We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900
Open access books available

185,000
International authors and editors

200M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



1

Mouse Models of Experimental Atherosclerosis as a Tool for Checking a Putative Anti-Atherogenic Action of Drugs

Jacek Jawien
Jagiellonian University School of Medicine, Chair of Pharmacology, Krakow, Poland

1. Introduction

Studies concerning the pathogenesis of atherosclerosis entered a new phase at the turn of the 21st century. The 20th century was the age of cholesterol and lipoproteins, which has been concluded in a number of clinical studies carried out on a large scale, and they demonstrated unequivocally that normalization of hypercholesterolemia significantly decreased the incidence and mortality of coronary artery disease (Prevention of cardiovascular events and death with pravastatin in patients with coronary heart disease and a broad range of initial cholesterol levels. The Long-Term Intervention with Pravastatin in Ischaemic Disease (LIPID) Study Group. 1998; Scandinavian Simvastatin Survival Study Group. Randomised trial of cholesterol lowering in 4444 patients with coronary heart disease: The Scandinavian Simvastatin Survival Study (4S). 1994) Nearly to the end of the nineties, atherosclerosis had been assumed to develop as the so-called chronic response to injury (response-to-injury hypothesis) that resulted in the loss of endothelial cells which line the inner side of the vessels (Ross & Glomset, 1976).

Atherosclerosis had been considered first of all a degenerative disease (Ross et al., 1977; Ross et al. 1984; Ross, 1986). However, approximately 20 years ago, the trials started to focus to a large extent on another pathogenetic mechanism of atherosclerosis, not considered so far – the inflammatory process.

2. The first indications

In 1986, with the use 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 (Jonasson et al., 1986). Several years later it was shown that these lymphocytes "recognize" as antigens the oxidized molecules of low-density lipoproteins (LDL) – oxLDL (Stemme et al., 1995). Moreover, the correlation between atherosclerosis and the presence of at least two types of infectious microorganisms: *Chlamydia pneumoniae* and *Herpes simplex virus* were observed (Thom et al., 1991; Hendrix et al., 1990). It raised the question if the inflammatory process participate in atherosclerosis. Speculations of this kind were initially received with great scepticism because there was no spectacular and unequivocal evidence of a significant role of inflammation in atherosclerosis.

This evidence was delivered by a new technique – 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.

3. Additional evidence for the presence of inflammation in atherosclerosis

The newest model of atherosclerosis (described precisely at the end of the paper) enabled the investigators to create apoE-knockout mice, an ideal animal model to test the influence of singular proteins participating in the inflammatory response on the development of atherosclerosis. These studies showed, for example, that the absence of only one cytokine – interferon γ (IFN-γ), reduced atherosclerosis even by 60% (Gupta et al., 1997).
The overexpression of adhesive molecules (vascular adhesion molecule 1 and intercellular adhesion molecule 1) at sites with atheromatous changes was also observed in apoE‑knockout mice (Nakashima et al., 1998). Monocyte chemotactic protein was shown to play an important part in the progression of atheromatous lesions (Aiello et al., 1999; Ni et al., 2001). Moreover, it was observed that interleukin-18 knockout decreased atherosclerosis by 35% (Elhage et al., 2003; Tenger et al., 2005).
Inhibition of CD40 signaling reduced atherosclerosis (Mach et al., 1998). 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, platelets – with CD40L activates a number of transcription factors: NF-κB, AP-1, STAT-1 or Egr-1. Therefore, it influences, for example, the endothelial cell, which, in consequence, acquires proinflammatory and proatherosclerotic phenotype leading to the expression of adhesive molecules and tissue factor on its surface. It creates new possibilities of therapeutic approach, consisting in inhibition of the CD40–CD40L pathway (Welt et al., 2004; Alber et al., 2006; Tousoulis, et al. 2007). In mice the effect of CD40 is also antagonized by transforming growth factor β (Robertson et al., 2003).
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. It was demonstrated that transfer of T cells to these mice aggravated atherosclerosis even by 164% (Zhou et al., 2000).

4. 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 previous theory of atherosclerosis as a chronic response to injury) officially proclaimed that atherosclerosis was an inflammatory disease (Ross, 1999).
Whereas the deposition of atheromatous lipids and the accumulation of foam cells – macrophages filled with such lipids – in intima is the main morphological hallmark of atherosclerosis, the more subtle changes in the environment of the arterial wall, stimulated by the influx of inflammatory cells and local release of cytokines and other inflammatory mediators are currently recognized as the crucial causative factors of atherogenesis (Glass & Witztum, 2001; Binder et al., 2002).

Inflammation occurs in response to a factor that destabilizes the local homeostasis. The factors that cause Toll-like receptor dependent macrophage activation in the arterial wall include oxLDL, heat shock protein 60 (HSP60) and bacterial toxins (Hansson, 2005).

The first stage of atherogenesis consists in endothelial dysfunction (Ross, 1999). It involves first of all the regions of arterial bifurcations where the blood flow is not laminar. Hence, these localizations are prone to develop atherosclerosis. In such places LDL is stored in the subendothelial space. Low-density lipoprotein accumulation is increased if serum LDL level is elevated. Low-density lipoprotein is transported by passive diffusion and its accumulation in the vascular wall seems to depend on the interaction between apolipoprotein B of the LDL molecule and proteoglycans of the matrix (Boren et al., 1998).

There is evidence that unchanged LDL are "collected" by the macrophages too slowly to activate their transformation into foam cells. Therefore, it has been suggested that LDL molecule is "modified" in the vascular wall. The most significant modification is lipid oxidation, resulting in the formation of so-called "minimally oxidized" LDL (Gaut & Heinecke, 2001). The generation of these "aliens" for the body molecules leads to the development of inflammatory response, with participation of monocytes and lymphocytes in the first place (Fredrikson et al., 2003; Pentikäinen et al., 2000). The 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 (Lusis, 2000).

Before the "minimally oxidized" LDL have been phagocytised by the macrophages, they have to be modified into "highly oxidized" LDL. The scavenger receptors are responsible for the rapid uptake of the modified LDL (Suzuki et al., 1997).

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 (Hansson, 2001). The interaction between the immunological cells requires the presence of CD40 receptor on the surface of macrophages and its ligand CD40L on the surface of T lymphocytes (Schonbeck et al., 2000; Phipps et al., 2000). It is currently believed that the immunological response of Th1 type and its mediators: IFN-γ, TNF-α, interleukin-1, interleukin-12 as well as interleukin-18 accelerate atherosclerosis, whereas the response of Treg type and its mediators: interleukin-10 and TGF-β inhibit the development of atherosclerosis (Daugherty & Rateri, 2002; Laurat et al., 2001; Pinderski et al., 2002). Therefore, there has arisen an idea of vaccination as a future treatment against atherogenesis (Hansson, 2002).

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, proliferation of these cells and finally production of the extracellular matrix by the smooth muscles cells.

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 (Shishehbor & Bhatt, 2004).

5. Is atherosclerosis an autoimmunological disease?

The role of HSP60 as an initiator of atherogenesis is currently intensively investigated. Its "molecular mimicry" with HSP of *Chlamydia* has been observed (Wick et al., 1995).
Moreover, the anti-oxLDL antibodies resemble antiphospholipid antibodies, therefore the concept of atherosclerosis as an autoimmunological disease has been established (Hansson, 2001; Kobayashi K et al., 2007; Wick G et al., 2001). The investigators also emphasize a high pathogenetic similarity of atherosclerosis to rheumatoid arthritis (Shoenfeld et al., 2001).

6. The new experimental model of atherosclerosis

Since 1992 the mouse has become an excellent object for the studies on atherosclerosis, replacing the previous animal models. (Paigen et al., 1994; Moghadasian, 2002; Jawien et al., 2004).
Then, the first line of mice with a switched off gene for apolipoprotein E (apoE-knockout) was developed almost contemporaneously in two laboratories in the United States. (Piedrahita et al., 1992; Plump et al., 1992).
These mice were soon described as "reliable and useful, the best animal model of atherosclerosis in present times" (Meir & Leitersdorf, 2004).
During the generation of apoE-knockout mice (known also as apoE null or apoE deficient mice) the normal gene coding apolipoprotein E is replaced by a mutated gene which does not produce this molecule. Such mice are called apoE knockout because they have a knockout, switched-off, null or inactivated gene coding apolipoprotein E. For clarity, in the following sections of this paper we will use the most popular name: apoE-knockout mice.
The year 1992, in which apoE-knockout mice were invented by a homological exchange of genes, was a real breakthrough year in the studies on the pathogenesis of atherosclerosis (Savla, 2002).
The apoE-knockout mice were formed by homological recombination of embryonic stem cells. The changed cells were implanted into the blastocyst of a mouse of C57BL/6J strain which were subsequently implanted into the uterus. The offspring was a "chimera" that was next crossbred with a mouse of C57BL/6J strain (wild type), which led to the formation of apoE-knockout, homozygous mice in the second generation (Capecchi, 2001).
The inactivation of the gene coding apoE resulted in the formation of mice with a phenotype with a complete suppression of apoE, but with preservation of fertility and vitality (Breslow, 1996).
The apoE-knockout mice, in contrast to all of other animal models, develop atherosclerosis spontaneously, without high-cholesterol diet (Hansson et al., 2002).
The generation of such a model changed the nature of the studies on the pathogenesis of atherosclerosis and enabled the investigators to formulate a new definition of atherosclerosis as a chronic inflammation (Savla, 2002).
In a number of reports on atherogenesis published so far there has been a tendency to consider this process 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. In the review published in Nature Medicine hypercholesterolemia and inflammation were described as "partners in crime" (Steinberg, 2002).

The inflammatory concept of atherosclerosis has been formulated just in the recent years. However, it is currently an unquestionable achievement of science which also have specific therapeutic implications (Fan & Watanabe, 2003; Libby, 2000; Libby, 2002; Libby P et al., 2002; Jawien et al., 2006; Alpert & Thygesen, 2007).

7. Animal models of atherosclerosis

Atherosclerotic cardiovascular disease, the major cause of death in Western society, results from complex interactions among multiple genetic and environmental factors.
Numerous animal species have been used to study the pathogenesis and potential treatment of the lesions of atherosclerosis. The first evidence of experimental atherosclerosis came into view as early as in 1908 when Ignatowski (Ignatowski, 1908) reported thickening of the intima with formation of large clear cells in the aorta of rabbits fed with a diet rich in animal proteins (meat, milk, eggs). The most useful animal models have thus far been restricted to relatively large animals, such as nonhuman primates, swine, and rabbits. Hamsters and pigeons have been used occasionally but present problems peculiar to their species. Rats and dogs are not good models for atherosclerosis because they do not develop spontaneous lesions and require heavy modifications of diet to produce vascular lesion. Despite the fact that rabbits do not develop spontaneous atherosclerosis, they are useful because they are highly responsive to cholesterol manipulation and develop lesions in a fairly short time (Drobnik et al., 2000).
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 cost. The pig is a very good model - when fed with cholesterol, they reach plasma levels and atherosclerotic lesions that are quite similar to those seen in humans. Problems with the pig model are costs, the difficulties involved in maintaining the colonies and in their handling.
What has been traditionally lacking 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. The use of pig models of the disease initially revealed that monocyte infiltration was one of the primary cellular events in the atherogenic process (Gerrity, 1981).
Studies in monkeys and rabbits have been pivotal in defining the cellular events in the initiation and development of lesions (Faggiotto & Ross, 1984; Rosenfeld et al., 1987). In recent years, there has been an explosion in the number of *in vivo* studies that is largely attributable to the use of mouse models to study atherogenic mechanisms.

8. Mouse as a model of atherosclerosis

Mice are highly resistant to atherosclerosis. The only exception in mice is the C57BL/6 strain. 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 a chronic inflammatory state rather than a genetic predisposition.

The earliest mouse model of atherosclerosis was the diet - induced model that was first characterized during the 1960s in Wissler's laboratory. Special diet contained 30% fat, 5% cholesterol, and 2% cholic acid led to atherosclerosis in C57BL/6 mice. However, this was a very toxic diet on which the mice lost weight and often got sick with 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 (Paigen et al., 1985).

Although there were many uses of this model, there were also many disadvantages. The lesions are very small in mice at 4 to 5 months of age, in order of 200 to 1 000 square microns in the aortic root. The lesions are largely confined to the aortic root, and they usually do not develop beyond the early foam-cell, fatty-streak stage. The diet is also unphysiological with regard to its extremely high cholesterol content, 1.25%, and the presence of cholic acid. In addition, Lusis et al. have shown that this diet is in itself inflammatory, as leads to the induction of hepatic NF-kB activation and the expression of acute phase reactants, such as serum amyloid A (Liao et al., 1993).

Paigen et al. colleagues also developed assays that are widely used to quantify atherosclerosis in the mouse model. The most standard assay is the measurement of the cross-sectional lesion area in the aortic root (Paigen et al., 1987).

In this assay, 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 and is of significant use in the study of atherosclerosis, the pathology of the lesions are 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 were not reproducible, that most mice did not get lesions, and that lesion pathology did not resemble atherosclerosis in humans. However, the use of lower - fat diets solved the survival problem; the use of inbred strains rather than random - bred mice solved the reproducibility problem; the use of susceptible strains resulted in most mice getting lesions; and longer experimental times showed that lesions with fibrous caps were produced.

The following is a list of questions that can be used to judge the usefulness of animal models of atherosclerosis: 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 mouse as a model meets many of these criteria, but first it is important to acknowledge many important differences between mice and humans. 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 we know in humans is protective against atherosclerosis. Thus, mice fed their normal low-fat chow diet do not get atherosclerosis, while it is a common disease 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. 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.
There are many advantages of using mice for experimental atherosclerosis research, including their relative ease and thriftiness to acquire and maintain. Their generation time is short, at about 9 weeks, 3 weeks for gestation and about 6 weeks until sexual maturity. It is easy to breed very large cohorts for experimental studies, and mice can develop atherosclerosis in a very short timeframe, as discussed below. Classical genetics in the mouse is very well established and is aided immensely by the availability of hundreds of inbred strains. Moreover, in 2002, The Mouse Genome Sequencing Consortium published the culmination of international efforts - a high quality sequence and analysis of the genome of the C57BL/6J mouse strain (Waterson et al., 2002).
With the coming of age of molecular genetics, it is now possible to add exogenous transgenes into mice, which can also be done in many other species. However, uniquely in mice, it is also possible to knock out or replace endogenous genes; this is one of the main advantages of working in the mouse model. The major disadvantage of the mouse model is their small size, which makes it difficult but not impossible to perform surgical manipulations and *in vivo* imaging. But there have been recent advances in these techniques that have overcome many of the size limitations, such as the ability to perform imaging of abdominal atherosclerotic lesions in living mice, cardiac catheterization to determine cardiovascular function in free-ranging mice, and surgical ligature of coronary arteries giving rise to myocardial ischemia.

9. Apolipoprotein E–knockout mice: A breakthrough

It has been a longstanding 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 (Piedrahita et al., 1992).
They inactivated the apoE gene 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 stucture. ES 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 blastocyst injection with targeted lines. They gave strong chimeras, which transmitted the

disrupted apoE gene to their progeny. Mice homozygous for the disrupted gene were produced from the heterozygotes. The facts that homozygous animals have been born at the expected frequency and that they appeared to be healthy were important. They demonstrated that lack of apoE was compatible with normal development, and they also provided another tool for studies of the phenotypic consequences of apoE deficiency. At the same time another group created also apoE - deficient mice (Plump et al., 1992).

Mice homozygous or heterozygous for the disrupted apoE gene appeared healthy. No difference in their body weights 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 increased total plasma cholesterol levels, which were five times those of normal litter mates. These levels were unaffected by 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% the normal level. The triglyceride levels were 68% higher than those of 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.

Mice naturally have high levels of HDL and low levels of LDL, in contrast to humans who are high in LDL and low in HDL. In addition, mice apparently lack the cholesteryl ester transfer protein, en enzyme that transfers cholesterol ester from HDL to VLDL and LDL. Despite these differences, apoE - deficient mice have phenotypes remarkably similar to those of apoE - deficient humans.

A chronological analysis of atherosclerosis in the apoE - deficient mouse 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 (Nakashima et al., 1994). 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). EM 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 comprised primarily of foam cells with migrating smooth muscle cells. These fatty - streak lesions rapidly progress to advanced lesions, which are heterogeneous but are typically comprised 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 (Reddick et al., 1994).

Other characteristics of the lesions in the apoE - deficient mouse, such as indications of oxidative change, merit attention as well (Palinski et al., 1994).

The atherosclerotic lesions in this mouse contain oxidation - specific epitopes. In young lesions these epitopes are predominantly localized in macrophage - rich areas, whereas in advanced lesions they are localized in necrotic regions. In addition, high titers of antibodies against the oxidized epitopes are present in the plasma of the apoE - deficient mice.

The complexity of lesions in the apoE - deficient mouse, together with the benefits of using the mouse as a model of human disease, makes it a desirable system in which to study both

environmental and genetic determinants of atherosclerosis. Initial studies examined the effects of grossly different diets on susceptibility to atherosclerosis in this animal. These studies confirmed the validity of this mouse as a model of human atherosclerotic disease and laid the groundwork for future dietary studies.

Hayek et al. developed a more physiological than Paigen diet - "western-type" diet for mouse studies, which 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 plasma cholesterol, while apoE-deficient mice have over a three-fold elevation, to about 2 000 mg/dl, again, mostly in βVLDL, but there is also an increase in LDL (Plump et al., 1992).

The post-prandial clearance of intestinally derived lipoproteins is dramatically impaired in apoE - deficient mice. The apoE - deficient mouse responds appropriately to a human - like western - type diet (Nakashima et al., 1994). 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 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.

The genetic background has a major effect on atherosclerosis susceptibility in strains of apoE - deficient mice. For example, lesions from 16-week chow diet C57BL/6 apoE-KO were relatively larger than from FVB apoE-KO mice and in contrast to FVB mice there was evidence of early development of fibrous caps in these mice. In older mice, fibrous plaques from C57BL/6 apoE-KO mice were larger in size and had larger necrotic cores compared with FVB apoE-KO mice. Comparing humans and apoE - deficient mice, lesion progression and cell types are similar, as is the presence of oxidized lipoproteins. The major difference of this mouse model, as is the presence of oxidized lipoproteins. The major difference of this mouse model, as is the case for most of the other models of experimental atherosclerosis, is that plaque rupture is not observed, whereas plaque rupture is fairly common in humans and can lead to heart attacks. 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 (Nakashima et al., 1994).

Heterozygous apoE-deficient mice do not exhibit elevated plasma cholesterol levels on the chow or Western-type diet, suggesting that when mice are fed a physiological diet, a 50% decrease in apoE is not sufficient to influence fasting plasma lipids (Van Ree et al., 1994).
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.
In 1995 Kashyap et al. (Kashyap et al., 1995) described the successful correction of apoE deficiency in apoE-deficient mice by using an alternative approach involving systemic delivery to mouse liver of recombinant adenovirus vectors expressing human apoE. Thus, the single genetic lesion causing apoE absence and severe hypercholesterolemia is sufficient to convert the mouse from a species that is highly resistant to one that is highly susceptible to atherosclerosis (Breslow, 1994).
The method of measure atherosclerosis by using the aortic root atherosclerosis assay was originally developed by Paigen et al. (Paigen et al., 1987). The aortic root cross sectioning assay is 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 measures of atherosclerosis, such as the en face method, correlate with aortic root measurements. However, these methods are less amenable for studies using large numbers of mice and do not allow for inspection of lesion histology.

10. LDL receptor deficient mice

Gene targeting in embryonic stem cells has recently been used to create LDL receptor - knockout (LDLR-KO) mice, a model of familial hypercholesterolemia. LDL receptor - deficient mice was made in 1993 by Ishibashi et al. (Ishibashi et al.1993). 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. On this diet, and at that level of plasma cholesterol, LDL receptor - deficient mice do not get atherosclerosis. However, this is a very diet-responsive model. After these mice are fed the Paigen diet, their plasma cholesterol levels soar to about 1 500 mg/dl, and large atherosclerotic lesions form (Ishibashi et al., 1994). 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.

11. Other mouse models

Overexpression of human apoA-I in apoE - deficient mice increased HDL cholesterol levels twofold and substantially decreased fatty streak and advanced fibroproliferative lesion formation (Paszty et al. 1994; Plump et al., 1994).
By 4 months of age, all but 3-5% of apoE - deficient mice have had detectable fatty streaks that vary considerably in size; some are barely detectable, whereas others occlude as much as 8% of the aortic lumen. In apoE - deficient mice that overexpress human apoA-I, more than 50% of animals have no lesions by 4 months of age, and the animals that do develop

atherosclerosis have lesions that are barley detectable. By 8 months of age, apoE - deficient mice have lesions that are highly organized and that occlude on average 25% of the aortic lumen. Those apoE - deficient mice that overexpress human apoA-I have mainly immature fatty - streak lesion that occlude on average only 5% of the aortic lumen. Collectively, these data suggest that overexpression of apoA-I can diminish lesion size and slow the initiation of fatty streak formation.

More recently, apoE and LDL-receptor (LDLr) double – knockout (apoE/LDLr-DKO) mice have been created (Ishibashi et al., 1994), representing a new mouse model that develops severe hyperlipidaemia and atherosclerosis (Bonthu et al., 1997).

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 (Witting et al., 1999).

Thus, the apoE/LDLr-DKO mouse is a suitable model in which to study the anti-atherosclerotic effect of compounds without having to feed the animals an atherogenic diet.

To study the contribution of endothelial nitric oxide synthase (eNOS) to lesion formation Kuhlencordt et al. (Kuhlencordt et al., 2001). created apoE / eNOS double - knockout mice. It has occured 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. (Veniant et al., 2000) 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.

Therefore, gene - targeted mouse models has changed the face of atherosclerotic research (Savla U, 2002) and helped in creation of the new theory of atherosclerosis - as an inflammatory disease (Ross, 1999)

12. The experimental use of gene targeted mice

The apoE - deficient mouse model of atherosclerosis can then be used to: 1) identify atherosclerosis susceptibility modifying genes, by the candidate-gene and gene-mapping methods; 2) identify the role of various cell types in atherogenesis; 3) identify environmental factors affecting atherogenesis; and 4) assess therapies that might block atherogenesis or lesion progression.

ApoE-deficient mice have also been used to look for environmental and drug effects on atherosclerosis and to test novel therapies. One of the first observations was paradoxical effects of probucol on atherogenesis in both apoE-KO (Moghadasian et al., 1999) and LDL receptor deficient (Bird et al., 1998) mice. Probucol with strong antioxidant and cholesterol - lowering effects increased atherogenesis in apoE-KO mice by 3 folds (Moghadasian et al., 1999). Several other compounds reduced the extent and severity of atherosclerotic lesions without affecting plasma cholesterol levels in apoE–KO mice. For example, administration of antioxidant N,N'-diphenyl 1,4 - phenylenediamine (DPPD) to apoE-KO mice resulted in a significant decrease in atherosclerosis without reducing plasma cholesterol levels (Tangirala et al., 1995).

A marked reduction in atherosclerosis by dietary vitamin E was accompanied by no change in plasma cholesterol levels in apoE-KO mice (Pratico et al., 1998).
Likewise, antiatherogenic effects of the angiotensin - converting enzyme inhibitors (Hayek et al. 1998; Keidar et al. 2000; Hayek et al. 1999) or the angiotensin II receptor antagonist (Keidar et al. 1997) in apoE-KO mice were independent of plasma cholesterol lowering effects.
Since inflammation plays an important role in atherogenesis, during recent years it has become apparent that the 5-lipoxygenase (5-LO) pathway may take significant part in modifying the pathogenesis of atherosclerosis. Enzymes associated with the 5-LO pathway are abundantly expressed in arterial walls of patients afflicted with various lesion stages of atherosclerosis of the aorta and of coronary arteries. These data raised the possibility that antileukotriene drugs may be an effective treatment regimen in atherosclerosis (Mehrabian et al., 2002).
Of special interest for atherosclerosis is the arachidonate 5-LO which was originally identified in polymorphonuclear leukocytes, but which over-expression was recently demonstrated in macrophages, dendritic cells, foam cells, mast cells and neutrophils within atherosclerotic vessels. This enzyme generates an unstable epoxide intermediate compound leukotriene A4 (LTA4), which is an important precursor of LTB4, LTC4 and other cysteinyl leukotrienes. Initial observations and the use of drugs affecting the 5-LO metabolism were mainly connected with asthma and other inflammatory diseases (De Caterina & Zampolli, 2004).
However, a growing understanding of the role of inflammation in atherosclerosis has brought attention to the potential role of leukotrienes and their metabolism. In 2002 Mehrabian et al. identified the 5-LO as a crucial enzyme, contributing to atherosclerosis susceptibility in mice (Mehrabian et al., 2002; Spanbroek et al., 2003).
This observation, after a long pause (De Caterina R et al., 1988) has again focused the attention of researchers on the role of leukotrienes in the pathogenesis of atherosclerotic plaque (Radmark, 2003; Zhao & Funk, 2004; Zhao et al., 2004; Kuhn et al., 2005; Kuhn H, 2005; Lotzer et al., 2005; Back & Hansson, 2006; Radmark & Samuelsson, 2007). Therefore, the speculations have been risen that anti-asthmatic drugs could have beneficial effects on atherogenesis (Spanbroek & Habenicht, 2003; Wickelgren, 2004; Funk, 2005; Back, 2006).
Indeed, it has been recently demonstrated that the 5-LO substantially contribute to atherosclerosis in both mouse models and humans (Mehrabian & Allayee, 2003; Dwyer et al., 2004). Later Aiello et al. showed that LTB4 receptor antagonism reduced monocytic foam cells in mice (Aiello et al., 2002). Lotzer et al. pointed that macrophage-derived LTs differentially activate cysLT2-Rs via paracrine stimulation and cysLT1-Rs via autocrine and paracrine stimulation, during inflammation and atherogenesis (Lotzer et al., 2003).
Therefore, a hypothesis has been formulated that leukotriene-inhibiting drugs developed to treat asthma might protect the heart. There are numerous potential targets that could be useful in the intervention in leukotriene metabolism in atherosclerosis. Interestingly, the 18 kDa microsomal protein - five lipoxygenase activating protein (FLAP) was found to be critical for the regulation of 5-LO activity and biosynthesis of leukotrienes. The role of FLAP in atherosclerosis was additionally confirmed in humans by Helgadottir et al. (Helgadottir et al., 2004) who showed that genetic polymorphisms of FLAP are associated with myocardial infarction and stroke by increasing leukotriene production and inflammation in the arterial wall.

The 5-LO is abundantly expressed in atherosclerotic lesions of apoE and LDLR deficient mice, appearing to co-localize with a subset of macrophages but not with all macrophage-staining regions. Indeed, the results of our studies showed that the inhibition of FLAP by MK-886 or BAYx1005 can significantly prevent the development of atherosclerosis in gene-targeted apoE/LDLR-DKO mice (Jawien et al., 2006; Jawien et al., 2007).
Moreover, this study showed that cysteinyl leukotriene receptor blocker montelukast decreases atherosclerosis in apoE/LDLR-double knockout mice (Jawien et al., 2008). These results derived also from our numerous studies, concerned with atherosclerotic mice (Elhage et al., 2004; Elhage et al., 2005; Guzik et al., 2005; Jawien et al., 2005; Jawien et al., 2007).
The findings of the study concerning MK-886 were confirmed by Back et al. on their model of transgenic apoE-/- mice with the dominant negative transforming growth factor β type II receptor, which displays aggravated atherosclerosis (Back et al, 2007).
Colin D. Funk's research team questioned the hypothesis concerning leukotrienes, 5-LO and their role in atherogenesis in gene-targeted mice, stating that in mouse plaques there is no 5-LO overexpression detectable (Cao et al., 2008).
Finally, Poeckel & Funk in 2010, they tried to explain the whole complicated phenomenon.

13. Limitations of animal models

Animal models potentially bear the risk of compensatory mechanisms due to genetic modification of the target gene that render the results difficult to interpret. Another caveat is species differences between mice and humans. For instance, 5-LO expression in intimal atherosclerotic lesions varies between mice and humans; also, 5-LO and 12/15-LO appear to be differentially regulated in inflammatory cells of mice and humans with the murine 12/15-LO producing mainly 12-HPETE, while its human counterpart primarily synthesizes 15-HPETE. Notably, both products may have opposing effects in inflammation (Conrad DJ, 1999).
Moreover, atherogenesis in mice differs in several facets from the human pathology. Thus, T cells, whose presence in all stages of atherosclerotic lesions is acknowledged, are underrepresented in murine models of atherosclerosis (Daugherty & Hansson, as cited in Dean & Kelly, 2000; Roselaar et al., 1996).
Despite these shortcomings, animal models afford an invaluable means to study the effects of directed genetic overexpression, deletion or pharmacological inhibition of key enzymes of the LT cascade in a physiological setting that cannot be achieved in humans.
5-LO/LT pathway shows important disparities between murine and human atherosclerosis. Advanced human plaques show differences in 5-LO expression compared with mouse lesions. In human lesions, 5-LO (+) cells were identified in macrophages, DCs, mast cells, and neutrophils (Spanbroek et al, 2003) and notably, these 5-LO (+) cells are present in the neointimal region, whereas in mice, they are restricted to the adventitial layer (Zhao et al., 2004). With increasing age, these adventitial macrophages form clusters with T cells, independent of the severity of atherosclerosis. Intimal inflammatory reactions are connected to distinct adventitial inflammation responses, whereby B lymphocytes, plasma cells, and T cells conglomerate with macrophages. 5-LO (+) cells accumulate around new blood vessels, a common feature between mice and humans.
In human atherosclerotic plaque specimens, the quantity of 5-LO (+) cells even increased during progression from early to late phase coronary heart disease (Spanbroek et al., 2003).

Moreover, the elevated 5-LO activity was found to be associated with BLT1-mediated matrix metalloproteinase release from T cells, promoting plaque instability (Cipollone et al., 2005).
Human lesions demonstrate detectable expression levels for all major components of the LT cascade, i.e., FLAP, LTA_4 hydrolase, and LTC_4 synthase, as well as BLT_1/BLT_2 and $CysLT_1/CysLT_2$ receptors.
Taken together, in advanced human atherosclerosis, a role for 5-LO is likely, which is distinct from its role in early atherogenesis. This presence of the 5-LO/LT pathway in advanced lesions is not found in mouse models, which might be due to: (i) rapid progression of atheroma growth in mice vs. slower, often interrupted progression in humans (i.e., initial fatty streaks might remain dormant for many years in humans, until certain factors promote the progression of some lesions into an advanced state) (Libby, 2006; Libby & Sasiela, 2006) (ii) advanced human plaques display a higher degree of instability and risk to rupture than murine plaques; (iii) temporal dissociation in the Th1/Th2 'balance' at distinct lesion stages between mice and humans (Kus et al., 2009; Toton-Zuranska et al., 2010; Smith et al. 2010).

14. Future directions

During the last few years there has been a resurgent focus on the 5-LO/LT pathway as a potential target in coronary vascular disease (CVD). The complexity of the 5-LO/LT pathway participation in mechanisms contributing to CVD is evident based on the many studies (Poeckel & Funk, 2010). Limitations of these studies often result from the 'snapshot' punctual nature of analysing a single time point in CVD pathogenesis that makes it difficult to gain systematic insight into 5-LO-driven or -independent processes.
Murine and human CVD etiology differ with respect to the 5-LO/LT pathway, and even within murine studies, the nature of the applied model (for atherosclerosis, abdominal aortic aneurysm (AAA), or ischemia/reperfusion injury) influences the conclusions. Whereas a role for 5-LO-derived LTs in early stages of murine and human atherosclerosis, AAA, and reperfusion injury is cogent based on their effects in chemotaxis and induction of pro-inflammatory responses, the 5-LO pathway appears to play a distinct role in advanced human atherosclerosis, but not in advanced murine disease. Targeting specific leukotriene G protein-coupled receptors rather than upstream targets involved in LT synthesis may be a superior strategy for future CVD therapeutic interventions, based on extensive past experience with other pathways (e.g., via angiotensin II and adrenergic receptors), although this remains to be determined. Conditional knockouts and comprehensive translational studies should serve better than the traditional, simplistic 'one model' approach to understand the complex effects exerted by 5-LO products. Understanding the cytokine milieu during distinct stages of CVD progression will be crucial to elucidate how the expression of members of the 5-LO/LT pathway is regulated. There is little doubt that 5-LO plays important roles in many facets of CVD, but the challenge for future studies will be to clearly dissect these activities in a temporal and cell- and tissue- specific context in order to provide a solid basis for potential therapeutic interventions.

15. Acknowledgments

This article was supported by the grant form Polish Ministry of Science and Higher Education nr: N N401548340 for the years 2011-2012.

16. References

Aiello R.J., Bourassa P.A., Lindsey S., et al. (1999). Monocyte chemoattractant protein-1 accelerates atherosclerosis in apolipoprotein E-deficient mice. *Arterioscler Thromb Vasc Biol*, 19, 1518-1525.

Aiello R.J., Bourassa P.A., Lindsey S., Weng W., Freeman A. & Showell HJ. (2002) Leukotriene B4 receptor antagonism reduces monocytic foam cells in mice. *Arterioscler Thromb Vasc Biol*, 22, 443-449.

Alber H.F., 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.

Back M. (2006) Leukotrienes: potential therapeutic targets in cardiovascular diseases. *Bull Acad Natl Med*, 190, 1511-1518.

Back M. & Hansson G.K. (2006) Leukotriene receptors in atherosclerosis. *Ann Med*, 38, 493-502.

Back M., Sultan A., Ovchinnikova O. & Hansson GK. (2007) 5-lipoxygenase-activating protein. A potential link between innate and adaptive immunity in atherosclerosis and adipose tissue inflammation. *Circ Res*, 100, 946-949.

Binder CJ, Chang MK, Shaw PX, et al. (2002) Innate and acquired immunity in atherogenesis. *Nat Med*, 8, 1218-1226.

Bird D.A., Tangirala R.K., Fruebis J., Steinberg D., Witztum J.L., & Palinski W. (1998) Effect of probucol on LDL oxidation and atherosclerosis in LDL receptor - deficient mice. *J Lipid Res*, 39: 1079-1090.

Bonthu S., Heistad D.D., Chappell D.A., Lamping K.G., & Faraci F.M. (1997) Atherosclerosis, vascular remodeling, and impairment of endothelium - dependent relaxation in genetically altered hyperlipidemic mice. *Arterioscler Thromb Vasc Biol*, 17, 2333-2340

Boren J., Olin K., Lee I., et al. (1998) Identification of the principal proteoglycan-binding site in LDL. A single-point mutation in apo-B100 severly affects proteoglycan interaction without affecting LDL receptor binding. *J Clin Invest*, 101, 2658-2664.

Breslow J.L. (1994) Lipoprotein metabolism and atherosclerosis susceptibility in transgenic mice. *Curr Opin Lipidol*, 5: 175-184.

Breslow J.L. (1996) Mouse models of atherosclerosis. *Science*, 272, 685-688.

Cao R.Y., St Amand T., Grabner R., Habenicht A.J., & Funk C.D. (2008) Genetic and pharmacological inhibition of the 5-lipoxygenase/leukotriene pathway in atherosclerotic lesion development in apoE deficient mice. *Atherosclerosis*, 203, 395-400.

Capecchi M.R. (2001) Generating mice with targeted mutations. *Nature Med*, 7, 1086-1090.

Cipollone F., Mezzetti A., Fazia M.L., et al. (2005) Association between 5-lipoxygenase expression and plaque instability in humans. *Arterioscler Thromb Vasc Biol*, 25, 1665-1670.

Conrad D.J. (1999) The arachidonate 12/15 lipoxygenases. A review of tissue expression and biologic function. *Clin Rev Allergy Immunol*, 17, 71-89.

Daugherty A. & Hansson G.K. (2000) Lymphocytes in atherogenesis, In: *Atherosclerosis*, RT Dean R.T & Kelly D., pp. 230-249, New York, Oxford Press.

Daugherty A., & Rateri D.L. (2002) T lymphocytes in atherosclerosis. The Yin-Yang of Th1 and Th2 influence on lesion formation. *Circ Res*, 90, 1039-1040.

De Caterina R., Mazzone A., Giannessi D., Sicari R., Pelosi W., Lazzerini G., Azzara A., Forder R., Carey F. & Caruso D. (1988) Leukotriene B4 production in human atherosclerotic plaques. *Biomed Biochim Acta*, 47, S182-S185.

De Caterina R. & Zampolli A. (2004) From asthma to atherosclerosis - 5-lipoxygenase, leukotrienes, and inflammation. *N Engl J Med*, 350, 4-7.

Dwyer J.H., Allayee H., Dwyer K.M., et al. (2004) Arachidonate 5-lipoxygenase promoter genotype, dietary arachidonic acid, and atherosclerosis. *N Engl J Med*, 350, 29-37.

Elhage R., Jawien J., Rudling M., et al. (2003) Reduced atherosclerosis in interleukin-18 deficient apolipoprotein E-knockout mice. *Cardiovasc Res*, 59, 234-240.

Elhage R., Gourdy P., Brouchet L., et al. (2004) Deleting TCR alpha beta+ or CD+ T lymphocytes leads to opposite effects on site-specific atherosclerosis in female apolipoprotein E - deficient mice. *Am J Pathol*, 165, 2013-2018.

Elhage R., Gourdy P., Jawien J., et al. (2005) The atheroprotective effect of 17-estradiol depends on complex interactions in adaptive immunity. *Am J Pathol*, 167, 267-274.

Faggiotto A. & Ross R. (1984) Studies of hypercholesterolemia in the nonhuman primate. II. Fatty streak conversion to fibrous plaque. *Arteriosclerosis*, 4, 341-356.

Fan J. & Watanabe T. (2003) Inflammatory reaction in the pathogenesis of atherosclerosis. *J Atheroscler Thromb*, 10, 63-71.

Fredrikson G.N., Soderberg 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.

Funk C.D. (2005) Leukotriene modifiers as potential therapeutics for cardiovascular disease. *Nature*, 4, 664-672.

Gaut J.P. & Heinecke J.W. (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.

Glass C.K. & Witztum J.L. (2001) Atherosclerosis: the road ahead. *Cell*, 104, 503-516.

Gupta S., Pablo A.M., Jiang X., et al. (1997) IFN-gamma potentiates atherosclerosis in ApoE knock-out mice. *J Clin Invest*, 99, 2752-2761.

Hansson G.K. (2001) Immune mechanisms in atherosclerosis. *Arterioscler Thromb Vasc Biol*, 21, 1876-1890.

Hansson G.K. (2002) Vaccination against atherosclerosis: science or fiction? *Circulation*, 106, 1599-1601.

Hansson G.K. (2005) Inflammation, atherosclerosis, and coronary artery disease. *N Engl J Med*, 352, 1685-1695.

Hansson G.K., Libby P., Schonbeck U., & Yan ZQ. (2002) Innate and adaptive immunity in the pathogenesis of atherosclerosis. *Circ Res*, 91, 281-291.

Hayek T., Attias J., Coleman R. et al. (1999) The angiotensin - converting enzyme inhibitor, fosinopril, and the angiotensin II receptor antagonist, losartan, inhibit LDL oxidation and attenuate atherosclerosis independent of lowering blood pressure in apolipoprotein E deficient mice. *Cardiovasc Res*, 44, 579-587.

Helgadottir A., Manolescu A., Thorleifsson G., et al. (2004) The gene encoding 5-lipoxygenase activating protein confers risk of myocardial infarction and stroke. *Nat Genet*, 36, 233-239.

Hendrix M.G., Salimans M.M., van Boven C.P., & 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.

Ignatowski A.C. (1908) Influence of animal food on the organism of rabbits. *S Peterb Izviest Imp Voyenno-Med. Akad*, 16, 154-173.

Ishibashi S., Brown M.S., Goldstein J.L., Gerard R.D., Hammer R.E., & Herz J. (1993) Hypercholesterolemia in low density lipoprotein receptor knockout mice and its reversal by adenovirus – mediated gene delivery. *J Clin Invest*, 92, 883-893.

Ishibashi S., Herz J., Maeda N., Goldstein J.L., & Brown MS. (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.

Jawien J., Nastalek P. & Korbut R. (2004) Mouse models of experimental atherosclerosis. *J Physiol Pharmacol*, 55, 503-517.

Jawień J., Gajda M., Mateuszuk L., et al. (2005) Inhibition of nuclear factor-kappaB attenuates artherosclerosis in apoE/LDLR - double knockout mice. *J Physiol Pharmacol*, 56, 483-489.

Jawien J., Gajda M., Rudling M., et al. (2006) Inhibition of five lipoxygenase activating protein (FLAP) by MK-886 decreases atherosclerosis in apoE/LDLR double knockout mice. *Eur J Clin Invest*, 36, 141-146.

Jawien J., Gajda M., Olszanecki R. & Korbut R. (2007) BAYx1005 attenuates atherosclerosis in apoE/LDLR - double knockout mice. *J Physiol Pharmacol* 58: 583-538.

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.

Jawien J., Gajda M., Wolkow P.P., Zuranska J., Olszanecki R. & Korbut R. (2008) The effect of montelukast on atherogenesis in apoE/LDLR - double knockout mice. *J Physiol Pharmacol*, 59, 633-639.

Jawień J. (2008) New insights into immunological aspects of atherosclerosis. *Pol Arch Med Wewn*, 118, 127-131.

Jawień J. (2009) The putative role of leukotrienes in experimental atherogenesis. *Pol Arch Med Wewn*, 119, 90-93.

Jawień J. & Korbut R. (2010) The current view on the role of leukotrienes in atherogenesis. *J Physiol Pharmacol*, 61: 647-650.

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.

Kashyap V.S., Samantarina-Fojo S., Brown D.R. et al. (1995) Apolipoprotein E deficiency in mice: gene replacement and prevention of atherosclerosis using adenovirus vectors. *J Clin Invest*, 96, 1612-1620.

Keidar S., Attias J., Smith J., Breslow J.L, & Hayek T. (1997) The angiotensin-II receptor antagonist, losartan, inhibits LDL lipid peroxidation and atherosclerosis in apolipoprotein E – deficient mice. *Biochem Biophys Res Comm*, 236, 622-625.

Keidar S., Attias J., Coleman R., Wirth K., Scholkens B., & Hayek T. (2000) Attenuation of atherosclerosis in apolipoprotein E - deficient mice by ramipril is dissociated from

its antihypertensive effect and from potentiation of bradykinin. *J Cardiovasc Pharmacol* 35, 64-72.

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.

Kuhlencordt P.J., 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.

Kuhn H. (2005) Biologic relevance of lipoxygenase isoforms in atherogenesis. *Expert Rev Cardiovasc Ther*, 3, 1099-1110.

Kuhn H., Romisch I. & Belkner J. (2005) The role of lipoxygenase isoforms in atherogenesis. *Mol Nutr Food Res*, 49, 1014-1029.

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.

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.

Liao F., Andalibi A., deBeer F.C. et al. (1993) Genetic control of inflammatory gene induction and NF kappa B-like transcription factor activation in response to an atherogenic diet in mice. *J Clin Invest*, 91, 2572-2579.

Libby P. (2000) Changing concepts of atherogenesis. *J Intern Med*, 247, 349-358.

Libby P. (2002) Inflammation in atherosclerosis. *Nature*, 420, 868-874.

Libby P., Ridker P.M., & Maseri A. (2002) Inflammation and atherosclerosis. *Circulation*, 105, 1135-1143.

Libby P. (2006) Atherosclerosis: disease biology affecting the coronary vasculature. *Am J Cardiol*, 98, 3Q-9Q.

Libby P. & Sasiela W. (2006) Plaque stabilization: can we turn theory into evidence? *Am J Cardiol*, 98, 26P-33P.

Lotzer K., Spanbroek R., Hildner M., et al. (2003) Differential leukotriene receptor expression and calcium responses in endothelial cells and macrophages indicate 5-lipoxygenase dependent circuits of inflammation and atherogenesis. *Arterioscler Thromb Vasc Biol*, 23, e32-e36.

Lotzer K., Funk C.D. & Habenicht A.J. (2005) The 5-lipoxygenase pathway in arterial wall biology and atherosclerosis. *Biochim Biophys Acta*, 1736, 30-37.

Lusis A.J. (2000) Atherosclerosis. *Nature*, 407, 233-241.

Mach F., Schönbeck U., Sukhova G.K., et al. (1998) Reduction of atherosclerosis in mice by inhibition of CD40 signalling. *Nature*, 394, 200-203.

Mehrabian M., Allayee H., Wong J., Shi W., Wang X.P., Shaposhnik Z., Funk C.D. & Lusis A.J. (2002) Identification of 5-lipoxygenase as a major gene contributing to atherosclerosis susceptibility in mice. *Circ Res*, 91, 120-126.

Mehrabian M. & Allayee H. (2003) 5-lipoxygenase and atherosclerosis. *Curr Opin Lipidol*, 14, 447-457.

Meir K.S., Leitersdorf E. (2004) Atherosclerosis in the apolipoprotein E – deficient mouse. A decade of progress. *Arterioscler Thromb Vasc Biol*, 24, 1006-1014.

Moghadasian M.H., McManus B.M., Godin D.V., Rodrigues B., & Frohlich J.J. (1999) Proatherogenic and antiatherogenic effects of probucol and phytosterols in

apolipoprotein E - deficient mice. Possible mechanisms of action. *Circulation*, 99, 1733-1739.

Moghadasian M.H. (2002) Experimental atherosclerosis: a historical overview. *Life Sci*, 70, 855-865.

Nakashima Y., Plump A.S., Raines E.W., Breslow J.L., & Ross R. (1994) ApoE - deficient mice develop lesions of all phases of atherosclerosis throughout the arterial tree. *Arterioscler Thromb*, 14: 133-140.

Nakashima Y., Raines E.W., Plump A.S., 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.

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.

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.

Paigen B., Morrow C., Brandon C., et al. (1985) Variation in susceptibility to atherosclerosis among inbtred strains of mice. *Atherosclerosis* 57: 65-73.

Paigen B., Morrow A., Holmes P.A., Mitchell D., & Williams R.A. (1987) Quantitative assessment of atherosclerotic lesions in mice. *Atherosclerosis*, 68, 231-240.

Paigen B., Plump A.S. & Rubin E.M. (1994) The mouse as a model for human cardiovascular disease and hyperlipidemia. *Curr Opin Lipidol*, 5, 258-264.

Palinski W., Ord V.A., Plump A.S., Breslow J.L., Steinberg D., & Witztum J.L. (1994) ApoE - deficient mice are a model of lipoprotein oxidation in atherogenesis. Demonstration of oxidation – specific epitopes in lesions and high titers of autoantibodies to malondialdehyde - lysine in serum. *Arterioscler Thromb*, 14, 605-616.

Paszty C., Maeda N., Verstuyft J., & Rubin E.M. (1994) Apolipoprotein AI transgene corrects apolipoprotein E deficiency - induced atherosclerosis in mice. *J Clin Invest*, 94, 899-903.

Pentikäinen M.O., Öörni K., Ala-Korpela M., & Kovanen P.T. (2000) Modified LDL-trigger of atherosclerosis and inflammation in the arterial intima. *J Intern Med*, 247, 359-370.

Piedrahita J.A., Zhang S.H., Hagaman J.R., Oliver P.M. & Maeda N. (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.

Phipps R.P. (2000) Atherosclerosis: the emerging role of inflammation and the CD40-CD40L system. *Proc Natl Acad Sci*, 97, 6930-6932.

Pinderski L.J., Fischbein M.P., 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.

Plump A.S., Smith J.D., 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.

Plump A.S., Scott C.J., & Breslow J.L. (1994) Human apolipoprotein A-I gene expression increases high density lipoprotein and suppresses atherosclerosis in the apolipoprotein E - deficient mouse. *Proc Natl Acad Sci USA*, 91, 9607-9611.

Poeckel D. & Funk CD. (2010) The 5-lipoxygenase/leukotriene pathway in preclinical models of cardiovascular disease. *Cardiovasc Res*, 86, 243-253.

Pratico D., Tangirala R.K., Rader D.J., Rokach J., & Fitzgerald G.A. (1998) Vitamin E suppresses isoprostane generation in vivo and reduces atherosclerosis in apoE -deficient mice. *Nature Med*, 4, 1189-1192.

Prevention of cardiovascular events and death with pravastatin in patients with coronary heart disease and a broad range of initial cholesterol levels. The Long-Term Intervention with Pravastatin in Ischaemic Disease (LIPID) Study Group. (1998) *N Engl J Med*, 339, 1349-1357.

Radmark O. (2003) 5-lipoxygenase-derived leukotrienes. Mediators also of atherosclerotic inflammation. *Arterioscler Thromb Vasc Biol*, 23, 1140-1142.

Radmark O. & Samuelsson B. (2007) 5-lipoxygenase: regulation and possible involvement in atherosclerosis. *Prostaglandins Other Lipid Mediat*, 83, 162-174.

Reddick R.L., Zhang S.H., & Maeda N. (1994) Atherosclerosis in mice lacking apoE. Evaluation of lesional development and progression. *Arterioscler Thromb*, 14, 141-147.

Robertson A.K., Rudling M., Zhou X., et al. (2003) Disruption of TGF-beta signaling in T cells accelerates atherosclerosis. *J Clin Invest*, 112, 1342-1350.

Roselaar S.E., Kakkanathu P.X. & Daugherty A. (1996) Lymphocyte populations in atherosclerotic lesions of apoE -/- and LDL receptor -/- mice. Decreasing density with disease progression. *Arterioscler Thromb Vasc Biol*, 16, 1013-1018.

Rosenfeld M.E., Tsukada T., Chait A. et al. (1987) Fatty streak expansion and maturation in Watanabe heritable hyperlipidemic and comparably hypercholesterolemic fat-fed rabbits. *Arteriosclerosis* 1987, 7, 24-34.

Ross R. & Glomset J.A. (1976) The pathogenesis of atherosclerosis. *N Engl J Med*, 295, 369-377.

Ross R., Glomset J., & Harker L. (1977) Response to injury and atherogenesis. Am J Pathol, 86, 675-684.

Ross R., Faggiotto A., Bowen-Pope D., & Raines E. (1984) The role of endothelial injury and platelet and macrophage interactions in atherosclerosis. *Circulation*, 70, 77-82.

Ross R. (1986) The pathogenesis of atherosclerosis – an update. *N Engl J Med*, 314, 488-500.

Ross R. (1999) Atherosclerosis – an inflammatory disease. *N Eng J Med*, 340, 115-126.

Savla U. (2002) At the heart of atherosclerosis. *Nat Med*, 8, 1209.

Scandinavian Simvastatin Survival Study Group. Randomised trial of cholesterol lowering in 4444 patients with coronary heart disease: The Scandinavian Simvastatin Survival Study (4S). 1994. *Lancet* 344: 1383-1389.

Schonbeck U., Sukhova G.K., Shimizu K., et al. (2000) Inhibition of CD40 signaling limits evolution of established atherosclerosis in mice. *Proc Natl Acad Sci USA*, 97, 7458-7463.

Shishehbor M.H. & Bhatt D.L. (2004) Inflammation and atherosclerosis. *Curr Atheroscler Rep*, 6: 131-139.

Shoenfeld Y., Sherer Y., & Harats D. (2001) Atherosclerosis as an infectious, inflammatory and autoimmune disease. *TRENDS Immunol*, 22, 293-295.

Smith D.D., Tan X., Tawfik O., Milne G., Stechschulte D.J. & Dileepan K.N. (2010) Increased aortic atherosclerotic plaque development in female apolipoprotein E-null mice is

associated with elevated thromboxane A2 and decreased prostacyclin production. *J Physiol Pharmacol*, 61, 309-316.

Spanbroek R., Grabner R., Lotzer K., et al. (2003) Expanding expression of the 5-lipoxygenase pathway within the arterial wall during human atherogenesis. *Proc Natl Acad Sci USA*, 100, 1238-1243.

Spanbroek R. & Habenicht A.J. (2003) The potential role of antileukotriene drugs in atherosclerosis. *Drug News Perspect*, 16, 485-489.

Steinberg D. (2002) Atherogenesis in perspective: hypercholesterolemia and inflammation as partners in crime. *Nat Med*, 8, 1211-1217.

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.

Suski M., Olszanecki R., Madej J., et al. (2011) Proteomic analysis of changes in protein expression in liver mitochondria in apoE knockout mice. *J Proteomics*, 74, 887-893.

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.

Tangirala R.K., Casanada F., Miller E., Witztum J.L., Steinberg D., & Palinski W. (1995) Effect of the antioxidant N,N' - diphenyl 1,4-phenylenediamine (DPPD) on atherosclerosis in apoE - deficient mice. *Arterioscler Thromb Vasc Biol*, 15, 1625-1630.

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.

Thom D.H., Wang S.P., Grayston J.T., et al. (1991) Chlamydia pneumoniae strain TWAR antibody and angiographically demonstrated coronary artery disease. *Arterioscler Thromb*, 11, 547-551.

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.

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.

Van Ree J.H., van der Broek W., Dahlmans V. et al. (1994) Diet-induced hypercholesterolemia and atherosclerosis in heterozygous apolipoprotein E - deficient mice. *Atherosclerosis*, 111, 25-37.

Veniant M.M., Withycombe S., & Young SG. (2000) Lipoprotein size and atherosclerosis susceptibility in Apoe-/- and Ldlr-/- mice. *J Clin Invest*, 106, 1501-1510.

Waterson R.H., Lindblad-Toh K., Birney E., et al. (2002) Initial sequencing and comparative analysis of the mouse genome. *Nature*, 420, 520-562.

Welt F.G., Rogers S.D., 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.

Wick G., Schett G., Amberger A., et al. (1995) Is atherosclerosis an immunologically mediated disease? *Immunol Today*, 16: 27-33.

Wick G., Perschinka H., & Millonig G. (2001) Atherosclerosis as an autoimmune disease: an update. *TRENDS Immunol*, 22, 665-669.

Wickelgren I. (2004) Gene suggests asthma drugs may ease cardiovascular inflammation. *Science*, 303: 941.

Witting P.K., Pettersson K., Ostlund-Lindqvist A.M., Westerlund C., Eriksson A.W., & Stocker R. (1999) Inhibition by a coantioxidant of aortic lipoprotein lipid peroxydation and atherosclerosis in apolipoprotein E and low density lipoprotein receptor gene double knockout mice. *FASEB J*, 13, 667-675.

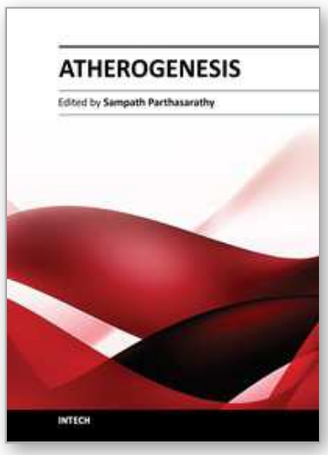
Zhang S.H., Reddick R.L., Piedrahita J.A., & Maeda N. (1992) Spontaneous hypercholesterolemia and arterial lesions in mice lacking apolipoprotein E. *Science*, 258, 468-471.

Zhao L. & Funk C.D. (2004) Lipoxygenase pathways in atherogenesis. Trends Cardiovasc Med, 14, 191-195.

Zhao L., Moos M.P., Gräbner R., Pédrono F., Fan J., Kaiser B., John N., Schmidt S., Spanbroek R., Lötzer K., Huang L., Cui J., Rader D.J., Evans J.F., Habenicht A.J. & Funk C.D. (2004) The 5-lipoxygenase pathway promotes pathogenesis of hyperlipidemia dependent aortic aneurysm. Nat Med, 10, 966-973.

Zhou X., Nicoletti A., Elhage R., Hansson G.K. (2000) Transfer of CD4(+) T cells aggravates atherosclerosis in immunodeficient apolipoprotein E knockout mice. *Circulation*, 102, 2919-2922.

IntechOpen



Atherogenesis

Edited by Prof. Sampath Parthasarathy

ISBN 978-953-307-992-9
Hard cover, 570 pages
Publisher InTech
Published online 11, January, 2012
Published in print edition January, 2012

This monograph will bring out the state-of-the-art advances in the dynamics of cholesterol transport and will address several important issues that pertain to oxidative stress and inflammation. The book is divided into three major sections. The book will offer insights into the roles of specific cytokines, inflammation, and oxidative stress in atherosclerosis and is intended for new researchers who are curious about atherosclerosis as well as for established senior researchers and clinicians who would be interested in novel findings that may link various aspects of the disease.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Jacek Jawien (2012). Mouse Models of Experimental Atherosclerosis as a Tool for Checking a Putative Anti-Atherogenic Action of Drugs, Atherogenesis, Prof. Sampath Parthasarathy (Ed.), ISBN: 978-953-307-992-9, InTech, Available from: http://www.intechopen.com/books/atherogenesis/mouse-models-of-experimental-atherosclerosis-as-a-tool-for-checking-a-putative-anti-atherogenic-acti

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

© 2012 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the Creative Commons Attribution 3.0 License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

IntechOpen

IntechOpen