Review paper

Apolipoprotein E knockout mice: an experimental model to study inflammatory mechanisms of atherosclerosis and to screen a putative anti-atherogenic properties of drugs

JACEK JAWIEŃ[1], RAFAŁ OLSZANECKI[1], BERNADETA NOWAK[2], JANUSZ MARCINKIEWICZ[2]

[1]Chair of Pharmacology, Jagiellonian University Medical College, Krakow, Poland
[2]Department of Immunology, Jagiellonian University Medical College, Krakow, Poland

Abstract

Based on the current knowledge it is well ascertained that inflammation backgrounds the pathogenesis of atherosclerosis. Still, some open questions remain.

Animal model is an important tool to study atherogenesis. Nowadays, genetically modified mice play a pivotal role. Wild mice are highly resistant to atherosclerosis, whereas "gene-targeted" modified mice can spontaneously (even without use of high cholesterol diet) develop atherosclerosis. The best example are apolipoprotein E (apoE)-knockout mice.

In this review we will discuss usefulness of apoE-knockout mice to study the pathogenesis of atherosclerotic lesions, especially the immune mechanisms of atherogenesis.

Key words: *atherosclerosis, apoE-knockout mice, LDL receptor-knockout mouse, animal models.*

(Centr Eur J Immunol 2012; 37 (1): 36-44)

Introduction

In 1986, with the development of monoclonal antibodies, the small cells with round nucleus present in the atheromatous plaque, known before as "small monocytes", were demonstrated to be T lymphocytes [1]. Several years later it was shown that these lymphocytes "recognize" the oxidized molecules of low-density lipoproteins (LDL) – oxLDL [2]. In 1990, it has been also demonstrated that the development of atherosclerotic plaques correlates with the presence of at least two types of infectious microorganisms; *Chlamydia pneumoniae* and *Herpes simplex virus* were observed [3, 4]. These findings posed the question about the involvement of the inflammatory process in atherosclerosis. Speculations of this kind were initially received with great skepticism because of the lack of spectacular, unequivocal evidences of significant, causal role of inflammation in atherogenesis. Such evidence was delivered by the use of a new technique of gene targeting, for the invention of which Mario R. Capecchi (Italy), Martin J. Evans (United Kingdom) and Oliver Smithies (USA) received the Nobel Prize in Physiology or Medicine in 2007.

Gene targeting enabled to create apolipoprotein E (apoE)-knockout mice (described later in the text), which soon turned out to be very good model to test the involvement of inflammation and immune mechanisms in the development of atherosclerosis. As it was evidenced in seminal paper of Gupta *et al*., the genetic deletion of only one cytokine in apoE knockout mice – interferon γ (IFN-γ), reduced atherosclerosis by 60% [5].

Over the last 15 years, many reports clearly showed the involvement of various inflammatory mechanisms in the development of atherosclerotic plaques in apoE knockout mice: the overexpression of adhesion molecules (vascular adhesion molecule 1 and intercellular adhesion molecule 1) at sites with atheromatous changes was observed [6], the monocyte chemotactic protein (MCP-1) was shown to play an important part in the progression of atheromatous lesions [7, 8]. It was also observed that knockout of interleukin-18 decreased atherosclerosis by 35% [9, 10].

Correspondence: Jacek Jawień, Chair of Pharmacology, Jagiellonian University Medical College, Grzegorzecka 16, 31-531 Krakow, Poland, phone +48 12 421 11 68, fax +48 12 421 72 17, e-mail: mmjawien@cyf-kr.edu.pl

Early report on apoE-knockout mice showed that inhibition of CD40 signaling reduced atherosclerosis [11]. This was explained by the fact that ligation of CD40 molecule [tumor necrosis factor α (TNF-α) receptor superfamily member] – found in the atheromatous plaque on endothelial cells, vascular smooth muscle cells, antigen-presenting cells and platelets – with CD40L activates a number of transcription factors involved in inflammatory responses: NF-κB, AP-1, STAT-1 or Egr-1. Therefore, CD40-CD40L ligation may influence the endothelial cells, which, in consequence, acquire proinflammatory and proatherosclerotic phenotype leading to the expression of adhesion molecules and tissue factor on their surface. This finding created new possibilities of therapeutic approach, consisting in inhibition of the CD40–CD40L pathway [12-14].

Finally, in apoE-knockout mice with severe combined immunodeficiency (SCID) atherosclerosis was reduced by 70% in comparison to the control group, due to a significantly lower number of lymphocytes in mice with SCID. Moreover, transfer of T cells to these mice aggravated atherosclerosis even by 164% [15].

Atherosclerosis as an inflammatory process

These and other facts made the investigators realize unequivocally that inflammation was essential for atherogenesis. Therefore, in 1999, just before his death, Russell Ross (the author of the "chronic response to injury" theory of atherosclerosis) officially proclaimed that "atherosclerosis is an inflammatory disease" [16].

However, inflammation occurs in response to factors that destabilize the local vessel wall homeostasis.

The first stage of atherogenesis consists in endothelial dysfunction. It predominantly involves all the regions of arterial bifurcations where the blood flow is not laminar. Disturbed blood flow, causes up-regulation of TLR2 on endothelial cells favoring inflammatory phenotype [17]. Hence, these localizations are prone to develop atherosclerosis. In such places LDL is accumulated in the subendothelial space. Clearly, lipoprotein accumulation is increased if the serum LDL level is elevated. Low density lipoproteins are transported by passive diffusion and their accumulation in the vascular wall seems to depend on the interaction between apolipoprotein B of the LDL molecule and proteoglycans of the matrix [18].

There is evidence that unchanged LDLs are "collected" by the macrophages too slowly to activate their transformation into foam cells. Therefore, it has been suggested that LDL molecule is "modified" within the vascular wall. The most significant modification is lipid oxidation, resulting in the formation of so-called "minimally oxidized" LDL [19]. The generation of these "alien molecules" leads to the development of inflammatory response, with predominant participation of monocytes and lymphocytes [20, 21]. The local inflammation is triggered by accumulation of the minimally oxidized LDLs in the subendothelial space, thus stimulating the endothelial cells to produce a number of proinflammatory molecules [22].

Hyperlipidemia causes macrophage accumulation in the blood vessels. Before the "minimally oxidized" LDLs have been engulfed by the macrophages, they have to be modified into "highly oxidized" LDL. The macrophage scavenger receptors are responsible for the rapid uptake of the highly modified LDL [23]. Oxidized molecules of low-density lipoproteins, and also heat shock protein 60 (HSP60) (endogenous TLR-ligands) and/or bacterial toxins (exogenous TLR-ligands) might trigger Toll-like receptor-dependent macrophage activation in the arterial wall [24, 25].

During the following phase macrophages "present the antigen" to T lymphocytes. This antigen may be a fragment of oxidized LDL "digested" by the macrophages, HSP60, β2-glycoprotein I or the fragments of bacterial antigens [26]. The interaction requires the presence of CD40 receptor on the surface of macrophages and its ligand CD40L on the surface of T lymphocytes [27, 28]. It is currently believed that the immunological response of Th1 type and its mediators (IFN-γ, TNF-α, interleukin-1, interleukin-12, interleukin-18) accelerate atherosclerosis, whereas the response of Treg type and its mediators (interleukin-10 and TGF-β) inhibit the development of atherosclerosis [29-31]. Therefore an idea of vaccination aroused as a future treatment against atherogenesis [32, 33].

The next phase of atherogenesis is the development of fibrous atheroma. The deposition of extracellular cholesterol and its esters is then intensified as well as the migration of smooth muscle cells from media to intima layer of artery wall, their proliferation and finally excessive production of the extracellular matrix.

A stable atheromatous plaque is most commonly covered with a fairly thick fibrous layer, protecting the lipid nucleus from contact with the blood. In an unstable plaque there is a big lipid nucleus with a fairly thin fibrous layer. In atheromatous plaque, changed as described above, the proinflammatory factors produced by T lymphocytes (such as IFN-γ) seem to play a crucial role. They decrease production of the extracellular matrix by smooth muscles and at the same time increase production of the metalloproteinases by macrophages [34].

In a number of reports published so far there has been a tendency to consider atherogenesis as the effect of dyslipidemia or inflammation alone. It is an erroneous dichotomy. It should be emphasized that atherosclerosis results from both lipid disorders and enhanced inflammation. Therefore, atherosclerosis is a chronic inflammatory disease, in most cases initiated and aggravated by hypercholesterolemia. Thus, hypercholesterolemia and inflammation were described as "partners in crime" [35].

There are numerous studies reporting that certain strains of probiotic bacteria reduce cholesterol level both in rodents and in humans [36-38]. Prebiotics administration (for 16

weeks) to ApoE–/– mice resulted in the reduction of atherosclerotic lesion size [39]. Well documented are anti-inflammatory and immunoregulating properties of probiotics reviewed elsewhere [40, 41]. We have shown that not only whole probiotic bacteria, but exopolysaccharide (EPS), the component of the cell wall of *Lactobacillus rhamnosus* has immunomodyfying and anti-inflammatory properties, both *in vitro* [42] and *in vivo* [43]. Our preliminary study suggest that *L. rhamnosus* EPS decreases atherosclerosis development in apoE–/– mice fed high-fat diet (unpublished data).

Several lines of evidence point to the important role of autoimmune processes in the development of atherosclerosis. The role of HSP60 as an initiator of atherogenesis is currently intensively investigated. Its "molecular mimicry" with HSP of *Chlamydia* has been recognized [44]. Moreover, the fact that anti-oxLDL antibodies resemble antiphospholipid antibodies, strengthen the concept of atherosclerosis as an autoimmune disease [26, 45, 46]. The investigators also emphasize similarities in pathogenesis of atherosclerosis and rheumatoid arthritis [47].

The inflammatory concept of atherosclerosis, formulated just in the recent years, is an unquestionable achievement of science which also have specific therapeutic implications [48-54].

Animal models of atherosclerosis

The first evidence of experimental atherosclerosis came into view as early as in 1908 when Ignatowski [55] reported thickening of the intima with formation of large clear cells in the aorta of rabbits fed a diet rich in animal proteins (meat, milk, eggs).

The most useful animal models have for a long time been restricted to relatively large animals, such as nonhuman primates, swine, and rabbits. Hamsters and pigeons have been occasionally used, but present problems peculiar to their species. Rats and dogs do not develop spontaneous lesions and require heavy modifications of diet to produce vascular lesion. Although rabbits do not develop spontaneous atherosclerosis they are highly responsive to cholesterol manipulation and develop lesions in a fairly short time. However, the lesions are much more fatty and macrophage-rich (inflammatory) than the human lesions and plasma cholesterol levels are extraordinarily high (very dissimilar to humans). Pigs and monkeys are better suited to model human atherosclerotic lesions. However, nowadays monkeys are not widely used due to obvious species-specific concerns (risk of extinction) and costs. The pigs are a very good model – when fed with cholesterol, they reach plasma levels and atherosclerotic lesions that are quite similar to those seen in humans. However, costs, the difficulties involved in maintaining the colonies and in their handling make this model inconvenient [56].

What has been lacking for long time was a small, genetically reproducible, murine model of atherosclerosis. Such a model could help to overcome the many problems and deficiencies of larger animals and, in particular, would permit studies of possible therapies that require relatively large numbers of animals.

Until 1992, the majority of atherosclerotic research focused on mechanisms in rabbits, with a lesser number of studies in pigs and nonhuman primates. These large animal models have provided invaluable insight. Studies in monkeys and rabbits have been pivotal in defining the cellular events in the initiation and development of lesions [57]. In recent years, there has been an explosion in the number of *in vivo* studies what is largely attributable to the use of mouse models to study atherogenic mechanisms.

Of mice and men – mouse as a model of atherosclerosis

Overall, mice are highly resistant to atherosclerosis. The only exception is the C57BL/6 strain. These mice develop atherosclerosis when fed a very high cholesterol diet containing cholic acid, however, the vascular lesions in the C57BL/6 differ from the human condition in the histologic nature and location and are possibly attributed to an aggressive, chronic inflammatory state caused by cholic acid administration.

The earliest mouse model of atherosclerosis was the diet-induced model characterized during the 1960s in Wissler's laboratory. Special diet containing 30% fat, 5% cholesterol, and 2% cholic acid led to atherosclerosis in C57BL/6 mice. However, this was a very toxic causing lost weight and often morbid respiratory infections. Paigen *et al*. modified this diet by blending it one part to three parts with a 10% fat diet to yield what is called the "Paigen diet" which consists of 15% fat, 1.25% cholesterol, and 0.5% cholic acid [58].

Paigen and colleagues also developed assays that are widely used to quantify atherosclerosis in the mouse models. The measurement of the cross-sectional lesion area in the aortic root is the most standard assay [58]. Freshly perfused and isolated hearts are fixed in formalin, embedded in gelatin, frozen, and cut into thin sections at anatomically defined sites in the aortic sinus and valve region. These sections are stained for lipids, and the lesion area is measured microscopically.

Although this model has been widely employed, the pathology of the lesions is not ideally suited as a model for human atherosclerosis. This shortcoming led many investigators to downplay the role of the mouse as a good model of atherosclerosis. Lesion formation in the diet-induced model is largely limited to the aortic root after feeding the Paigen diet for periods of 14 weeks to 9 months. The lesions are quite small, only several hundred to a few thousand square micrometers, and they consist almost entirely of macrophage foam cells with little evidence for smooth muscle cell involvement. Thus, this model is largely limited to

the fatty streak stage and does not progress to resemble human intermediate lesions.

For many years the mouse was not used as an experimental model for atherosclerosis research because of the beliefs that mice could not survive on high-fat atherogenic diets, that lesions are not reproducible (most mice do not develop lesions), and that lesion pathology do not resemble atherosclerosis in humans.

To judge the usefulness of animal model for atherosclerosis research the following questions should be asked: 1) what is the nature of the experimental lesions and their similarity to human lesions; 2) is the plasma lipoprotein profile and metabolism similar to metabolism in humans; 3) what is the time frame necessary for lesions to form, and how long does it take to breed the animals for the studies; 4) what is the cost of acquiring and maintaining the animals; 5) what is the ability to perform in vivo manipulations and imaging; and 6) what is the ability of the model to take advantage of classical and molecular genetic approaches?

The average lifespan of a mouse is about 2 years, compared to about 75 years in humans. Mice weigh much less, about 30 grams for the adult. The lipid profile in the mouse is very different from that in humans, who carry about 75% of their plasma cholesterol on LDL. Mice carry most of their cholesterol on high-density lipoprotein (HDL), which in humans is considered protective. Mice fed their normal low-fat chow diet do not develop atherosclerosis, while it is a common in humans (one difference, which is an advantage of all animal models, is the ability to control the environment and diet in mouse studies, which is impossible for long-term human studies). Also, the mouse immune system, although well understood and readily manipulated, diverges in many ways from that of humans (see below). Human genetic studies are limited in range to various types of association studies. With mice, on the other hand, many additional kinds of genetic experiments are possible, including breeding and genetic engineering [56].

Apolipoprotein E knockout mice – a breakthrough in atherosclerotic research

It has been a long-standing goal of many investigators around the world to create better mouse models for lipoprotein disorders and atherosclerosis and to identify genes that may modify atherogenesis and lesion progression.

In 1992 apoE-deficient mice were generated by inactivating the apoE gene by targeting [59]. The apoE gene was inactivated in mouse embryonic stem (ES) cells by homologous recombination. Two targeting plasmids were used, pJPB63 and pNMC109, both containing a neomycin-resistance gene that replaced a part of the apoE gene and disrupted its structure. Embryonic stem cell colonies targeted after electroporation with plasmids were identified by the polymerase chain reaction (PCR) followed by genomic Southern analysis. Chimeric mice were generated by injecting targeted cells into blastocysts. They gave strong chimeras, which transmitted the disrupted apoE gene to their progeny. Mice homozygous for the disrupted gene were obtained from the heterozygotes. As the homozygous animals have been born at the expected frequency and they appeared to be healthy, Piedrahita *et al*. demonstrated that the lack of apoE did not interfere with normal development of mice. At the same time another group also created apoE-deficient mice [60]. Mice homozygous or heterozygous for the disrupted apoE gene appeared healthy. No difference in their body weight compared to normal mice was observed.

However, significant phenotypic differences between normal animals and the homozygous mutants were observed in their lipid and lipoprotein profiles. The apoE-knockout mice had markedly (5 times) increased total plasma cholesterol levels, regardless of the age or sex of the animals. Although the total plasma cholesterol levels were greatly elevated in the mutants, the high density lipoprotein (HDL) cholesterol levels were only 45% of the normal level. The triglyceride levels were 68% higher than those in normal animals. (These apoE-deficient mice have had a dramatic shift in plasma lipoproteins from HDL, the major lipoprotein in control mice, to cholesterol-enriched remnants of chylomicrons and VLDL).

A chronological analysis of atherosclerosis development in the apoE-deficient mice has shown that the sequential events involved in lesion formation in this model are strikingly similar to those in well-established larger animal models of atherosclerosis and in humans [61]. Animals as young as 5-6 weeks of age have monocytic adhesions to the endothelial surface of the aorta that can be appreciated readily with electron microscopy (EM). Electron microscopy also has demonstrated transendothelial migration of blood monocytes in similarly aged mice. By 6-10 weeks of age, most apoE-deficient mice have developed fatty-streak lesions primarily consisting of foam cells with migrating smooth muscle cells. These fatty-streak lesions rapidly progress to advanced lesions, which are heterogeneous but are typically composed of a necrotic core surrounded by proliferating smooth muscle cells and varying amounts of extracellular matrix, including collagen and elastin.

These lesions have well-formed fibrous caps made up of smooth muscle cells and extracellular matrix that often have groups of foam cells at their "shoulders". It is not uncommon for the inflammatory lesion to erode deep into the medial wall of the aorta, and some of these animals develop aortic aneurysms. Many of the lesions found in older mice develop calcified foci [62].

The diet developed by Hayek *et al*. has been considered a more physiological than Paigen diet; this "western-type" diet for mouse studies is similar in composition to an average American diet of several years ago, consisting of 21% fat by weight, 0.15% cholesterol, and no cholic acid. When fed this diet, wild-type mice have a two-fold elevation in

Table 1. Comparison of lipid levels between wild mice and apoE-knockout mice

Animals	Total cholesterol in mg/dl ±SD	HDL cholesterol in mg/dl ±SD	Triglyceride in mg/dl ±SD
normal	86 ±20	73 ±28	73 ±36
apoE-knockout mice	434 ±129	33 ±15	123 ±51

plasma cholesterol, while apoE-deficient mice have over a three-fold elevation, to about 2000 mg/dl, again, mostly in βVLDL, but there is also an increase in LDL [60] (Table 1). The post-prandial clearance of intestinally derived lipoproteins is dramatically impaired in apoE-deficient mice. The apoE-deficient mice respond appropriately to a human-like western-type diet [61]. On this diet, lesion formation is greatly accelerated and lesion size is increased. In 10-week old animals fed this diet for only 5 weeks, lesions are 3-4 times the size of those observed in mice fed a low-fat diet. In addition, monocytic adhesions and advanced lesions develop at a significantly earlier age. The results of this dietary challenge demonstrate that the mouse model responds in an appropriate manner, i.e. increased fat leads to increased plasma cholesterol, which in turn leads to increased atherosclerosis. Moreover, the data suggest that in addition to its histological similarity to humans, the mouse model exhibits a response to environmental cues resembling that of humans.

Lesions in the apoE-deficient mouse, as it is in humans, tend to develop at vascular branch points and progress from foam cell stage to the fibroproliferative stage with well-defined fibrous caps and necrotic lipid cores, although plaque rupture has not been observed in apoE-deficient mice or in any other mouse model. Progression of lesions appears to occur at a faster rate than in humans atherosclerosis; the rapidity of lesion progression can be advantageous in many experimental situations.

Comparing humans and apoE-deficient mice, lesion progression and cell types involved are similar, as is the presence of oxidized lipoproteins. However, the plaque rupture, which is typical for all animal models of this disease and fairly common in humans, is not observed in apoE-knockout mice. One potential reason for the lack of plaque rupture in mice is that the diameter of the aorta is less than 1 mm, which is even smaller than the diameter of the major coronary arteries in humans. As the vessel diameter decreases, the surface tension increases exponentially; thus, in the mouse there may be so much surface tension that plaque rupture would not be likely to occur.

ApoE-knockout mice are considered to be one of the most relevant models for atherosclerosis since they are hypercholesterolemic and develop spontaneous arterial lesions. The apoE-deficient mouse contained the entire spectrum of lesions observed during atherogenesis and was the first mouse model to develop lesions similar to those of humans. This model provided opportunity to study the pathogenesis and therapy of atherosclerosis in a small, genetically defined animal.

As has been already mentioned, the method of measurement of atherosclerosis by using the "aortic root" atherosclerosis assay was originally developed by Paigen *et al.*

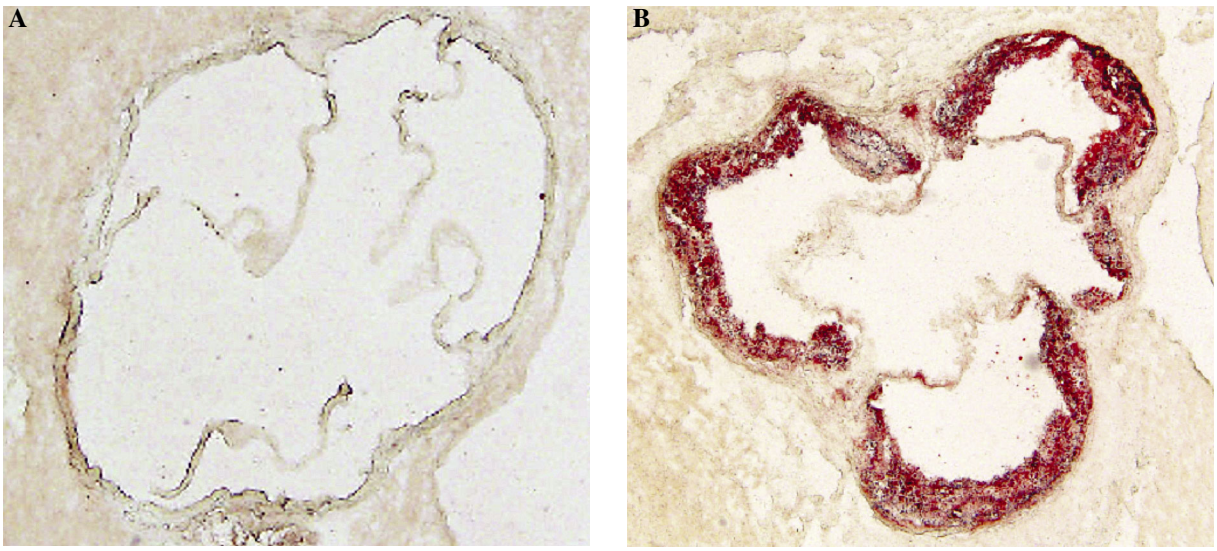

Fig. 1. Representative micrographs showing oil-red O stained "aortic roots" ("cross-sections") from wild mice (**A**) and apoE-knockout mice (**B**)

[58]. The aortic root "cross sectioning" assay (Fig. 1), widely used in murine studies of atherosclerosis, allows for coincident inspection of lesion histology, and is amenable in studies using large numbers of mice. Alternative measurement of atherosclerosis, such as the "en face" (Fig. 2) method also correlates with aortic root measurements. However, this method is less amenable for studies using large numbers of mice and does not allow for inspection of lesion histology.

Low-density lipoproteins receptor-deficient mice

Gene targeting in embryonic stem cells has been used to create LDL receptor-knockout (LDLR-KO) mice, a model of familial hypercholesterolemia. Low-density lipoproteins receptor-deficient mice were made in 1993 [63]. These mice have a more modest lipoprotein abnormality than the apoE-deficient mice, with increases in LDL and VLDL cholesterol leading to a total plasma cholesterol of about 250 mg/dl on a chow diet. Under such conditions LDL receptor-deficient mice do not get atherosclerosis. However, this is a very diet-responsive model. After these mice were fed the Paigen diet, their plasma cholesterol levels soar to about 1500 mg/dl, and large atherosclerotic lesions form [64]. It has also been shown that feeding the less toxic western-type diet also leads to the development of large lesions, with plasma cholesterol levels of about 400 mg/dl. The lesion pathology in this model is not as well characterized as in the apoE-deficient model, but it does appear similar in that the lesions can progress beyond the foam-cell fatty-streak stage to the fibro-proliferative intermediate stage.

Newest mice models

More recently, apoE and LDL-receptor (LDLr) double-knockout (apoE/LDLr-DKO) mice have been created [64], representing a new mouse model that develops severe hyperlipidaemia and atherosclerosis [65]. It has been reported that, even on a regular chow diet, the progression of atherosclerosis is usually more marked in apoE/LDLr-DKO mice than in mice deficient for apoE alone [66]. Thus, the apoE/LDLr-DKO mouse is a suitable model to study the anti-atherosclerotic effect of agents, without having to feed the animals an atherogenic diet.

To study the contribution of endothelial nitric oxide synthase (eNOS) to lesion formation apoE/eNOS double-knockout mice were created [67]. It turned out that chronic deficiency of eNOS increases atherosclerosis in apoE KO mouse model. Furthermore, in the absence of eNOS, peripheral coronary disease, chronic myocardial ischemia, heart failure, and an array of vascular complications develop that have not been observed in apoE-KO animals.

Recently, Veniant *et al*. [68] managed to even up the cholesterol levels in chow-fed apoE-KO mice and LDLR-KO mice. They did so by making both mouse models homozygous for the apolipoprotein B-100 allele, which ameliorates the hypercholesterolemia in the setting of apoE deficiency but worsens it in the setting of LDLR deficiency. Moreover, the LDLR-KO Apob100/100 mice developed extensive atherosclerosis even on a chow diet. So far this model seems to be the best as concerns the development of atherosclerosis in mice.

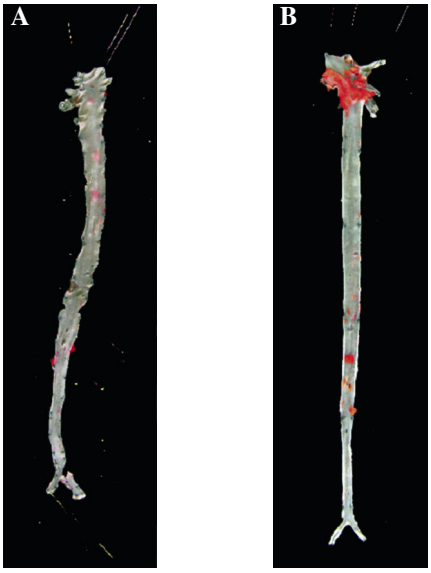

Fig. 2. Representative Sudan-IV stained "en face" preparations of aortas from wild mice (**A**) and apoE-knockout mice (**B**)

Immune mechanisms of atherosclerosis

The possibility that immune system might be involved in atherogenesis emerged with the discovery of T cells and macrophages in human atheromata [1]. Identification of activation markers of almost all cell types involved in innate and adaptive immunity in atheromatous plaques suggest that immune cells in lesions are functionally significant [69].

Although the specific antigen has not been clearly identified, both cellular and humoral immune responses are generated in humans and experimental animals, possibly towards LDL and HSP60. Based on the studies in mice, powerful pro-atherogenic activity has been attributed to Th-1 cells producing pro-inflammatory cytokines: IFN-γ and TNF-α [70]. On the other hand, atheroprotective signals are delivered by regulatory T cells (Tregs) mainly by anti-inflammatory cytokines, IL-10 and TGF-β, which countervail the pro-inflammatory cytokines [71]. Hence, both stimulatory and inhibitory mechanisms operate during atherogenesis. Progress and challenges in translating the biology of atherosclerosis has been described by Libby *et al*. [72].

In general, studies over past 20 years have proved that apoE-deficient mouse model of atherosclerosis can be successfully applied to: 1) identify atherosclerosis susceptibil-

ity modifying genes, by the candidate-gene and gene-mapping methods; 2) identify the role of various cell types and mediators (including immune cells and various cytokines) in atherogenesis; 3) identify environmental factors affecting atherogenesis; and 4) assess multiple therapies that might partially block atherogenesis or lesion progression [33, 52, 56, 73-77].

Acknowledgements

This article was supported by the grant from Polish Ministry of Science and Higher Education nr: N N401 548340 for the years 2011-2012 and nr: N N401 042438 (2010-2013).

References

1. Jonasson L, Holm J, Skalli O, et al. (1986): Regional accumulations of T cells, macrophages, and smooth muscle cells in the human atherosclerotic plaque. Arteriosclerosis 6: 131-138.
2. Stemme S, Faber B, Holm J, et al. (1995): T lymphocytes from human atherosclerotic plaques recognize oxidized low density lipoprotein. Proc Natl Acad Sci USA 92: 3893-3897.
3. Thom DH, Wang SP, Grayston JT, et al. (1991): Chlamydia pneumoniae strain TWAR antibody and angiographically demonstrated coronary artery disease. Arterioscler Thromb 11: 547-551.
4. Hendrix MG, Salimans MM, van Boven CP, Bruggeman CA (1990): High prevalence of latently present cytomegalovirus in arterial walls of patients suffering from grade III atherosclerosis. Am J Pathol 136: 23-28.
5. Gupta S, Pablo AM, Jiang X, et al. (1997): IFN-gamma potentiates atherosclerosis in ApoE knockout mice. J Clin Invest 99: 2752-2761.
6. Nakashima Y, Raines EW, Plump AS, et al. (1998): Upregulation of VCAM-1 and ICAM-1 at atherosclerosis-prone sites on the endothelium in the ApoE-deficient mouse. Arterioscler Thromb Vasc Biol 18: 842-851.
7. Aiello RJ, Bourassa PA, Lindsey S, et al. (1999): Monocyte chemoattractant protein-1 accelerates atherosclerosis in apolipoprotein E-deficient mice. Arterioscler Thromb Vasc Biol 19: 1518-1525.
8. Ni W, Egashira K, Kitamoto S, et al. (2001): New anti-monocyte chemoattractant protein-1 gene therapy attenuates atherosclerosis in apolipoprotein E-knockout mice. Circulation 103: 2096-2101.
9. Elhage R, Jawien J, Rudling M, et al. (2003): Reduced atherosclerosis in interleukin-18 deficient apolipoprotein E-knockout mice. Cardiovasc Res 59: 234-240.
10. Tenger C, Sundborger A, Jawien J, Zhou X (2005): IL-18 accelerates atherosclerosis accompanied by elevation of IFN-gamma and CXCL16 expression independently of T cells. Arterioscler Thromb Vasc Biol 25: 791-796.
11. Mach F, Schönbeck U, Sukhova GK, et al. (1998): Reduction of atherosclerosis in mice by inhibition of CD40 signaling. Nature 394: 200-203.
12. Welt FG, Rogers SD, Zhang X, et al. (2004): GP IIb/IIIa inhibition with eptifibatide lowers levels of soluble CD40L and RANTES after percutaneous coronary intervention. Catheter Cardiovasc Interv 61: 185-189.
13. Alber HF, Frick M, Suessenbacher A, et al. (2006): Effect of atorvastatin on circulating proinflammatory T-lymphocyte subsets and soluble CD40 ligand in patients with stable coronary artery disease – a randomized, placebo-controlled study. Am Heart J 151: 139.
14. Tousoulis D, Antoniades C, Nikolopoulou A, et al. (2007): Interaction between cytokines and sCD40L in patients with stable and unstable coronary syndromes. Eur J Clin Invest 37: 623-628.
15. Zhou X, Nicoletti A, Elhage R, Hansson GK (2000): Transfer of CD4(+) T cells aggravates atherosclerosis in immunodeficient apolipoprotein E knockout mice. Circulation 102: 2919-2922.
16. Ross R (1999): Atherosclerosis – an inflammatory disease. N Engl J Med 340: 115-126.
17. Dunzendorfer S, Lee HK, Tobias PS (2004): Flow-dependent regulation of endothelial Toll-like receptor 2 expression through inhibition of SP1 activity. Circ Res 95: 684-691.
18. Borén J, Olin K, Lee I, et al. (1998): Identification of the principal proteoglycan-binding site in LDL. A single-point mutation in apo-B100 severely affects proteoglycan interaction without affecting LDL receptor binding. J Clin Invest 101: 2658-2664.
19. Gaut JP, Heinecke JW (2001): Mechanisms for oxidizing low-density lipoprotein. Insights from patterns of oxidation products in the artery wall and from mouse models of atherosclerosis. Trends Cardiovasc Med 11: 103-112.
20. Fredrikson GN, Söderberg I, Lindholm M, et al. (2003): Inhibition of atherosclerosis in apoE null mice by immunization with apoB-100 peptide sequences. Arterioscler Thromb Vasc Biol 23: 879-884.
21. Pentikäinen MO, Oörni K, Ala-Korpela M, Kovanen PT (2000): Modified LDL – trigger of atherosclerosis and inflammation in the arterial intima. J Intern Med 247: 359-370.
22. Lusis AJ (2000): Atherosclerosis. Nature 407: 233-241.
23. Suzuki H, Kurihara Y, Takeya M, et al. (1997): A role for macrophage scavenger receptors in atherosclerosis and susceptibility to infection. Nature 386: 292-296.
24. Hansson GK (2005): Inflammation, atherosclerosis, and coronary artery disease. N Engl J Med 352: 1685-1695.
25. Hermansson A, Ketelhuth DF, Strodthoff D, et al. (2010): Inhibition of T cell response to native low-density lipoprotein reduces atherosclerosis. J Exp Med 207: 1081-1093.
26. Hansson GK (2001): Immune mechanisms in atherosclerosis. Arterioscler Thromb Vasc Biol 21: 1876-1890.
27. Schönbeck U, Sukhova GK, Shimizu K, et al. (2000): Inhibition of CD40 signaling limits evolution of established atherosclerosis in mice. Proc Natl Acad Sci USA 97: 7458-7463.
28. Phipps RP (2000): Atherosclerosis: the emerging role of inflammation and the CD40-CD40L system. Proc Natl Acad Sci USA 97: 6930-6932.
29. Daugherty A, Rateri DL (2002): T lymphocytes in atherosclerosis: the yin-yang of Th1 and Th2 influence on lesion formation. Circ Res 90: 1039-1040.
30. Laurat E, Poirier B, Tupin E, et al. (2001): In vivo downregulation of T helper cell 1 immune responses reduces atherogenesis in apolipoprotein E-knockout mice. Circulation 104: 197-202.
31. Pinderski LJ, Fischbein MP, Subbanagounder G, et al. (2002): Overexpression of interleukin-10 by activated T lymphocytes inhibits atherosclerosis in LDL receptor-deficient mice by altering lymphocyte and macrophage phenotypes. Circ Res 90: 1064-1071.

32. Hansson GK (2002): Vaccination against atherosclerosis: science or fiction? Circulation 106: 1599-1601.
33. Jawien J, Gajda M, Wołkow P, et al. (2008): The effect of montelukast on atherogenesis in apoE/LDLR-double knockout mice. J Physiol Pharmacol 59: 633-639.
34. Shishehbor MH, Bhatt DL (2004): Inflammation and atherosclerosis. Curr Atheroscler Rep 6: 131-139.
35. Steinberg D (2002): Atherogenesis in perspective: hypercholesterolemia and inflammation as partners in crime. Nat Med 8: 1211-1217.
36. Kawase M, Hashimoto H, Hosoda M, et al. (2002): Effect of administration of fermented milk containing whey protein concentrate to rats and healthy men on serum lipid and blood pressure. J Dairy Sci 83: 255-263.
37. Hlivak P, Odraska J, Ferencik M, et al. (2005): One-year application of probiotic strain Enterococcus faecium M-74 decreases serum cholesterol levels. Bratisl Lek Listy 106: 67-72.
38. Xie N, Cui Y, Yin YN, et al. (2011): Effects of two Lactobacillus strains on lipid metabolism and intestinal microflora in rats fed a high-cholesterol diet. BMC Complement Altern Med 11: 53.
39. Rault-Nania MH, Gueux E, Demougeot C, et al. (2006): Inulin attenuates atherosclerosis in apolipoprotein E-deficient mice. Br J Nutr 96: 840-844.
40. Adams CA (2010): The probiotic paradox: live and dead cells are biological response modifiers. Nutr Res Rev 23: 37-46.
41. Oelschlaeger TA (2010): Mechanisms of probiotic actions – a review. Int J Med Microbiol 300: 57-62.
42. Ciszek-Lenda M, Nowak B, Sróttek M, et al. (2011): Immunoregulatory potential of exopolysaccharide from Lactobacillus rhamnosus KL37: effects on the production of inflammatory mediators by mouse macrophages. Int J Exp Pathol 92: 382-391.
43. Nowak B, Ciszek-Lenda M, Śróttek M, et al. (2011): Lactobacillus rhamnosus exopolysaccharide ameliorates arthritis induced by the systemic injection of collagen and lipopolysaccharide in DBA/1 mice. Arch Immunol Ther Exp, submitted.
44. Wick G, Schett G, Amberger A, et al. (1995): Is atherosclerosis an immunologically mediated disease? Immunol Today 16: 27-33.
45. Kobayashi K, Tada K, Itabe H, et al. (2007): Distinguished effects of antiphospholipid antibodies and anti-oxidized LDL antibodies on oxidized LDL uptake by macrophages. Lupus 16: 929-938.
46. Wick G, Perschinka H, Millonig G (2001): Atherosclerosis as an autoimmune disease: an update. Trends Immunol 22: 665-669.
47. Shoenfeld Y, Sherer Y, Harats D (2001): Artherosclerosis as an infectious, inflammatory and autoimmune disease. Trends Immunol 22: 293-295.
48. Fan J, Watanabe T (2003): Inflammatory reactions in the pathogenesis of atherosclerosis. J Atheroscler Thromb 10: 63-71.
49. Libby P (2000): Changing concepts of atherogenesis. J Intern Med 247: 349-358.
50. Libby P (2002): Inflammation in atherosclerosis. Nature 420: 868-874.
51. Libby P, Ridker PM, Maseri A (2002): Inflammation and atherosclerosis. Circulation 105: 1135-1143.
52. Jawien J, Csanyi G, Gajda M, et al. (2007): Ticlopidine attenuates progression of atherosclerosis in apolipoprotein E and low density lipoprotein receptor double knockout mice. Eur J Pharmacol 556: 129-135.
53. Alpert JS, Thygesen K (2006): A call for universal definitions in cardiovascular disease. Circulation 114: 757-758.
54. Hansson GK, Hermansson A (2011): The immune system in atherosclerosis. Nat Immunol 12: 204-212.
55. Ignatowski AC (1908): Influence of animal food on the organism of rabbits. S Peterb Izviest Imp Voyenno-Med Akad 16: 154-173.
56. Jawień J, Nastałek P, Korbut R (2004): Mouse models of experimental atherosclerosis. J Physiol Pharmacol 55: 503-517.
57. Faggiotto A, Ross R, Harker L (1984): Studies of hypercholesterolemia in the nonhuman primate. I. Changes that lead to fatty streak formation. Arteriosclerosis 4: 323-340.
58. Paigen B, Morrow A, Holmes PA, et al. (1987): Quantitative assessment of atherosclerotic lesions in mice. Atherosclerosis 68: 231-240.
59. Piedrahita JA, Zhang SH, Hagaman JR, et al. (1992): Generation of mice carrying a mutant apolipoprotein E gene inactivated by gene targeting in embryonic stem cells. Proc Natl Acad Sci USA 89: 4471-4475.
60. Plump AS, Smith JD, Hayek T, et al. (1992): Severe hypercholesterolemia and atherosclerosis in apolipoprotein E-deficient mice created by homologous recombination in ES cells. Cell 71: 343-353.
61. Nakashima Y, Plump AS, Raines EW, et al. (1994): ApoE-deficient mice develop lesions of all phases of atherosclerosis throughout the arterial tree. Arterioscler Thromb 14: 133-140.
62. Reddick RL, Zhang SH, Maeda N (1994): Atherosclerosis in mice lacking apoE. Evaluation of lesional development and progression. Arterioscler Thromb 14: 141-147.
63. Ishibashi S, Brown MS, Goldstein JL, et al. (1993): Hypercholesterolemia in low density lipoprotein receptor knockout mice and its reversal by adenovirus – mediated gene delivery. J Clin Invest 92: 883-893.
64. Ishibashi S, Herz J, Maeda N, et al. (1994): The two-receptor model of lipoprotein clearance: tests of the hypothesis in "knockout" mice lacking the low density lipoprotein receptor, apolipoprotein E, or both proteins. Proc Natl Acad Sci USA 91: 4431-4435.
65. Bonthu S, Heistad DD, Chappell DA, et al. (1997): Atherosclerosis, vascular remodeling, and impairment of endothelium-dependent relaxation in genetically altered hyperlipidemic mice. Arterioscler Thromb Vasc Biol 17: 2333-2340.
66. Witting PK, Pettersson K, Ostlund-Lindqvist AM, et al. (1999): Inhibition by a coantioxidant of aortic lipoprotein lipid peroxidation and atherosclerosis in apolipoprotein E and low density lipoprotein receptor gene double knockout mice. FASEB J 13: 667-675.
67. Kuhlencordt PJ, Gyurko R, Han F, et al. (2001): Accelerated atherosclerosis, aortic aneurysm formation, and ischemic heart disease in apolipoprotein E/endothelial nitric oxide synthase double knockout mice. Circulation 104: 448-454.
68. Véniant MM, Withycombe S, Young SG (2001): Lipoprotein size and atherosclerosis susceptibility in Apoe-/- and Ldlr-/- mice. Arterioscler Thromb Vasc Biol 21: 1567-1570.
69. Hansson GK, Libby P (2006): The immune response in atherosclerosis: a double-edged sword. Nat Rev Immunol 6: 508-519.
70. Andersson J, Libby P, Hansson GK (2010): Adaptive immunity and atherosclerosis. Clin Immunol 134: 33-46.
71. Ait-Oufella H, Salomon BL, Potteaux S, et al. (2006): Natural regulatory T cells control the development of atherosclerosis in mice. Nat Med 12: 178-180.

72. Libby P, Ridker PM, Hansson GK (2011): Progress and challenges in translating the biology of atherosclerosis. Nature 473: 317-325.
73. Olszanecki R, Jawień J, Gajda M, et al. (2005): Effect of curcumin on atherosclerosis in apoE/LDLR – double knockout mice. J Physiol Pharmacol 56: 627-635.
74. Jawień J (2008): New insights into immunological aspects of atherosclerosis. Pol Arch Med Wewn 118: 127-131.
75. Franczyk-Zarów M, Kostogrys RB, Szymczyk B, et al. (2008): Functional effects of eggs, naturally enriched with conjugated linoleic acid, on the blood lipid profile, development of atherosclerosis and composition of atherosclerotic plaque in apolipoprotein E and low-density lipoprotein receptor double-knockout mice (apoE/LDLR-/-). Br J Nutr 99: 49-58.
76. Kus K, Gajda M, Pyka-Fosciak G, et al. (2009): The effect of nebivolol on atherogenesis in apoE- knockout mice. J Physiol Pharmacol 60: 163-165.
77. Toton-Zuranska J, Gajda M, Pyka-Fosciak G, et al. (2010): AVE 0991-angiotensin-(1-7) receptor agonist, inhibits atherogenesis in apoE-knockout mice. J Physiol Pharmacol 61: 181-183.