Plaque Attack

One Hundred Years of Atherosclerosis in The American Journal of Pathology

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Research articles on atherosclerosis have been well represented in The American Journal of Pathology (AJP), with more than 500 articles published since 1925. An initial focus on descriptive studies led to the proposal that atherosclerosis occurs as a response to vascular injury. With time, this view was modified by a greater understanding of the roles played by lipids and integrity of the vessel wall’s constituent cells and matrix. AJP has been a major contributor to the field, publishing numerous seminal research papers and review articles on the latest advances in atherosclerosis. This Centennial Review highlights these myriad contributions. (Am J Pathol 2012, 180:2184–2187; http://dx.doi.org/10.1016/j.ajpath.2012.04.003)

Atherosclerosis is a recurring theme throughout the history of The American Journal of Pathology (AJP). More than 500 articles directly relating to the disease have been published in the AJP since its debut in 1925; in fact, the first issue featured a paper on the topic. Throughout the next 25 years, however, the rate of publication averaged only about one article per year. Instead, studies of infectious diseases dominated, a reminder that in the preantibiotic era, only a fortunate few avoided microbes long enough to worry about heart disease. In these early years, most of the articles on atherosclerosis centered on examination of human lesions from autopsy specimens, with a smattering of studies using rudimentary animal models. The pace of publication in the field quickened in the 1950s, aided by the introduction of electron microscopy for dissection of the fine structure of atheromas and the development of better techniques to analyze the contents of plaques. Today, articles on vascular biology, atherosclerosis, and endothelial biology are so numerous that they merit their own section in AJP’s Table of Contents.

The Response-to-Injury Theory

The “response-to-injury” hypothesis is now widely accepted as the mechanistic basis of the pathogenesis of atherosclerosis, and it has helped to weave together the many threads of research over the years. The idea that atherosclerosis results from injury to arterial walls traces back to Rudolph Virchow in 1856, but it took more than a century before the basic molecular and cellular details that constitute the modern view were established. Russell Ross, a past president of the American Society for Investigative Pathology (ASIP) and a long-time member of the AJP’s Editorial Board, first formulated the response-to-injury theory with his colleague John Glomset in 1973 and elaborated on it in an AJP review article in 1977. In essence, the hypothesis states that atherosclerosis begins with injury to the endothelial lining of arteries. Although injury was initially presumed to cause endothelial denudation, subsequent experimentation has shown that the damage can be very subtle, resulting in only dysfunction of the endothelium without overt loss. Such altered endothelium recruits circulating mononuclear cells into the arterial wall, where subsequent uptake of lipoproteins by the leukocytes fosters their activation and sets the stage for a chronic inflammatory response. Factors released by cells in the developing lesion then promote influx and proliferation of smooth muscle cells, as well as heightened synthesis of extracellular matrix. The ultimate outcome is an intimal plaque that insidiously encroaches on the vessel lumen and may abruptly rupture, leading to thrombotic occlusion.

What, then, is the initial insult to the endothelium that sets the whole process in motion? In an era of pervasive...
advertising (and intense, competitive marketing for a host of statins), almost everyone, scientist or not, would likely answer that at least a part of the equation is too much “bad” cholesterol. The association between hypercholesterolemia and atherosclerosis has long been recognized, but we now know that a host of other metabolic disturbances can potentially augment the effects of cholesterol. Indeed, the first article on atherosclerosis in *AJP* dealt with precisely this topic. Entitled “The Effect of Certain Metabolic Changes on the Aorta of Rabbits and Guinea Pigs,” it was written by Otto Saphir.4 (It is noteworthy how many of the early papers were penned by just one or two investigators, conjuring up the image of a lone pathologist hunched over his, or rarely her, microscope late into the night.) Unfortunately, none of the metabolic perturbations that Saphir introduced (thyroxine, acidosis, quinine) resulted in atherosclerotic lesions. Nevertheless, his article stands as a good example of the early use of animals to study this disease. In fact, rabbits proved key to recognizing a causal relationship between hypercholesterolemia and atherosclerosis, as feeding them a diet rich in fat produces lesions that are similar (but certainly not identical) to human atheromas. The cholesterol-fed rabbit features prominently in a number of studies published in *AJP*, although its use must have been rather controversial: papers that used the model abound with statements akin to, “It is realized that the mechanisms of atherogenesis in the rabbit may be different from those in man.”5,p877 The desire for a more faithful mimic of the human disease is evident from the wide variety of animals examined to this end in *AJP* publications. The menagerie reads like a ship’s manifest from Noah’s ark: rats, swine, chickens, hamsters, dogs, White Carneau pigeons, a variety of non-human primates, and even trout have had their vessels sliced and diced in the quest to recapitulate human atherosclerosis.

The Emergence of Alternative Hypotheses

Several investigators in the 1930s, 1940s, and 1950s in fact doubted the validity of the cholesterol-fed rabbit model and openly questioned whether hyperlipidemia had any causative role in human atherogenesis. Rather, they posited that lipids might accumulate in lesions secondary to other factors, such as loss of flexibility of the vessel.6 Zeek7 even speculated that hypercholesterolemia could be a result of atherosclerosis, with blood-borne lipids emanating from ruptured plaques. Another theory from that time, dubbed the encrustation hypothesis, suggested that plaques grow primarily due to repeated incorporation of thrombi by the vascular wall. McLetchie8 put this hypothesis to the test by inducing clots in rabbits with cobra venom factor. Although his results were equivocal, organization of thrombi on an injured vessel wall is still considered one of the mechanisms by which plaque accurses. McLetchie’s concluding remarks, made in 1952, showed considerable prescience: “The vascular endothelium is (currently) considered to play a passive role . . . I have doubts about the confinement of the endothelium to a passive role . . . It appears obvious that little advance can be made without more precise knowledge of the general biology of blood vessels, in particular their reactions to stress and the relationship of the endothelium to the normal fluidity of the blood.”8,p422-424

Despite the limitations of the rabbit model, many investigators put it to good use, aided by advances in methods for microscopy in the 1950s. Duff et al9 devised a technique to view the surface of whole mounts of intact aortic intima, which permitted analysis of very early lesions in the cholesterol-fed rabbit. In hindsight, two of their observations are remarkable. First, they noted that the endothelium overlying early lesions appears normal, a foreshadowing of the idea that endothelial dysfunction, rather than overt damage, can lead to disease. Second, they observed mononuclear cells on the surface of early lesions and speculated that such cells crawl through the endothelium to enter the intima, where collections of so-called histiocytes were observed. The authors were circumspect regarding identification of these mononuclear cells and ended their article thus: “That monocytes can enter the tissues, forming phagocytes, is generally agreed. There is no agreement that lymphocytes can do so . . . The evidence offered by the present study does not justify our entering the controversy.”9,p859

Lessons Learned from Electron Microscopy

It would be another 20 years or so before the macrophage regained its proper place in the spotlight. In the meantime, electron microscopy drew attention to another important cellular player, the smooth muscle cell. In 1960 and 1965, Parker and Odland published integrated studies examining the evolution of lesions in cholesterol-fed rabbits via electron microscopy, light microscopy, histology, and biochemistry (to characterize lipids). Most importantly, they found that lesions are rich in cells that have the ultrastructural characteristics of smooth muscle cells.10–12 These investigators were led to conclude that much of the cellularity in atherosclerotic plaque is in fact smooth muscle that originated from the media, a then-radical concept that is now accepted as fact. They also raised the possibility that many of the characteristic lipid-laden foam cells in lesions—previously thought to represent overstuffed macrophages—derived from smooth muscle cells. Similar conclusions were ultimately reached by Geer et al13 in their study of the fine structure of atherosclerotic lesions in human specimens. The contemporary interest in this line of investigation is underscored by the fact that the paper by Geer and colleagues was one of the 20 most highly cited in *AJP* for that decade. Electron microscopy also provided evidence that endothelial cells in cholesterol-fed rabbits are altered morphologically, and it was posited that they might be ferrying lipids into the growing lesions.10–12 Thus, the stage was set for Ross’s response-to-injury hypothesis; indeed, Parker and Odland11 ended one of their papers with the statement, “it would seem that the accumulation of smooth muscle cells within the intima represents a response to various types of stimuli, perhaps best generally characterized as injury.”p211

As mentioned, Ross and colleagues5 outlined their concept of the hypothesis in a review article published in *AJP* in 1977. At that time, they proposed endothelial denudation as
the injury that incites atherosclerosis, with subsequent aggregation of platelets at the damaged site. This view was based on their in vitro experiments, demonstrating that platelets elaborate factors that stimulate migration and proliferation of smooth muscle cells. But how did this scenario fit with observations that the endothelium in early atheromas often appears intact, even using methods as sensitive as electron microscopy? Also unresolved were the origin and role of foam cells and the mechanistic relationships that could connect hypercholesterolemia, as well as other metabolic disturbances (eg, homocysteinemia or diabetes), to atherogenesis. It took another generation of technical innovations to supply the answers. In particular, advances in genetic engineering, culture of primary cells, and cellular imaging led to important breakthroughs. In terms of imaging, the burgeoning ability to identify distinct cell types via specific markers proved especially valuable. In fact, four papers that applied this approach to atherosclerosis are among the most highly cited AJP articles of the 1980s. Considerable evidence had accumulated to suggest that some of the lipid-laden foam cells in atherosclerotic lesions were in fact smooth muscle cells. However, a population lacking ultrastructural features of smooth muscle was also noted. Schaffner et al helped to resolve the origin of foam cells by isolating them from cholesterol-fed rabbits and monkeys and demonstrating that many bore receptors for immunoglobulins and complement proteins and could phagocytize opsonized red blood cells, all features of macrophages. In experiments using cholesterol-fed swine, electron microscopy revealed cells with the enzymatic characteristics of monocytes adhering to endothelium and apparently migrating into the intima of the aorta even before lesions could be discerned. Other investigators used immunocytochemical techniques to delineate the cellular composition of human atherosclerotic plaques. These studies made it clear that both macrophages and T lymphocytes are present in early lesions and that substantial numbers of plaque-associated T cells display markers of activation. Improved fixation procedures for electron microscopy that preserve extracellular lipids refined the chronology of pathogenesis even further: in cholesterol-fed rabbits, extracellular fats accumulate in the aortic intima well before monocytes infiltrate.

**Immune Mechanisms of Injury**

At the same time, the accuracy of McLetchie’s prescient conclusion in 1952 that the endothelium must play more than a passive role in atherogenesis was becoming increasingly apparent. A 1988 article by Jordan Pober, on the occasion of his receiving ASIP’s Warner-Lambert/Parke-Davis Award, reviewed the growing evidence that endothelial cells are key players in many immune processes, including recruitment of circulating leukocytes. During this time, significant advances in tissue-culture techniques permitted dissection of the interactions between endothelium and monocytes in vitro, without having to sort through a multitude of confounding variables in the intact animal. Tissue-culture experiments also furnished a means for formally testing the direct involvement of lipids and lipoproteins in atherogenesis and helped to eliminate any notion that they were merely collateral players. Accordingly, studies published in AJP using in vitro approaches demonstrated that cholesterol and low-density lipoproteins (LDL) directly enhance the adhesion of monocytes to endothelial cells and perturb the metabolic functions of the latter. Moreover, monocytes isolated from hypercholesterolemic rats were shown to produce factors that attract smooth muscle cells and foster their proliferation. In 1984, Kruth reported the presence of unesterified (free) cholesterol in human atherosclerotic lesions, suggesting an important role for free cholesterol in the development of disease and foreshadowing subsequent work that would demonstrate roles for free cholesterol in inflammation and plaque formation. By the end of the 1980s, a much clearer picture had thus emerged of how atheromas are initiated and progress. In 1993, Russell Ross published another review article in AJP that refined the response-to-injury hypothesis, largely codifying the present-day view of the pathogenesis of atherosclerosis. The review was based on the lecture that Dr. Ross delivered on the occasion of receiving ASIP’s Rous-Whipple Award in recognition of his outstanding scientific career and contributions to the field. To quote, “. . . the lesions of atherosclerosis represent a specialized form of a protective, inflammatory-proliferative response to various forms of insult to the artery wall. Depending upon the nature and duration of the insult, the protective response may become excessive and over many years in its excess become a disease state.” (The emphasis is that of Dr. Ross.)

**Future Trajectory of Atherosclerosis Research**

Of course, our current picture of atherogenesis is clear only in broad strokes, and many details still await illumination. As such, the topic remains of great interest to authors and readers of AJP. The need for additional animal models that will enable analysis of the intricacies of the disease is a recurring theme. The Watanabe rabbit, which lacks LDL receptors, is a model of human familial hypercholesterolemia and is discussed in an AJP article that was part of a series on animal models of human disease. Other recent papers describe rodent models that incorporate multiple etiologies, such as diabetes and hypercholesterolemia, to mirror more closely the complexity that underlies the human illness. LDL receptor-deficient mice have been exploited to assess how lowering lipids affects regression of early or advanced atherosclerotic lesions. Notably, models of atherosclerosis in genetically-engineered mice, discussed in a 2004 AJP review article, have been instrumental in dissecting immunological and molecular details of pathogenesis and will be the subject of a future article in this Centennial series. Insights provided by basic research—much of it described in the pages of AJP over the past century—have led to a host of effective treatments for atherosclerosis. Because of the mechanistic link established between hypercholesterolemia and atherosclerotic disease, st-
Concluding Remarks

This survey of the last 100 years demonstrates that AJP has been a long-standing and integral collaborator in the dissemination of our understanding of atherosclerosis. With its broad focus on cellular and molecular mechanisms of disease encompassing a wealth of model systems, both in vitro and in vivo, the AJP attracts some of the best contributions of breakthroughs in the field.

References

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