Circulation Research

ATHEROSCLEROSIS COMPENDIUM

How Far We Have Come, How Far We Have Yet to Go in Atherosclerosis Research

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We have made enormous strides in atherosclerosis research over the last century. The story of the cholesterol hypothesis provides a model whereby decades of fundamental research bore fruit when translated to the clinic.[1] This victory of basic science intertwined with clinical development merits revisiting as it furnishes a pathway that can inspire us and prod us to achieve a similar goal with remaining risk factors beyond cholesterol (cover Figure) but more quickly and adding to the effectiveness of treatment of cholesterol.

The story of cholesterol in atherosclerosis begins experimentally with the work of Anichkov and Chalatow,[1,2] who demonstrated the creation of fatty lesions in the arteries of rabbits that consumed a diet enriched in cholesterol. Windaus[3] chemically characterized cholesterol in atherosclerotic plaques. A technological advance spurred the next chapter in the cholesterol story. The advent of the ultracentrifuge equipped Lindgren et al[4] in the middle of the 20th century to characterize the lipoproteins that bear cholesterol and other lipids in the bloodstream. This work ushered an era of rapid progress in the characterization of different classes of lipoproteins, their correlation with disease states, and their chemical characterization. Lindgren et al[4] recognized LDLs (low-density lipoproteins) and HDLs (high-density lipoproteins)—a classification that permitted them and many others to pinpoint LDL as a risk factor for atherosclerotic cardiovascular disease.

The unraveling of the LDL receptor pathway provided the next boost to cholesterol research.[5] The discovery by Endo et al[6] of natural products that inhibit hydroxymethylglutaryl coenzyme A, the rate-limiting step in the synthesis of sterols, a pathway elucidated by Bloch,[7] opened the door to the development of effective new treatments for atherosclerotic cardiovascular disease by statins. The discovery by industrial investigators of ezetimibe, which acts, in part, by inhibiting cholesterol absorption in the small intestine, led to the discovery of the important role of the cholesterol transfer protein NPC1L1 (Niemann-Pick C1-like 1 protein) in enterocytes. Inhibition of NPC1L1 can lower LDL cholesterol independently of statins.[8,9] The observations of Catherine Boileau in kindreds with autosomal dominant hypercholesterolemia, coupled with the biochemical observations of Nabil Seidah, led to the cholesterol-regulating role of PCSK9 (proprotein convertase subtilisin/kexin type 9). This protein became the target of another series of LDL cholesterol therapeutics, both neutralizing antibodies and a small interfering RNA.[10] Large-scale clinical trials validated the ability of PCSK9 inhibition to improve cardiovascular outcomes.[11,12] This evolution from basic discovery to clinical translation took all in all more than a century but provided us with the tools to lower LDL cholesterol markedly and enabled enormous inroads into the prevention and treatment of cardiovascular diseases.

But, the battle, although joined, is not yet won. A considerable residual burden of cardiovascular disease persists despite substantial LDL lowering and other highly effective preventive measures ranging from smoking cessation through treatment of hypertension. Coronary heart disease remains a leading cause of disability and death in the developed world.[13] With the epidemiological transition in the developing world, the epidemic of atherosclerotic disease has spread globally, threatening vast numbers of individuals. The waning of many communicable diseases permits survival such that atherosclerotic cardiovascular disease can develop later in life. In both the developed world and countries in development, the global increase in aging also fuels atherosclerotic cardiovascular disease.[14]

Key Words: allergy and immunology ■ atherosclerosis ■ coronary artery disease ■ thrombosis ■ endothelium

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For Sources of Funding and Disclosures, see page 1110.

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Nonstandard Abbreviations and Acronyms

ARIC	Atherosclerosis Risk in Communities
BET	bromodomain and extraterminal domain
CD	cluster of differentiation
HDL	high-density lipoprotein
LDL	low-density lipoprotein
MESA	Multi-Ethnic Study of Atherosclerosis
NPC1L1	Niemann-Pick C1-like 1 protein
PCSK9	proprotein convertase subtilisin/kexin type 9

Perhaps if human populations had lifelong LDL cholesterol concentrations of a newborn or of many nonprimate animal species, atherosclerosis would be an orphan disease. Yet, the seed of atherogenesis is already sown for many adults and concerningly, many children as well. Moreover, the burgeoning pandemic of obesity, diabetes mellitus, and attendant dysmetabolism provides further alarm that even in an era of conquest of LDL cholesterol, we have not yet vanquished atherosclerosis.

We have developed an impressive armamentarium of high-technology approaches to treating established atherosclerosis (eg, percutaneous or surgical revascularization) and heart failure (eg, transplantation and circulatory assist devices). Indeed, ischemic cardiomyopathy—a consequence of the ravages of atherosclerotic cardiovascular disease—persists as the most common cause of heart failure—a syndrome that drains human well-being and healthcare resources. The current treatment of advanced heart failure, vascular and valvular disease, and arrythmias too often requires the use of resource-intensive interventions or devices. Such wonders of modern medicine include heart transplantation, mechanical circulatory assist devices, deployment of stents or artificial valves, or ablation of arrythmias. But these technology-intensive treatments of late-stage disease actually reflect a failure of prevention or lack of deeper understanding of the disease processes that necessitate deployment of these treatments dubbed "halfway technologies" by the prescient physician-essayist Lewis Thomas. He pointed out that treating advanced cardiovascular disease with such late-stage high-technology interventions presents ethically puzzling and impossibly expensive challenges to human health and healthcare systems.[15] He called for more basic research to address disease at an earlier stage and more fundamental levels.

Where can we now turn to continue progress in combatting atherosclerotic cardiovascular disease in an era of mastery over LDL cholesterol and in possession of an impressive armamentarium of interventions for late-stage disease? The model of investment in basic research (as in the case of LDL) and an imperative drive to rapid clinical translation provides a promising path toward this goal. Access of therapies and adherence to pharmaceutical therapies and lifestyle measure represents another critical challenge, a pertinent subject for investigation by behavioral economics and social sciences, a keenly important companion to the fruits of basic research.

This compendium presents a report on progress in atherosclerosis research since last summarized in these pages in 2016.[16] The advances reported in this current collection of expert articles range from the microscale to the macroscopic view. The technical advances include a rapidly accelerating tool kit for studying noncoding RNAs and single-cell RNA expression.[17,18] These technical advances promise to identify new targets for therapies beyond the traditional risk factor control measures. Single-cell RNA sequencing of atherosclerotic lesions has begun to reveal a surprising number of cell clusters representing different cell types and populations within traditionally defined lesional cell types.[17] How to harness this expanded catalogue of cell types to increase fundamental understanding of disease and aid the development of new treatments presents a key challenge going forward. The extensive research on noncoding RNAs has advanced considerably and has led to strategies to target functional atherosclerosis-relevant microRNAs as potential therapies.

On the macroscopic scale, the concept of phenotype stacks and big data promises transformation in the way that we identify patients at risk and target therapies in a more personalized manner than one size fits all.[19] Multigene risk assessment, facilitated by the scientific and technological advances of rapid, affordable, and widespread genotyping, furnishes another opportunity for a more precision-oriented approach to risk stratification and targeting of therapeutic interventions.[20] Expansion of genetic discovery and prediction efforts that encompass greater representation from populations of non-European genetic ancestries presents an important goal for the future.

In addition to these technical innovations, a more traditional candidate-based approach has identified new targets beyond LDL. Those highlighted in contributions to this compendium include CD (cluster of differentiation) 31—a molecule expressed by leukocytes, platelets and endothelial cells—that has become a potential therapeutic target.[21] Recent research has revealed that CD31 agonists promote the healing of injured arteries in animal experiments. The rapidly developing field of epigenetics is also leading to new therapeutic avenues, for example, therapies that target the BET (bromodomain and extraterminal domain)-containing protein family that regulates gene transcription by reading the specific histone marks that allow euchromatin to transition to the open state needed for transcription. BETs serve as epigenetic reader proteins by binding to acetylated lysine residues

Figure. Atherosclerosis: progress and opportunities.
Artistic version of the results of a single-cell RNA sequencing experiment showing different clusters of cells isolated from a mouse atherosclerotic plaque. This rendition evokes the application of emerging technologies to the study of atherosclerosis. Surrounding the cell clusters, the icons represent various risk factors—traditional and emerging. Traditional risk factors are represented by a blood pressure cuff, a cigarette, and a test tube containing blood from a person with hyperlipidemia. The computer workstation at the **bottom** represents the potential of big data, artificial intelligence, and advanced computational analytic approaches to deal with the massive amounts of data generated by contemporary molecular technologies. Genetic approaches provide the tools for a more precise identification of patients at risk and the potential of targeting therapies in a more personalized fashion based on application of informatic technologies. (Illustration Credit: Ben Smith.)

on histone tails and facilitating the assembly of transcription complexes and the transcriptional machinery.[22]

We are witnessing a renaissance of intermediary metabolism as it relates to aspects of atherosclerosis biology and therapeutics that reach beyond the traditional tools. Recently, bempedoic acid received approval by the Food and Drug Administration as an adjunct to diet and maximally tolerated statin therapy for the treatment of adults with heterozygous familial hypercholesterolemia or established atherosclerotic cardiovascular disease who require additional LDL cholesterol lowering. Bempedoic acid—an ATP citrate lyase inhibitor—prevents the tricarboxylic acid cycle intermediate citrate from conversion to acetyl coenzyme A—a precursor of cholesterol biosynthesis and other cellular processes. These advances prompt us to dust off our college biochemistry texts, as this venerable subject assumes new prominence in an era of metabolomic research and heightened understanding of heterogeneity in microenvironments within tissues such as the atherosclerotic plaque.[23] The increasing realization that cellular metabolites act not only intracellularly but can exchange among lesional cells and alter their functions represents another timely concept covered in this compendium.[23]

The involvement of inflammation in atherosclerosis has advanced from theory to a proven reality since the publication of the last *Circulation Research* compendium on atherosclerosis. In light of the CANTOS trial (Canakinumab Anti-Inflammatory Thrombosis Outcomes Study),[24,25] neutrophil extracellular traps—a fairly recently recognized set of structures that link host defenses, innate immunity, cell death, and thrombosis—may also provide new therapeutic targets and ways of subtyping of patients susceptible to specific therapeutic interventions.[26] Lifestyle furnishes the foundation of atherosclerosis prevention and remains a cornerstone of management of risk of recurrent atherothrombotic events. Recent experimental work in mice has expanded the fundamental understanding of how measures such as sleep, psychosocial stress, and voluntary exercise can modify atherosclerosis. Nonpharmacological measures

to mitigate this disease intersect with inflammatory pathways and innate immunity by muting hematopoiesis.[27] Summarizing one family of pharmacological targets, interleukin-1 and allied cytokines and the NLRP3 (NLR family pyrin domain containing 3) inflammasome, a panel of experts provides an update on this family of inflammatory mediators.[28] The role of adaptive immunity in atherosclerosis, well established experimentally, has spurred a number of attempts to advance vaccination strategies to the clinic.[29] This compendium provides an update on the status of these translational undertakings and discusses the remaining obstacles to the development of a potentially effective and safe atherosclerosis vaccination strategy.

As noted above, atherosclerosis has extended its reach beyond the middle aged white male that was the typical patient with coronary heart disease in the middle of the last century. The Coronary Drug Project launched by the US federal government in the mid-1960s studied over 8000 individuals, all male, in middle age (30–64 years). Studies of atherosclerosis have seldom enrolled substantial numbers of participants of women or of ethnic minorities. In the half century since design of the Coronary Drug Project, the demographics of atherosclerosis has shifted dramatically, as has our profession's commitment to inclusion and diversity. More recent federal projects such as the MESA (Multi-Ethnic Study of Atherosclerosis) and the ARIC Study (Atherosclerosis Risk in Communities) have striven to enroll a more diverse population. Yet, we lag in experimental studies to provide sufficient attention to sex as a biological variable. This compendium includes a contribution that addresses this important gap in both clinical and basic studies and aims to stimulate a more balanced and informative approach to experimental designs in the atherosclerosis field going forward.[30] Future personalized medicine approaches depend critically on increased understanding of sex as a biological variable in atherosclerosis and cardiovascular disease.

This compendium purposefully has avoided an encyclopedic approach. We have emphasized areas of atherosclerosis research that have progressed substantially and matured since the 2016 compendium, published just a few years ago. It is a credit to our enterprise that we have made substantial progress worthy of report in so few intervening years. We have deliberately omitted some topics that have received high-level attention in recent authoritative reviews, for example, the microbiome[31,32] or environmental risk factors such as air pollution.[33]

While we have made much progress and should take just pride in the advances made to date, we must not relent or shrink from the task ahead. We must harness the power of the new technologies including those highlighted in the series, to identify and exploit new targets. We must strive to move beyond catalogues to elucidate novel mechanisms. We must not allow ourselves to be complacent and satisfied with laboratory work alone. We must hasten to bring the fruits of cell culture studies and animal experiments to the clinic and to use human data to guide further mechanistic studies. We should emulate the success of the conquest of LDL as a risk factor for atherosclerosis, but we owe it to the public and our patients to take less than another century to do so.

ARTICLE INFORMATION

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Acknowledgments

We thank Dr Amélie Vromman, Dr Testsushi Nakao, Dr Rajat Gupta, and Shamsudheen Karuthedath Vellarikkal for their contribution to the cover of the Compendium.

Sources of Funding

P. Libby is funded by the National Heart, Lung, and Blood Institute (R01HL080472 and 1R01HL13489), the American Heart Association (18CSA34080399), and the RRM Charitable Fund. K.E. Bornfeldt reports National Institute of Health grants P01HL092969, R01HL126028, R01HL127694, R21AI135447, R01HL149685, and P30DK017047.

Disclosures

P. Libby is an unpaid consultant to, or involved in clinical trials for, Amgen, Kowa Pharmaceuticals, Novartis, AstraZeneca, Esperion Therapeutics, Ionis Pharmaceuticals, Merck, Pfizer, and Sanofi-Regeneron. P. Libby is also a member of a scientific advisory board for Amgen, Kowa Pharmaceuticals, Novartis, Corvidia Therapeutics, DalCor Pharmaceuticals, Olatec Therapeutics, Medimmune, and XBiotech, Inc. P. Libby is on the Board of Directors for XBiotech, Inc, and receives laboratory funding from Novartis. The other author reports no conflicts.

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