky contacts. Apo1 passes endothelium through intercellular contacts or using transcytosis and appeared inside interstitium (7). It contacts with the baso-lateral PM of ECs and takes cholesterol and fatty acids from it. Next, HDL interacts with the PM of tissue cell and takes cholesterol when it is necessary. Then, ApoA1 is transformed into HDL of two types (8, 9). After this, HDLs are absorbed by lymph capillaries and delivered to the blood (yellow arrows). Finally, HDLs pass through pores (yellow arrow near 1) of sinusoidal endothelium and reach scavenger receptors of the PM of hepatocytes (1). Abbreviations: APM, apical PM; ChM, chylomicron; HDL, high-density lipoprotein; LDL, low-density lipoproteins; PM, plasma membrane; VLDL, very LDL.

removed from the VLDL because of the action of LPL and CETP enzymes, the composition of the molecule changes, and it becomes intermediate-density lipoprotein. Close proximity of an HDL particle to the membrane leads to immediate transfer of cholesterol. Red blood cells are rich in cholesterol and act as exceptionally potent acceptors for cholesterol, as shown using cholesterol-loaded mouse peritoneal macrophages. In humans, red blood cells account for about 45% of the blood volume and their cholesterol concentration is comparable to that of circulating lipoproteins. Phospholipids represent about 60–70% of total red blood cells lipids, while about 25% are free cholesterol. Finally, LDLs and HDL enter the liver

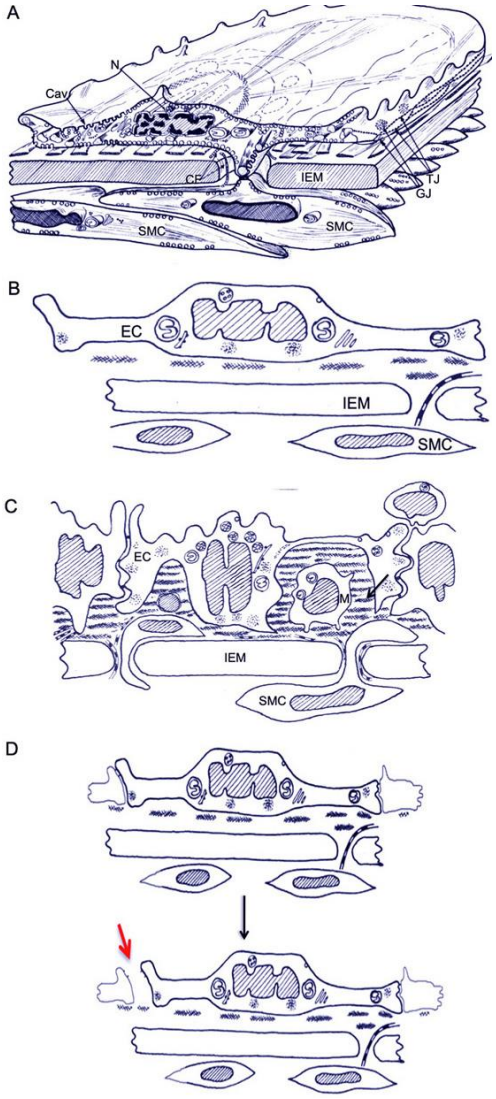

Figure 2. Scheme of the structure of aorta intima in rats (A, B) and its alteration in hypertensive animals and in area of (C, D). (B) Simplified scheme of the aortic intima. (C) Accumulation of multilayered basement membrane (arrow) below endothelial cells. Movement of SMC from media into intima (thick arrow). Macrophages (M) in intima. (D) Effect of deregulation of endothelial contractility through eNOS and other stimuli. Excessive EC contraction could open tight junctions (to the right). Red arrow shows the open contact between ECs. Abbreviations: Cav, caveola; CF, collagen fibrils; EC, endothelial cell; GJ, gap junction; IEM, internal elastic membrane; M, macrophage; N, nucleus; SMC, smooth muscle cell; TJ, tight junction.

through corresponding receptors to be subsequently degraded into FFAs and cholesterol (16) (Figure 1).

In endocardium, arteries, venules, veins and lymphatic vessels, capillaries of lymph nodes, heart, lung, esophagus, thymus, ovaries, dense bone, connective, connective and adipose tissue (17), and nervous tissues (neurons and glia), blood capillaries contain continuous (closed) endothelium (18) (Figure 2, 3). ECs in secreting mammary gland are of closes type (19). In kidney peritubular capillaries of the cortex and medulla of suprarenal glands, pituitary gland, intestine, thyroid glands, endothelium is fenestrated. In capillaries and endothelium is fenestrated. In kidney glomerular capillaries, endothelium is porous. In liver sinusoids, endothelium is discontinuous (18).

3. ATHEROGENESIS

3.1. Models of atherogenesis and epidemiology

Models of atherosclerosis are based on mice, rats, rabbits, hamsters, guinea pigs, pigeons, chickens, quail, other birds, swines, cats, dogs, and non-human primates (20-28). Mice, rats, cats and dogs have a high level of HDL and it is very difficult to induce hypercholesterolemia or atherosclerosis in these animals. Pigs and monkeys are better suited to model human atherosclerotic lesions (2,29,30). Watanabe heritable hyperlipidemic rabbits express non-functional LDL receptors, which recognize some VLDL remnants, but not LDL. In the rabbit blood, LDL and VLDL remnants are accumulated whereas the metabolism of ChM is normal (31). Mice without ApoE, LDL receptors or scavenger receptors are sensitive to atherosclerosis (6,32-36). The scavenger receptor B1–deficient mouse accumulates very large HDL particles in the circulation (37). ApoE- and LDL-receptor double - knockout mice have been created (35). However, usually under normal diet, the lesions do not develop beyond the early foam-cell, fatty-streak stage (29,30). For instance, CD36/SRA double knockout mice contain lipid-laden macrophages in vessel wall atherosclerotic plaques (9,36,38). In ApoE-/- mice, SMCs in atherosclerotic plaques are exclusively derived from the local vessel wall not from STEM cells (39).

On the other hand, studies of familial hypercholesterolemia (FH) revealed that problems with LDLR account for 80–85% of FH cases,

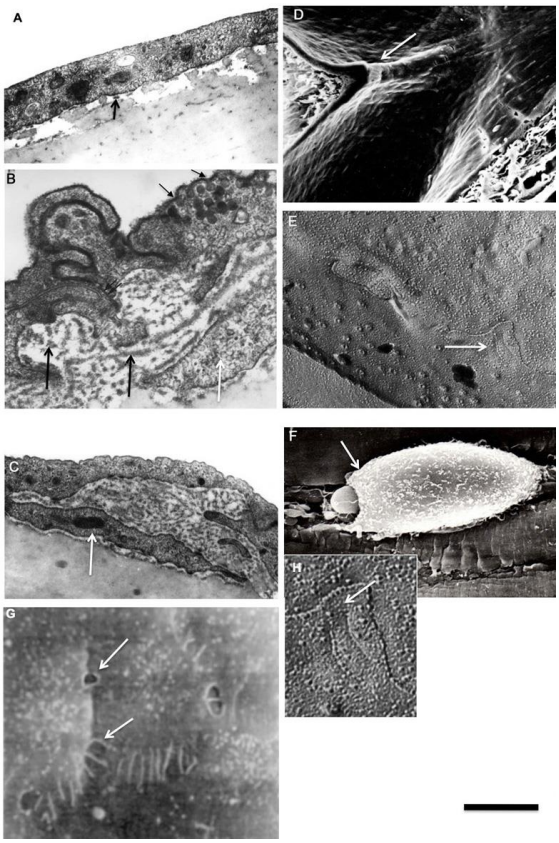

Figure 3. Ultra-structure of atherosclerosis (originals). (A) Structure of aortic endothelium under normal conditions. BM (arrow) is perforated. (B, C) Structure of intima in the area of hemodynamic stress. Accumulation of layers of the BM below endothelial cells (black arrows). Pericyte-like cell (white arrow) is present below ECs. (B) Ruthenium red stains caveolae. (D) Divider of the blood blow in rat aorta. Scanning electron microscopy (SEM). (E, H) Platinum replicas of endothelial cell prepared after freeze-fracture. White arrows show the opening within rows of intramembrane particles forming tight junction. (E) Evaporation from low-left corner. (H) Evaporation from the right side. (F) During mitosis inter-endothelial contacts became open and platelets attached to BM. (G) SEM of open contacts (white arrows) between ECs at the sites of altered flow pattern according to Cunningham and Gotlieb (2005) in the human aorta (sample from Kolpakov *et al*., 1996). Scale bars: 700 nm (A, E); 350 nm (B, H); 1.4 μm (C); 1 mm (D); 4.7 μm (F); 1.8 μm (G); 350 nm (H).

impairment of ApoB100 function cause 5–10% of the cases, PCSK9 – 2% of the cases and LDL receptor adaptor protein 1 accounting for less than 1% of the cases. Mutations in ApoE, signal transducing adaptor family member 1, lysosomal acid lipase, ABCG5 or ABCG8 genes can also generate the FH-like phenotype, but its frequency is very low (40,41). The sub-endothelial retention of atherogenic apoB-containing lipoproteins is the initiating event in atherogenesis (42). LDL-derived cholesterol accumulates in macrophages to form foam cells (9). EC damage and accumulation of LDL promote the recruitment of monocytes (43), which migrate into intima, absorb LDL and form foam cells.

3.2. Initial events

According to the current consensus, accumulation of lipoproteins in the arterial intima is a key element in the onset and development of atherosclerosis. The initial step is a fatty streak lesion due to accumulation of macrophages inside intima. Then plaques progress to advanced lesions, composed of lipid droplets, foam cells, macrophages, and lymphocytes (4). These cells accumulate LDL and become foam cells. For instance, already newborn Watanabe rabbits have fat strips (44). Moreover, lipid accumulation was observed in the intima of the fetal human aorta from normal mothers (45). Then, smooth muscle cells (SMCs) migrate to the intima from the media and also are transformed into foam cells. Human macrophages take up native and modified LDLs by fluid-phase pinocytosis and also form foam cells (9,46-51). Importantly, hormones are important for atherogenesis (52,53). SMCs have no significant number of LDL or scavenger receptors. Therefore, the level of their clathrin-dependent endocytosis is low. If in SMCs, absorbed lipid vesicles, which are accumulated in the intima after apoptosis of foam cells, by pinocytosis. It was shown that VLDL-gold complex perfused *in situ* was transcytosed across endothelium by plasmalemma vesicles which induces a progressive accumulation of extracellular densely packed uni- or multilamellar vesicles took place within the intima (54). On the other hand, uptake of lipids by pericytes or SMC is very difficult because in human intima, these cells are surrounded with BM. Thus, it is necessary to uncoat their PM in order to give them a possibility to uptake lipids from interstitium via endocytosis and pinocytosis. One of such possibilities could be the break of intercellular contacts. Indeed, the disruption of the contacts among subendothelial cells in the fatty streaks and in the areas of high hemodynamic stress is observed (Figure 2, 3). Thinning and arborisation of contact-forming cellular processes were also demonstrated

Table 1. Size of lipid particles in blood

Type of Apolipoproteins	Minimal diameter (nm)	Maximal diameter (nm)
ChM		>120 nm
VLDL	30	100
LDL	21	100
HDL	3/8	15
Albumin		3.6

(55,56). Disruption of cellular communications between sub-endothelial cells stimulates consumption of lipids by these cells (57). On the other hand, lipid accumulation in the subendothelial cells of human aortic intima impairs cell-to-cell contacts. For instance, the incubation of cultured human aortic sub-endothelial cells with desialilated LDL, LDL immobilized on latex, and LDL-free latex microspheres induced the alterations in cell-to-cell contacts similar to those occurring in a fatty streak *in situ* (58).

Transport of LDL through continuous (closed) endothelium is the main problem of the cholesterol transport and atherosclerosis models. Of interest, delivery of FFAs labeled with isotope to the adipose tissue is very fast (19). It is not clear whether tight junctions are always impermeable. The transport of LDL and LDL through continuous endothelium is the main unresolved issue of modern atherosclerosis models and even normal ECs. How do LDLs penetrate through a continuous layer of EC in the arteries if they are larger than any pores and any transcytosis carrier? What role does EC damage play? The answer to this question should be sought in the mechanisms of lipid transport and lipoprotein transcytosis. BM of endothelial cells also must be permeable for lipid particles. All these lipid particles cannot pass through tight junctions (TJs) between ECs due to their size (Table 1). In the mouse lymphoid endothelial cell line, orosomucoid, albumin, insulin and LDL were transcytosed from the apical (luminal) to basal (abluminal) side by a receptor-mediated pathway. Specific LDL transcytosis involved transport of intact LDL. This is a newly described transcellular passage of LDL via lysosomes (59). However, nobody observed LDL inside the cytoplasm of ECs and between them and BM and inside interstitium *in situ*.

Now the role of caveolae as transendotelial transport carriers is questioned. There are hot debates about this. However, caveolae cannot transport ChMs, VLDL and even LDL due to incompatibility of their diameter with the size of these cargoes (Table 1). But the main evidence against the role of caveolae as transport carriers is the observation that in fast-frozen ECs of capillaries in pancreatic islets of Langerhans, none caveolae with a narrow neck is found. Moreover, there are no isolated vesicles with diameter equal to that of caveolae (60). Also there is no clear evidence that membrane fusion-dependent transcytosis is involved in delivery of ChMs, VLDL and LDL to tissue cells. Neither NEM nor filipin caused reductions in albumin or LDL clearance across the peritoneal capillaries. Toxic effect of NEM increases transcytosis of albumin and LDL (61) On the other hand, experiments with NEM and filipin demonstrated that membrane fusion is important for transcytosis of large particles (62).

Finally, in lymph obtained form legs, the concentration of ApoB is 5-10% of its total concentration in serum (63). In contrast, lymph is enriched in HDL. Indeed, in the peripheral (i.e. from leg) lymph, the concentration of HDLs is higher than in blood, which means that HDL either specifically penetrates into the interstitium or forms there. In normal human interstitial fluid, there are more than 50 HDL particles to one LDL particle (37). Elimination of HDL from interstitium occurs through lymph capillaries (10). If LDL would pass through ECs, lymph capillaries also should absorb these particles. Indeed, large lipid particles are actively absorbed from interstitium into the lymphatic capillary lumen (15).

ChMs, VLDLs, LDLs and even HDLs cannot be transported through continuous

endothelium because of size restrictions. LDLs were not found in interstitium, in EC cytoplasm and in lymph. However, delivery of lipids from blood to the tissue through continuous endothelium is fast (19). In order to solve these contradictions, we hypothesize that in reality there is no transcytosis of the whole ChMs, VLDL or LDL and even HDL through ECs. In contrast, the passage occurs by insertion of FFA and cholesterol into lipid bilayer, diffusion along it and then lipids absorption by ApoA1, which is in interstitium. ChMs, VLDL hit ECs and contact with the APM of ECs and reach the lipid bilayer. Indeed, contact ChMs with the APM of EC is shown (64).

Lipase is localized on the APM of ECs and transforms triglycerides into FFA and glycerol. Then lipids are transferred to this lipid bilayer. VLDL and ChMs give cholesterol and FFAs to the external leaflet of the endothelial APM. Then these lipids jump/flip-flop to the cytosolic leaflet of the APM, diffuse through breaks in continuous tight junctions, and reach the BLPM. After this transfer ChMs and VLDL are converted into LDL. Accumulated lipids could be stored in caveolae because caveolin and caveolae regulate temporal accumulation of cholesterol and FFAs in the APM and then within the BLPM. Simultaneously Apo A1 is secreted (moving along the secretory pathway) from enterocytes and hepatocytes as the unfolded molecule, which passes through endothelial contact and tight junction because its wideness in lower than the maximal diameter of macromolecules able to pass through TJs. ApoA1 could be transported through inter-endothelial contacts if it is not yet folded. Indeed, it was shown that ECs could bind, internalize, and transport apoA-I in a specific manner (65). Then ApoA1, which already has appeared inside interstitium, extracts ChSt and FFAs from the BLPM and form HDLs, which then deliver the lipids to the PM of tissue cells. Then, HDLs are absorbed with lymph capillaries. If there is an excess of cholesterol in tissue cells, ApoA takes it from the BLPM of tissue cells and removes it from tissue in form of HDL into the bloodstream through lymphatic drainage (10). Lymph capillaries actively absorb lipid particles (10,15). In the blood HDLs are delivered to liver sinusoids and captured by scavenger receptors of the hepatocyte BLPM and endocytosed. Excessive cholesterol is processed by hepatocyte mitochondria into bile acids and thrown into the intestine. This explains why concentration of LDL in lymph is low whereas concentration of HDL is high.

Despite experimental evidence that HDL particles function in an anti-atherosclerotic manner, clinical studies have failed to demonstrate that plasma concentrations of high-density lipoproteins (HDLs) are directly correlated with the intensity of atherogenesis (10). For instance, recent clinical trials designed to test whether increasing plasma levels of HDLs is therapeutically beneficial did not demonstrate their efficacy Moreover, genetic studies did not confirm that in humans, higher levels of plasma HDLs associates with protection from cardiovascular disease. In randomized trials, strategies that effectively raise plasma HDL levels have not reduced clinical cardiovascular events (10,37). The possible exclusive role of HDL in the uptake of cholesterol excess through interstitium explains why the augmentation of HDL concentration does not improve atherogenesis. However, this issue should be examined more carefully.

3.3. Endothelial damage

The important factor of atherosclerosis development is damage of endothelial cells (66). For instance, irradiation of the aorta with x-rays accentuates the development of abdominal aortic arteriosclerosis in the dog in a way that is indistinguishable from the naturally occurring disease in this species (67,68). After irradiation, the feeding of dogs with a large amount of cholesterol leads to the development of atherosclerosis (69-72). The maintenance of physiological levels of nitric oxide (NO) produced by eNOS represents a key element for vascular endothelial homeostasis. On the other hand, NO overproduction, due to the activation of iNOS under different stress conditions, leads to endothelial dysfunction (Figure 2D) and, in the late stages, to the development of atherosclerosis (73,74). For instance, in hyperlipidemic Watanabe rabbits, atherosclerotic lesions are also associated with increased immune reactivity for inducible nitric oxide synthase and endothelin-

1 in thoracic aortic intimal cells (75).

There are several indications that altered flow pattern is one of the most important factors, which impair endothelial cells (76). Watanabe newborn rabbits have already some damage of ECs in aorta (44). Turbulent blood flow modulates endothelial transcriptional and post-transcriptional programs (73). LDLs penetrate through continuous endothelium into intima in places of altered flow pattern or hemodynamic load. Even when LDL concentration is high, atherosclerosis was never observed in muscular arteries. Also although, pulmonary artery is of elastic type, but there is no atherosclerosis even when concentration of LDL in blood is high. On the other hand, atherosclerosis plaques are developed mostly at the site of altered flow pattern, where the EC pattern is altered (55,77). In atherosclerosis susceptible regions where altered flow pattern of blood flow is observed, ECs display a cuboidal morphology (78). In human subjected to perfusion fixation of aorta immediately after death due to mechanical injury in the sites with high oscillatory flow hemodynamic, we found small areas of endothelial detachment and widened inter-endothelial contacts (Figure 3G). There we also observed ECs with cilia and multinuclear ECs. However, no any difference in the rate of proliferation was observed at the flow divider (56). Also in rats, in these areas the mitotic index is normal (79). There, ECs have ellipsoidal shape, aligned coaxially. In the human aorta, plaques are at the sites of arterial branching (80, 81). Cryo-damage of the arterial wall accelerates the development of atherosclerotic lesions in arterial vessels of Watanabe hyperlipidemic rabbits (82,83).

Within the atherosclerotic lesions of the interior carotid artery, endothelial layer was preserved but displayed pronounced irregularities in endothelial architecture including appearance of cuboidal cells. Some endothelial cells were covered by numerous microvilli and/or contained "craters" disrupting continuous surface of the endothelium. Platelets and leukocytes adhering to endothelium were frequently observed. There were also areas of the vessel lumen with endothelial denudation (84). The atherosclerotic vessel wall has a large gap between ECs (85). Thus, turbulent flow pattern events of blood flow promotes atherogenesis. In different models of atherogenesis, endothelial damage is important for although only when the concentration of LDL is high (82).

It is established that elevated plasma cholesterol level is unique in being sufficient to drive the development of atherosclerosis, even in the absence of other known risk factors (86). However, it should be accompanied by local alteration of endothelium. LDLs are captured by intima within the area, where the altered flow pattern is evident. Concentration of LDL in the plaques is five times more than in the unaffected parts of the vessels (87). However, normally continuous endothelium lining arteries is impermeable for LDL. Thus, it should be temporal mechanisms ensuring LDL penetration. EC contacts could be affected by turbulence. One of such mechanisms could be increased dynamics of inter-endothelial contacts in these areas. Indeed, wholes within inter-endothelial contacts were found between endothelial cells lining early atheroma (84) and areas subjected to high mechanical stress (56) (Figures 3, 4).

When the epithelial tissue grows, TJs could break and then undergo reparation (88). In this case, why LDLs do not go back to the blood during the next fluctuation of inter-endothelial contact? The reason could be the high development in these zones of multi-layered basement membrane, which has higher affinity to LDL (89). Why BM is so thick in these areas? The reason could be that BM is synthesized during endothelial spreading. Indeed, we found multi-layer BM in area of hemodynamic stress. However, also after multiple re-endothelization even in the absence of cells division we found accumulation of such BM in such areas (90). Moreover increased mechanical influence in rats with arterial hypertension induces accumulation of BM below ECs in rat aorta (91,92). High mechanical or altered flow pattern induces spreading of EC and induces synthesis of BM components. Similar effect was observed during senescence (93)

During mitosis of ECs inter-endothelial contacts became wider (94) (Figure 3F, 4). For instance, newborn Watanabe rabbits exhibiting initial atherosclerosis events in aorta due to elevated level

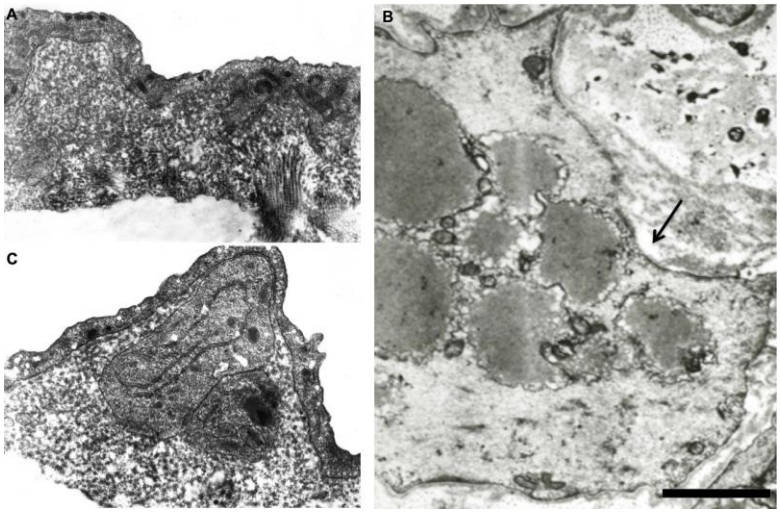

Figure 4. Ultra-structure of the aorta of normal and hypertensive rats as well as of the aorta of the rabbit subjected to cholesterol feeding. (A) Accumulation of multilayered BM below aorta ECs of rat with arterial hypertension. (B) SMC with lipid granules has the PM with areas not covered with BM (arrow). (C) Appearance in the intima of the foam cell (lipid droplets in lysosomes) derived from smooth muscle cell. Arrow shows the PM of this smooth muscle cells not covered with the basement membrane. Scale bars: 1.4 μm (A, C); 1 μm (B).

of LDL in blood have a lot of dividing ECs suitable for penetration of VLDLs into intima (44) (Figure 4B). Local holes in endothelial tight junction barrier function could be important for permeability. These leaks occur as cell boundaries elongate, correspond to visible breaks in the tight junction (88). In polarized cells, the integrity of tight junctions is preserved during mitosis (95).

3.4. Oxysterols

Elevated plasma cholesterol level is considered as unique in being sufficient to drive the development of atherosclerosis, even in the absence of other known risk factors. However, oxysterols are even more important for atherogenesis. Indeed, attempts to reproduce atherosclerosis experimentally using various damaging artery substances (adrenaline, digitalis, barium chloride, pathogenic bacteria) usually were unsuccessful. However, when cholesterol from scientific suppliers were additionally purified the development of atherosclerotic lesson became less developed Oxidized cholesterol in the diet as well as dietary and lifestyle factors that promote the endogenous oxidation of cholesterol likely play important roles in the development of atherosclerosis. Oxidative modifications in LDL drive the initial formation of fatty streaks (96). Cholesterol in ChMs and LDL is accessible for oxygen (96). Under normal physiological conditions, oxysterols are found in low levels (97,98). After addition of only 5% oxidized cholesterol to the chow diet containing only 0.33% cholesterol was added was the percentage of aortic area covered by fatty streaks increased 2-fold (99). Pohjantahti *et al*. (100) summarized 50 studies and concluded that the elevation of oxidized low-density lipoprotein is a key event in the development of atherosclerosis. Oxidized LDLs produce significant impairment in the modulation of the eNOS/iNOS machinery induces the EC oxidative stress (74). Apoptosis in vascular cells could be induced by 7-ketocholesterol (41). The negative results related to the promoting role of oxidized cholesterol (77) could be a result of specific experimental conditions. Thus, oxidized LDLs represent the major candidate to trigger endothelial dysfunction leading to atherosclerosis.

Antioxidant treatment with various agents reduces the development of atherosclerosis in both non-primate and primate hypercholesterolemic animal models. Feeding rabbits a high-cholesterol diet supplemented with antioxidants prevented the intimal thickening of aortas, even though their blood continued to have a cholesterol level 40-fold higher than control rabbits, but their plasma oxysterols

levels decreased significantly compared to the rabbits fed without antioxidants (96,101). In contrast, elimination of anti-oxidation substances from the food stimulates development of atherosclerosis (102). Epidemiologic data in humans also supports a protective role for antioxidant supplementation. Despite this, prospective clinical trials with antioxidant vitamins such as vitamin E and beta carotene, in patients with preexisting atherosclerosis have thus far been disappointing (6). Why oxidized LDL potentiate atherogenesis is unknown. One of the reasons could be impairment of intracellular transport (see below)

3.5. Atherogenic serum

About 20% of subjects without clinical signs of heart disease have an atherogenic serum (103). The atherogenic factor can be removed by passing the blood plasma through a sorbent with immobilized LDL. This suggests that this factor is antibodies (104,105). People with initial stages of atherosclerosis exhibited alteration in glycosylation of Apo proteins (77). The scavenger receptors CD36 and SRA were identified as important mediators of modified LDL uptake (9). Modified LDL, remnant lipoproteins, triglyceride rich lipoprotein lipolysis products, dysfunctional HDL are involved in the changes induced in EC morphology (reduced glycocalyx, overdeveloped endoplasmic reticulum, Golgi apparatus and basement membrane), loose intercellular junctions, increased oxidative and inflammatory stress, nitric oxide/redox imbalance, excess Lp transport and storage, as well as loss of anti-thrombotic properties, all of these being characteristics of endothelial dysfunction (106). The list of other risk factors includes oxidative stress, inflammation, infection and others (28).

The autoimmune hypothesis of atherosclerosis pathogenesis was first formulated by Klimov *et al*. (1). Autoimmune complexes including LDL as an antigen were detected in blood and in the vascular wall. Then impaired glycosylation of Apo B and E and antibody against LDL were found (77). Accumulation of both IgG and IgM against lipids and LDL are associated with an increased risk of future cardiovascular disease. Persons with cardiovascular disease contain anti-oxidized low-density lipoprotein immunoglobulin. Anti-phosphorylcholine IgM with high their affinity protects against CVD (107). Also oxygenated LDL is immunogenic and induces the generation of autoantibodies that form circulating immune complexes. Oxidized LDL and their immune complexes contribute to the formation of foam cells (108,109). Why different types of atherogenic serum induce alterations typical for atherosclerosis is unknown. However, one should take into consideration that isolation of subendothelial cells leads to the dissolution of their BM and makes them sensitive to modified LDL. Therefore these results have to be considered cautiously.

3.6. Human intima and atherosclerosis

The reason why in experimental animals the atheroma narrows the vascular lumen whereas in human such phenomenon is rare could be the following. Structure of the intima in large arteries in humans and rabbit is different (55,110). In small animals intima consists of only endothelium and its BM. Therefore, atheroma narrows the vascular lumen. In contrast, in humans and large animals intima contains also pericytes, cells and possibly SMCs (55,110). In human arteries of elastic type, there is no necessity for penetration of SMC into intima because there are stellate cells of SMC or pericyte origin, which could serve as a substrate for the development of atherosclerosis and collagen fibrils. Human intima contains many cells of different type (45). Most (84-93%) of the intimal cells exhibit antigens of smooth muscle cells and pericyte-like stellate cells (87). Pericyte-like cells have been identified in the inner intima (111). Importantly, cultures of subendothelial cells from human aortic intima that contained a mixed cell population made up mainly of typical and modified SMCs (112). In spite of this, until now human aortic intima is considered as being compose of endothelium and its basement membrane (6,73).

SMC can secrete fibrils suitable for the formation of elastic fibers. However, SMCs synthesize procollagen I extremely rarely due to problems with its secretion as a mega-cargo (66,113,114). Indeed, *in situ* (although not in cell culture), SMCs are surrounded by their basement membrane (BM) and there is not free surface of the

plasmalemma for such secretion. It is not cleat whether pericytes can synthesize significant amount of procollagen of I type and layers of elastic fibers *in situ* due to similar problems (115). In human atherosclerotic plaques the synthesis of type I procollagen is unregulated (116). It seems that in order to acquire the ability to secrete procollagen-I SMCs and pericytes should break their intercellular contacts and eliminate their BM surrounding them under normal conditions. In rat aorta, the flow dividers do not contain lipids and resembles fibrous cap of the plaque (80,81,117). Migration of SMC even inside intima and especially from media into intima is a very slow process. It is observed only below ECs (117). The regenerative response of medial SMC to extensive freeze injury proceeds in the form of intimal thickening. SMCs are travelling in the sub-endothelial layer from the edges where SMCs have not been damaged. No migration of SMC inside the medial layer, consisting only of elastic and collagen fibers were observe.

Small animals are rather resistant to atherosclerosis be cause they have not substrate for this, namely intima populated with stellate cells. Rats and mice are particularly resistant. Their intima consists of only one layer of ECs. Barn rats and red rats eat almost any food, but still they prefer high-quality food, such as meat or fresh grain. Black rats generally prefer vegetables, fruits and grain. They are reluctant to accept meat or fish. Rats are not averse to profit carrion. Rarely, they can eat carrion. However, a rat eats the dead animals, unless there's nothing to eat. In small animals, SMCs migrate into intima from media, whereas in human and large animals this is not necessary because SMCs are already there. Moreover migration of SMCs into already populated intima or even after cellular destruction is very slow and need strong/severe stimuli. In order to test this hypothesis is necessary to feed large animals with human food for years. However, this is very expensive and out of the ability of one person. In small animals, monocytes first migrate, then SMCs migrate and then foam cells appear. In human, there is no migration of SMCs from the media and therefore there is no large protrusion of plaque into the lumen. Small predators have no intima and have a high content of ApoA1 (77).

It is not clear also why atherosclerosis are more often in old people and people with hypertension? One of the reasons could be formation of multilayer BM. Indeed, multilamellar network-like BM is formed in the area of hemodynamic stress, in old rats, rats with arterial hypertension, and after repetitive re-endothelization (56,90-93). In all these cases, repeated endothelial regeneration induces appearance the formation not only of the multi-layered BM but also of multinuclear EC clusters (90, 118). An age-dependent increase in BM thickness has also been reported for the human vascular BMs (119). In old rats and in hypertensive animals, and after repetitive injuries (after several rounds of de-endothelization), re-endothelization was even faster (56,90-93). At ageing, the integrity and continuity of the endothelial monolayer is preserved, on the surface local intimal pits, craters and micro-defects appear, adhesiveness of endothelium to leucocytes increases. The type of the senescent remodeling in the endothelial layer revealed predisposes to development of atherosclerosis (120). Repeated lesions also produce an increase in the heteromorphism degree of the endothelial layer. However, in the rat aorta in places of increased hemodynamic stress, the mitotic index is not increased (79). Consequently, there is no pronounced regeneration of EC there. Of interest, multilayer matrix accelerates EC migration even when mitotic activity of ECs was inhibited. For instance, blockade of endothelial proliferation with gamma-irradiation of the vessel before the last cryo-destruction, an increased rate of EC migration has been revealed (90). Spreading of ECs leads to the beginning of synthesis of BM (92).

If we take into consideration that within the areas with the highly altered flow pattern, BM is multilayered and also that after repetitive injuries, in old animals and rats with arterial hypertension BM is also multilayered the obvious hypothesis could be that regeneration of damaged endothelium led to the thickening of BM. Even when mitotic activity is blocked, spreading and migration of endothelial cells was not affected and led to the thickening of BM. Thus, not mitosis per se, but spreading of ECs triggers synthesis and secretion of BM components (114,121). Multilayered BM has higher affinity to LDL especially to the atherogenic LDL (89). Thus, intimal

alterations related to age and arterial hypertension are based on the ability of endothelial cells to synthesize and then transport components of the basement membrane as mega-cargos through the Golgi complex (114). The extracellular matrix of the sub-endothelium, particularly proteoglycans, is thought to play a major role in the retention of atherogenic lipoproteins (122). Multi-layered BM binds more LDL after contraction of ECs due to altered flow pattern and then ECs spread faster when BM is better developed. In the arterial intima, LDL may become trapped in the extracellular matrix where they may be modified (89). The atherogenicity of apoB-containing low-density lipoproteins (LDL) is linked to their affinity for artery wall proteoglycans. LDLs bind to the extracellular matrix thus being deposited in vessels leading to atherosclerosis under conditions of excess LDL (123). Indeed, mice expressing proteoglycan-binding-defective LDL developed significantly less atherosclerosis than mice expressing wild-type control LDL (124). In early stages of atherogenesis, beta-VLDL-gold complexes or deposits of ApoB and ApoE were detected in close association with extracellular liposomes. With the appearance of intimal macrophage-derived foam cells, the immunoperoxidase reaction product, revealing the presence of the two apolipoproteins, could also be seen in intracellular lipid inclusions (125). Altogether this explains the role of age and arterial hypertension for atherosclerosis.

3.7. Lysosome-ER transport

The important question is why in intima, macrophages form lipid droplets in lysosomes instead of the ER membrane? It is known that once in the bloodstream, VLDL is transformed into LDL (41). LDLs are captured by ApoE receptors and HDLs are captured by scavenger receptors. After internalization and delivery of LDL to late endosomes/lysosomes LDL cholesterol esters are hydrolyzed to release free cholesterol and FFAs. Free cholesterol and FFAs are generated following degradation of ingested lipids in the lysosome. Accumulation of cellular cholesterol leads to activation of several transcription factors, which regulate expression of their target genes important for efflux of free cholesterol and scavenger receptors. In lysosomes, there are no enzymes for the digestion of cholesterol and FFAs. This is possible only by mitochondria. Therefore, according to the current consensus, free cholesterol is transferred from lysosomes to the ER and then to mitochondria (41,126). Coordinated actions of Niemann-Pick type C (NPC) 1 and NPC2 proteins are necessary for the delivery of cholesterol into lysosomal membrane (127,128). In the acidic environment of lysosomes FFAs become neutral and can be introduced into the bilayer of lysosomes, Exit of LDL-cholesterol from lysosomes is blocked by a class of drugs called hydrophobic amines or progesterone (37). This suggests that low pH is important. Of interest, LDL-cholesterol that leaves lysosomes could be quickly found in the plasmalemma (129).

Cholesterol is insoluble in water. Pulling one cholesterol molecule out of a lipid bilayer into water required an energy barrier of 80–90 kJ/mol corresponding to hydrolysis of about 1.5 ATP molecule (130,131). Thus, only lipid transfer proteins can deliver cholesterol ad FFAs from membrane of lysosomes into membrane of the ER. For this it is also necessary to have transfer of cholesterol and FFAs from the luminal leaflet of lysosomal membrane to its cytosolic monolayer. Numerous (now more than 131) lipid- and sterol-transfer proteins have been identified to mediate directional cholesterol transfer at membrane contact sites formed between two closely apposed organelles (127,128).

Oxidation of cholesterol head makes it more hydrophilic and the molecule loses its ability to do flip-flops. The excess of cholesterol and presence of even small amount of oxysterols could block these mechanisms and during year this small damage could result in accumulation of cholesterol in lysosomes. The presence of oxysterols changes membrane fluidity (131). It is possible that oxysterols inhibit function of lipid transfer proteins operating between membrane of lysosome and membrane of the ER attached to lysosome. We hypothesized that if ChMs remained in the blood for a long time, they could be oxidized. This explains why lipid granules in foam cells are derived from lysosome. Is there information supporting this hypothesis? Yes, there is. For instance, without protein OCBP, the oxysterol transfer protein, lipids are accumulated in lysosome (Figure 4B). In contrast, when the amount of ER-

lysosome contacts site where this protein is localized, increased, the phenomenon disappeared (132). The accumulation of oxidized LDL could result in the conversion of macrophage into foam cells that lose the ability to phagocytosis particles (98,133). Indeed, in agreement with this hypothesis, when the amount of OCBP, the oxysterols transfer-protein becomes low lipids accumulated in LAMP2-positive lysosomes. In contrast, when the area of lysosome-ER contact site increased this phenomenon became less evident (134).

3.8. Food "improvement" and intercellular transport in the development of atherosclerosis

The important aspect of atherogenesis is its dependency of the "improvement" of human food and this aspect could be explained from the (point of view of intracellular transport. Indeed, in the 19th century, pathologists did not notice a wide spread of atherosclerosis. In the beginning of 20th century the number of cases of atherosclerosis started to increase quickly Atherosclerosis has become more often in sixties of the 20th century. The mortality from infarct of heart in all economically developed countries (including Japan) has been increasing after the Second World War (135-137). Atherosclerosis started to affect even young people. For instance, according to Vihert and Drobkova (138), lipid spots and stripes in the thoracic and abdominal aorta in children under 10 years are found in 92.2% of cases in the age from 10 till 16 years – 100%, and to 25 years of life in the affected area of the aorta reaches 30-50% of its surface.

This spreading of atherosclerosis occurred in parallel with augmentation of the “quality” and amount of food consumption, especially during a single meal. Since 1960 till 2020 consumption of dairy (milk products) increased 2-fold. Since 1960 till 2000, consumption of animal oils per capita was not changes whereas consumption of vegetable oils increased 2.3-fold (139). There has been a considerable increase (62% during the period 1963-2009) in the available food consumption of meat worldwide, with the biggest increases in the developing countries (a threefold increase since 1963). Processed meats (which refers to post-butchering modifications of foods such as curing, smoking, or addition of sodium nitrate), account for 35.8% of all meat consumed in HIC (140). There was a wide spread of refrigerators and animal products industry long-term storage of meet, or milk and egg powders, bread products with animal fats. People started to eat more products where lipids were oxidized. These lipids were generated as a result of long-term storage. Moreover, flavoring additives and special cooking make food tastier and it is very difficult to stop absorbing it while eating. People began to eat more lipids at once. Animal products were stored after their drying. Therefore, there is the marked growth of purchases of all packaged foods and beverages (all categories of processing). For example, 58% of calories consumed by Mexicans come from packaged foods and beverages, which is similar throughout the Americas and even within the United States (139). The consumption of dried animal products and such products after prolonged storage started to increase dramatically after the Second World War and atherosclerosis started to increase quickly. Long storage of food induced accumulation of oxysterols in food. Seven-ketocholesterols is present in food after its prolong storage in the presence of oxygen. In ChMs and LDL, cholesterol is more accessible for oxygen (96). Among sources of 7-ketocholesterol could be dried milk, dried eggs, meet after its prolonged storage and other products containing cholesterol in contact with oxygen. Experimental data show that even a very small amount of oxysterols accelerated atherosclerosis.

Epidemiology studies established that those who consumed the highest levels of both unprocessed and processed red meat had the highest risk of all-cause of mortality, cancer mortality and cardiovascular disease mortality. Unprocessed red meat over the course of the study raised the risk of total mortality by 13%. An extra serving of processed red meat (such as bacon, hot dogs, sausage and salami) raised the risk by 20%. Replacement of red meet with fish, poultry, nuts, legumes, low-fat dairy and whole grains lower the risk of mortality by 7% to 19% (141). Also in Italy, scenarios for reducing beef consumption are consistent with significant health and environmental co-benefits on current and future generations (142). However, moderate meat consumption, up to ~100

g/day, was not associated with increased mortality from ischemic heart disease, stroke or total cardiovascular disease among either gender (143). Products with a high content of unsaturated fats (e.g. olive oil) could act as antioxidants and protect from atherosclerosis (139). Consumption of omega-3 fatty acids protects against atherosclerosis and even reduces the size of atherosclerosis plaques (139,144).

The Greenland Eskimos eat less animal and dairy fat, fewer eggs, and more polyunsaturated fats and monounsaturated fats. They have low level of atherosclerosis. However, after their movement to cities the level of atherosclerosis increased. Indeed, Greenland Eskimos living in Denmark at that time had much higher cholesterol and triglyceride levels (145). Also atherosclerotic disease of the aorta and atherosclerosis of the coronary arteries in Yakutsk was more frequently found in alien than the in igneous population (146). The natives of the polar region, having preserved the traditions and lifestyle of their ancestors, almost never occurs atherosclerotic diseases (147,148), whereas the Yakuts, living in the city of Yakutsk, was the high incidence of coronary artery disease (149). The number of cases of myocardial infarction and increased mortality from cardiovascular diseases were noted in the newcomers of the Far North where food are preserved for a long time and small amount of plant food is available (147). Altogether these data show the parallelism between the "improvement' of food and increase of a single meal on one hand and spreading of atherosclerosis. The reason of this parallelism remains enigmatic. One of the possible explanations could be the following.

The animals do not eat lipids in a large amount and after their prolonged storage in the presence of oxygen. Large animals, i.e. cows, giraffes, elephants, don't eat fat especially lipids derived from animals. They have intima similar to that in humans, but are susceptible only to arteriosclerosis without significant accumulation of lipids. In aorta of squirrel monkey, intima contains additional cellular elements such as SMC and additional elastic fibers (18). Tigers and other large predators eat only fresh meat. Humans like monkey are derived from the progenitor who ate only plants. Humans began to eat meat and fats and then to store and oxidize this food. After this atherosclerosis appeared. Larger animals have larger vessels and more turbulent flow, which means more damage to the EC. LDLs penetrate through the extended EC contacts and stick to the multi-layer PM. Due to less mechanical stress, damage is less pronounced in smaller arteries and small animals or when blood pressure is low. Aorta of large dogs, caws, baboons, ostriches, giraffes and elephants has intima with cells and suffer from arteriosclerosis, but they do not have atherosclerosis (25,27,150-154). In old dogs, one could find only arteriosclerosis, but not atherosclerosis, because there are no lipid components. X-irradiation is followed by the development of arteriosclerotic lesions similar to those that occur naturally in old dogs, namely, the development of intimal fibrosis and plaque formation (67, 68). In giraffes and in elephants of East Africa, signs of arteriosclerosis were found in areas of high hemodynamic stress (152-155). In elephants, arteriosclerotic plaques are similar to those in human arteriosclerosis (155).

Animals, who eat meat, have a high HDL/ApoA1 content. In large herbivorous animals that have developed intima in the arteries of the elastic type, arteriosclerosis occurs. Hypertension without consuming animal fat and a large amount of vegetable fat, gives arteriosclerosis, which can cause aneurysm and rupture of the aorta or other large vessel. Experiments with fat-fed elephants, chimpanzees, giraffes and other large animals excess animals were not carried out. It could be interesting to feed sheep's, cows, horses, ponies with cholesterol and especially oxycholesterol. It is possible that animals that feed on carrion, such as hyenas, have mutations that allow oxidized cholesterol to be digested. Large animals not eating animal fat have arteriosclerosis of arteries of elastic type, but without lipid components. Only during the last few thousands of their history some human started to each a lot of oil.

Moreover, our recent observations indicate that overloading of enterocytes with lipids increases the diameter of ChMs and led to mistakes of ApoB glycosylation (our unpublished observations; 12). Changes in transcytosis through the small intestinal

enterocytes of carps and hamsters were visible after their oil feeding (156). Normally, these animals don't eat a lot of clean oil. Overloading of the GC could alter the correctness of protein glycosylation there (157). Indeed, when Caco-2 cells from the patient with blood group 0(I) were overloaded with lipids, some ChMs exhibited labeling for antigen A (our unpublished observations). Thus, when human eat a lot of fat ChMs enlarged and their glycosylation is impaired. This could generate antibodies and desialilation of ChMs. This explains why Apo B and Apo E in LDL have sings of impaired glycosylation and why "improvement of food led to spreading of atherosclerosis.

4. SUMMARY AND FUTURE PERSPECTIVES

Secretion of lipids and particles, their transcytosis and endocytosis play a significant role in atherogenesis. Humans are large animals originated from herbivorous ancestors and originally focused on plant food, like monkeys. In the arteries of the elastic type they have a well-developed intima and eat a lot of cholesterol, part of which could be in oxidized state. Oxysterols accelerate formation of foam cells. We proposed also that nations that are genetically adapted to feeding milk, often suffer from atherosclerosis. The Chinese, Koreans and Japanese do not tolerate milk and are less affected by atherosclerosis.

Here, we tried to explain several previously unanswered questions:

1. Whether Effect of food on atherogenesis is phylogenetically dependent?

2. Why did atherosclerosis begin to spread in the early 20th century?

3. How do LDL and HDL pass through the continuous endothelial layer in significant amount? These particles due to size restrictions cannot pass through ECs in capillaries and arteries lined with closed endothelium.

4. Why elevation of HDL concentration alone did not decrease atherosclerosis development?

5. Why the concentration of ApoA1 in the lymph is significantly higher than in the blood?

6. Why lipid granules inside foam cells are formed from lysosome whereas in other cells these are formed inside membrane of the smooth endoplasmic reticulum (SER)?

7. What is the role of atherogenic transformations of LDL: desialization, opsonisation, aggregation by antibodies, etc?

8. What could be the role of impaired glycosylation and of auto-immunity?

9. Why in experimental animals the atheroma narrows the vascular lumen whereas in human such phenomenon is rare?

10. Why in foam cells, lipid droplets are formed from lysosomes rather than inside the ER membrane.

Our hypotheses provide the framework for the explanation of unclear questions within the field of atherosclerosis. However, they should be experimentally tested. The philosopher of science K. Popper (158) argued that the strength of a scientific theory rested on it being subject to the test of falsifiability, the application of criticism to the basis of the theory. In order to assess the applicability of these models, we used the Popper's principle, the formulation of prohibitive and supportive observations (114). The supportive observations for our hypothesis would be the following: 1) high ratio between ApoA1 and ApoB in interstitium in comparison to this ration in blood and its dynamics after feeding; 2) augmentation of the ratio between of the luminal and abluminal caveolae in endothelial cells of rabbit aorta after feeding with fat and cholesterol in comparison to

the situation after starvation; 3) appearance of atherosclerotic plaques in aorta of large mammals after their prolonged feeding with solution of cholesterol in vegetable oil. The absence of these facts-predictions would be prohibitive observations.

The main future directions in the field of atherosclerosis include examination of the following questions. Why did atherosclerosis begin to grow in the early 20th century? How do LDL and HDL pass through the continuous endothelial layer in significant amount? These particles due to size restrictions cannot pass through ECs in capillaries and arteries lined with closed endothelium. Why elevation of HDL concentration alone did not decrease atherosclerosis development? Why the concentration of ApoA1 in the lymph is significantly higher than in the blood? Why lipid granules inside foam cells are formed from lysosome whereas in other cells these are formed inside membrane of SER? The role of atherogenic transformations of LDL: desialization, opsonization and aggregation by antibodies, etc. is not clear. What could be the role of impaired glycosylation and of auto-immunity? How oxysterols affect lipid uptake by subendothelial cells? It is necessary to identify more proteins carrying oxysterols.

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Abbreviations: APM, apical plasma membrane; Apo, apolipoprotein; BM, basement membrane; ChM, chylomicrons; EC, endothelial cell; ER, endoplasmic reticulum; FFA, free fatty acid; GER, granular ER; HDL, high-density lipoprotein; LDL, low-density lipoproteins; NO, nitric oxide; SER, smooth ER; SMC, smooth muscle cell; TJ, tight junction; VLDL, very LDL.

Key Words: Atherosclerosis, LDL, HDL, VLDL, Transcytosis, Endothelial Cell, Chylomicron; Intima, Foam Cell, Lysosome, Review

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