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Review article

Translational atherosclerosis research: From experimental models to coronary artery disease in humans

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ABSTRACT

Atherosclerosis is the leading cause of death worldwide. Research on the pathophysiological mechanisms of atherogenesis has made tremendous progress over the past two decades. However, despite great advances there is still a lack of therapies that reduce adverse cardiovascular events to an acceptable degree. This review addresses successes, but also questions, challenges, and chances regarding the translation of basic science results into clinical practice, i.e. the capability to apply the results of basic and/or clinical research in order to design therapies suitable to improve patient outcome. Specifically, it discusses problems in translating findings from the most broadly used murine models of atherosclerosis into clinically feasible therapies and strategies potentially improving the results of clinical trials. Most likely, the key to success will be a multimodal approach employing novel imaging methods as well as large scale screening tools—summarized as "omics" approach. Using individually tailored therapies, plaque stabilization and regression could prevent adverse cardiovascular events thereby improving outcome of a large number of patients.

1. Atherosclerosis

Despite great advances in basic and clinical research, atherosclerosis still represents the major cause of death worldwide [1,2]. In addition, non-fatal myocardial infarction and stroke induce a large burden of morbidity with all its social and economic consequences [3–5]. Atherogenesis is a multifactorial process promoted by a plethora of risk factors [6]. Very briefly, one can differentiate between non-modifiable risk factors (such as gender, age, and genetic predisposition), and modifiable risk factors such as arterial hypertension, hyperlipidemia, diabetes mellitus, obesity, or tobacco consumption.

The long-standing history of research elucidating the mechanisms of atherogenesis starts with the early works of Rudolph v. Virchow in 1856, who identified the atherosclerotic plaque as analogue of the abscess, thereby revealing the crucial role of inflammation for atherogenesis [7]. Gerrity et al. could demonstrate the relevance of monocyte-derived macrophages for atherogenesis [8,9]. The crucial role of modified lipoproteins could be confirmed in many studies [10]. Williams and Tabas have summarized the work of many investigators in the response-to-retention hypothesis [11], which claims subendothelial retention of atherogenic lipoproteins to be the central pathogenic process in atherogenesis. They conclude that other contributory processes are either not individually necessary or are not sufficient [11]. Thus, all inflammatory processes resulting from the presence of lipoproteins are considered a consequence.

In fact, clinical studies have shown that lowering cholesterol reduces the likelihood of future adverse cardiovascular events (multiple statin trials [12], IMPROVE-IT [13]), while a number of trials specifically focussing on anti-inflammatory therapeutics have failed (ARISE [14], STABILITY [15], SOLID TIMI 52 [16]). Also, while statins have been shown effective in reducing cardiovascular events in patients with increased high-sensitivity C-reactive protein (CRP), Mendelian randomization could not confirm a causal involvement of CRP in cardiovascular disease [17]. Thus, the evidence that reducing inflammation reduces adverse cardiovascular events has not yet been given by randomized clinical trials. Currently, several studies specifically investigating the effects of anti-inflammatory drugs such as low-dose methotrexate or anti TNF antibodies are under way. Results of the CIRT [18] and the CANTOS [19] trials are expected in 2016 and 2017 and may change our understanding of the clinical relevance of inflammation in human atherosclerotic disease.

Cardiovascular medicine has lead to significantly improved

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outcome of patients suffering from cardiovascular events. E.g. in Germany, over the past two decades, mortality from coronary artery disease could be reduced by 28%. Mortality of acute myocardial infarction even decreased by 40% ("Heart Report 2014", German Heart Foundation). However, even with optimal interventional and medical therapy, 20% of patients suffer from recurrent acute coronary syndrome (ACS) within three years [20,21]. Considering the high prevalence of ACS, these numbers are unacceptably high and there is still need for improvement. In the following paragraphs, I will try to summarize some of the most important questions and challenges we are currently facing in atherosclerosis research. Furthermore, I will discuss some of the chances, which may be opened by novel approaches to understand and treat the fateful disease.

2. Successes in atherosclerosis research

The role of lipoproteins as triggers of atherogenesis has had tremendous impact on the way the disease is treated today. Native and modified lipoproteins affect atherogenesis at various levels. Thus, they facilitate monocyte attachment to and transmigration through the vascular endothelium [22]. Furthermore, lipid uptake by monocyte-derived macrophages induces foam cell formation, which is associated with the induction of various pro-inflammatory mechanisms and mediators [23,24]. Accordingly, based on the current international guidelines, lowering cholesterol is one of the main goals when treating patients with coronary artery disease [25,26]. These recommendations are based on a multitude of clinical studies, in most of which lowering LDL has been demonstrated to efficiently reduce the cardiovascular event rate [27].

Most of these studies investigated the role of statins as lipid-lowering agents. Statins were first described in 1976 [28]. Mechanistically, they inhibit the HMG-CoA reductase, which is the rate-limiting enzyme of cholesterol synthesis [29]. First clinical trials were conducted in 1980 with Iovastatin being the first statin marketed in the U.S. in 1987 [30]. While lipid-lowering seems to be the major effect through which statins prevent adverse cardiovascular events, numerous pleitropic effects have been postulated, many of which could be confirmed *in vitro* or in animal models [31].

Additional approaches to lower cholesterol have been developed over the past fifteen years: Ezetimibe is an inhibitor of intestinal cholesterol resorption, which may be prescribed in patients who do not tolerate statins due to side effects. In 2015, the IMPROVE-IT trial could demonstrate that addition of significantly reduces adverse cardiovascular events (notably without reducing all-cause mortality) [32].

As a very recent addition, PCSK9 antibodies have been introduced into clinical practice. Briefly, blocking PCSK9 using monoclonal antibodies prevents degradation of the LDL receptor thereby leading to fast and significant reduction of plasma LDL [33]. The role of PCSK9 was identified through a mutation associated with familial hypercholesterolemia [34]. It took about one decade after identification of the therapeutic target until clinical tools became available targeting PCSK9 [33]. Data proving that PCSK9 inhibition improves cardiovascular outcome are expected by 2017/18.

Taken together, the development of lipid-lowering drugs is one of the milestones, which have lead to substantial improvement of patient outcome.

3. Questions in atherosclerosis research

A large body of atherosclerosis research relies on murine disease models. In 1992, the *Apoe*$^{-/-}$ mouse was simultaneously described by Piedrahita et al. and Plump [35,36]. *Apoe*$^{-/-}$ mice spontaneously develop atherosclerosis on a standard chow diet; furthermore, atherogenesis and vascular wall inflammation are increased in these mice when fed a high-fat, Western-type diet. *Apoe*$^{-/-}$ mice are an excellent model to study human atherosclerosis as the plaques developing in these mice (especially in the brachiocephalic trunk) are very similar to those found in humans. Even though, they do not develop plaque rupture on a regular basis, *Apoe*$^{-/-}$ mice have become a broadly used model to study the mechanisms of plaque development *in vivo*. One year later, Ishibashi et al. described the *Ldlr*$^{-/-}$ mouse, which has since then become another generally accepted mouse model of atherosclerosis [37]. While *Apoe*$^{-/-}$ mice spontaneously develop atherosclerotic lesions, *Ldlr*$^{-/-}$ mice only do so after being fed a high fat diet. Furthermore, lesion development in *Ldlr*$^{-/-}$ mice takes much longer.

When searching for the terms "atherosclerosis" and "Apoe mouse" (or "atherosclerosis" and "Ldlr mouse") on PubMed, we get 4127 (1011) hits (as of 03 February 2016). Since 2010, 300 to 400 papers dealing with the *Apoe*$^{-/-}$ mouse model and atherosclerosis have been published per year. It is virtually impossible to refer *all* studies employing murine atherosclerosis models published during the last 22 years. A decade ago, Meir and Leitersdorf summarized the results of atherosclerosis research in the murine *Apoe*$^{-/-}$ mouse [38]. In 2007, Zadelaar et al. have summarized studies addressing the ability of pharmacological compounds to ameliorate atherosclerosis in mouse models including *Apoe*$^{-/-}$, *Ldlr*$^{-/-}$, and *Apoe3* Leiden mice [39]. Several recent review articles summarize our current understanding of the role of murine models of atherosclerosis, plaque progression, and plaque rupture [40–42].

There is a large number of papers investigating the role of specific cell types, exogeneous agents, or certain genes (either knocked out or overexpressed) in the different murine models. Many of them have given valuable insight in disease mechanisms: E.g. the important role of M-CSF, the major growth factor promoting monocyte macrophage differentiation, could be identified using the *Apoe*$^{-/-}$ mouse model [43]. Similarly, the importance of specific epitopes of oxidized LDL and antibodies directed against these epitopes was shown in the *Apoe*$^{-/-}$ mouse model [44]. Furthermore, a good example is a very elegant study identifying the important role of activated platelets for the disease process by repeated injection of activated platelets into *Apoe*$^{-/-}$ mice [45]. Another very interesting recently published paper could demonstrate that lesional macrophage accumulation in *Apoe*$^{-/-}$ mice is largely the consequence of local proliferation rather than increased monocyte recruitment [46]. A potentially useful model of plaque rupture that employs *Apoe*$^{-/-}$*Fbn1*$^{C1039G\pm}$ mice has recently beend described. These mice have a mutation in the fibrillin-1 gene leading to elastin fragmentation, which in turn results in a highly unstable plaque phenotype displaying intra-plaque hemorrhage, plaque neovascularization, and plaque rupture [47]. Other murine models of plaque destabilization involving arterial ligation or cast placement around specifically defined arteries have recently been compared demonstrating model-specific differences [48]. Taken together, we have gained valuable insight into basic mechanisms of atherogenesis by using murine models of atherosclerosis. One could add dozens of other findings, comprehensively summarized in several recent review articles [6,49].

By contrast, animal models may also lead to confusion: E.g. the role of the scavenger receptors SR-A and CD36 has been controversially discussed. Deficiency of CD36 or SRA-A or both have been associated with reduced atherogenesis in *Apoe*$^{-/-}$ mice [50,51], however, there was no additive effect on lesion development when both were knocked out [52]. In some of these studies, scavenger receptor deficiency was associated with reduction of foam cell formation or lesion complexity without affecting lesion size [53,54]. These different findings may be explained by differences in genetic backgrounds, time points assessed, and different methods