REVIEW IN DEPTH

Advances in the cell biology of atherogenesis

Edited by Alan Daugherty

Overview

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Atherosclerosis is the gradual, progressive, and active formation of lesions in vascular beds and is responsible for most of the major cardiovascular diseases. The authors of the reviews in this section have provided insights into the cellular components of atherosclerosis as well as some of the proposed mechanisms responsible for lesion formation. Atherosclerosis is an extremely difficult disease to study because of its chronicity and complex pathology. Even apparently simple questions, such as the evolution of the cellular components of atherosclerosis, have been difficult to define unequivocally. Many mechanisms in the atherogenic process have been extrapolated from cell culture studies. Most of the reviews, however, emphasize the increased use of in-vivo experiments and newer techniques to characterize the presence of cells, specific proteins, and gene expression within vascular lesions.

Michael Rosenfeld and Eva Pestel describe some of the cellular aspects in the evolution of atherosclerotic disease. Historically, the definition of the cellular components of atherosclerotic lesions has depended on classical pathological techniques on human samples derived with inadequate preservation. However, the evolution of cellular components of atherosclerotic lesions has been clarified by the availability of specific animal models of atherosclerosis; these animal models have permitted acquisition of vascular tissue at selected stages in the athero-

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sclerotic process. The availability of tissue combined with the development of cell-specific antibodies for immunocytochemistry has permitted the unequivocal designation of cell type at specific stages of the disease. Ross and colleagues [1,2] first demonstrated the cellular progression of atherosclerosis in cynomolgus monkeys and later in Watanabe heritable hyperlipidemic rabbits [3,4]. Both species showed similar overall changes. The initial response was adherence of monocytes to an intact endothelium at flow dividers within the vascular bed. Monocytes then traversed intact endothelium and accumulated in the intima, where they progressively became engorged with lipid to form foam cells. These foam cells progressed to lipid cores, which were encased in a fibrous cap. Gross fissuring of lesions and the subsequent formation of large thrombi, which is responsible for many of the clinical manifestations of atherosclerosis in man, is not a salient feature of animal models of the disease.

The extrapolation of the data derived from animal studies seems plausible given the initial results of the multicenter Pathobiological Determinants of Atherosclerosis in Youth (PDAY) trial [5]. PDAY is acquiring aortic and coronary vascular samples under well-defined conditions from young individuals who have died in traumatic circumstances. The initial results of the PDAY study seem to substantiate the major features of the animal experiments in terms of cellular constituents. The following reviews will discuss the cellularity of atherosclerotic lesions and the temporal recruitment of specific cell types into these lesions. Mechanisms discussed include the ability of macrophages to engorge with lipid and become foam cells, some of the thrombotic components of atherosclerotic lesions, and the potential importance of immune mechanisms within the pathological tissue.

The initial event in the development of atherosclerotic lesions is the recruitment, adhesion, and transmigration of monocytes at the site of the evolution of the disease. The factors controlling these events are discussed in the review by Mahamad Navab and colleagues. The recruitment of bloodborne cells to evolving atherosclerotic lesions appears to be relatively specific for monocytes, while it is generally considered that neutrophils are not involved. The adhesion of circulating cells to evolving atherosclerotic lesions requires the inducement of specific adhesion molecules at both the endothelial surface and the recruited monocytes. Many adhesion molecules have now been characterized, and several of these proteins have been identified in atherosclerotic lesions. Several pharmacological approaches to the inhibition of adhesion molecule interactions may be useful in defining the role of these proteins in the development of atherosclerosis. These compounds have been used effectively in cell culture experiments. Studies using L-arginine have provided indirect indications of the benefit of inhibiting monocyte adhesion. There are also several candidates for the induction of leukocyte recruitment and transmigration through the endothelium. Gerrity [6] first demonstrated that aortic extracts from hypercholesterolemic pigs were chemotactic for monocytes. One potential mediator of this monocyte recruitment could be lysophospholipids that are increasingly present in oxidized low-density lipoproteins (LDL) and in atherosclerotic tissue. More recently, it has been demonstrated that monocyte chemotactic protein-1 can be induced in monocytes by mildly modified LDL and is present in atherosclerotic tissues. As with the adhesion molecules, however, the relative importance of chemotactic agents is not known *in vivo*. Given the intense interest in the mechanisms of monocyte recruitment and with the increased availability of reagents to characterize these interactions, it is likely that increased emphasis will be placed on defining their importance in the early stages of atherogenesis.

Excessive lipid deposits are commonly present in macrophages of atherosclerotic lesions, although smooth muscle cells may also form foam cells. Considerable effort has been extended in defining the lipoproteins responsible for delivering the excessive cholesterol into cells. Since the early 1970s it has been recognized that native lipoproteins do not readily overload cells with cholesterol. However, specific modifications to lipoproteins, especially LDL, can lead to a marked change in the ability of macrophages to handle these particles. It is now established that oxidative modifications of LDL may have important implications for atherogenesis as discussed in the review by Jay Heinecke. Steinberg and colleagues [7] first recognized the ability of cultured cells to modify LDL in such a manner that they failed to interact with the LDL receptor, but promoted interactions with macrophages. This interaction of modified LDL with macrophages is mediated by a cell surface protein termed the 'scavenger receptor'. Subsequent work has revealed that these cell-determined modifications of LDL are via oxidative mechanisms. Numerous studies have demonstrated that oxidation of LDL causes gross changes in the physical, biochemical, and metabolic characteristics of LDL that render it 'atherogenic'. The importance of lipoprotein oxidation to the development of atherosclerosis has been enhanced by the finding of similarly oxidized particles from vascular lesions *in vivo*. This has included the ability to isolate LDL-like particles with properties consistent with oxidation from both human and animal models of atherosclerosis. Also, antibodies against specific oxidized epitopes have been developed and used to immunolocalize modified lipoproteins within tissue sections. Furthermore, a number of unrelated antioxidants have been shown to reduce the severity of atherosclerosis in animal models. Although oxidation is thought to be involved in the atherogenic process, one of the major questions that remains unresolved is the mechanism of this oxidation. Jay Heinecke has discussed some of the plausible cellular mechanisms for lipoprotein oxidation, which include the involvement of the superoxide ion, thiols, lipoxygenase, and myeloperoxidase. All of these mechanisms are at present speculative and will require further effort to determine whether they represent appropriate mechanisms of oxidation under in-vivo circumstances.

Lipoproteins are assumed to enter the intracellular space via specific cellular receptors. The receptor that recognizes LDL was characterized by the classic experiments of Goldstein and Brown in 1974 [8]. This receptor is undoubtedly important in the control of plasma cholesterol concentrations, as dramatically shown in familial hypercholesterolemia where LDL receptors are absent. However, the relevance of LDL receptors at the level of arterial tissue is undefined, and is at present considered to be unimportant. The major interests in arterial wall receptors focus on those that would recognize modified forms of lipoprotein, as discussed by Alan Daugherty. The major receptor of interest in this regard has been the scavenger receptor. Scavenger receptor protein was isolated, and the gene cloned, by Krieger and colleagues [9,10]. Scavenger receptors have now been characterized extensively for their ligand specificity and their modulation of activity on cells in culture. The presence of scavenger receptor mRNA and protein has recently been described in atherosclerotic tissue. At present, the specific role of scavenger receptors in the generation of atherosclerotic lesions is not known. Several groups, however, are genetically engineering mice either to overexpress or to knockout the scavenger receptor. Availability of these animals should provide a great insight into the possible role of scavenger receptors in the atherogenic process. Two other receptor classes have recently been identified as being able to recognize and metabolize modified lipoproteins. These include the Fcγ RII-B2 receptors and CD36. The description of these two further receptor classes potentially provides multiple mechanisms by which macrophages may become overloaded with cholesterol. The low-density lipoprotein receptor-related protein/α_2-macroglobulin receptor (LRP/α_2M) has also been proposed as a receptor for specific lipoprotein classes. LRP/α_2M is now known to have an extremely broad ligand specificity and may not only have effects on the lipid loading phenomena seen in atherosclerosis, but may also modulate the modeling of the extracellular matrix through its action on proteases and protease inhibitors.

Although considerable emphasis has been placed on the role of cholesterol in the development of atherosclerotic lesions, it is increasingly realized that the disease represents a chronic immune reaction. Sten Stemme and Göran Hansson have reviewed both the involvement of specific lymphocyte classes that are present in atherosclerotic lesions and the possibility that products of this cell type may modify the disease. Lymphocytes are present in virtually every stage of atherosclerotic lesion development, and there are specific characteristics of lymphocyte classes at different stages of the disease. In addition, it is well known that lymphocytes secrete a wide repertoire of cytokines that can have multiple effects on cellular activity. The wide array of these activities generates some confusion in terms of clearly defining whether deletion of a specific cytokine would be beneficial or detrimental to the development of atherosclerotic lesions. Although the presence of lymphocytes is a well characterized phenomenon in atherosclerotic lesions, the challenge now remains to determine whether this cell type is merely a bystander of the disease or an active component of disease progression.

The acute clinical manifestations of atherosclerosis are due to thrombotic complications that arise because of the presence of lesions. Josiah Wilcox has reviewed the mechanisms that could be responsible for the formation of acute thrombus following rupture of an atherosclerotic lesion. One of the major salient features that may be responsible for thrombus formation is the presence of tissue factor in high concentrations in the regions of atherosclerotic lesions. Tissue factor may be accessible to blood-borne elements following lesion rupture, as described by Davies and colleagues [11]. In addition to this well recognized phenomenon of thrombotic events causing some of the clinical manifestations of atherosclerotic diseases, thrombotic mechanisms may also be involved in the development of the lesion itself. Josiah Wilcox has reviewed the presence of some thrombotic mediators, especially platelet-derived growth factor, which has been shown to be present within both human and animal experimental models of atherosclerosis. Furthermore, he has provided evidence from recent experiments that a thrombus can evolve into an atherosclerotic lesion that bears gross similarities to lesions formed under hyperlipidemic conditions.

New technologies are making it increasingly easy to characterize some of the specific events in atherogenesis. Unfortunately, these advances are matched by the increasing complexity in the hypotheses of atherosclerotic lesion development. Development of the disease is no longer considered merely a simple deposition of excessive lipid, but rather is considered to be a network of excessive lipid deposition with immune modulators and thrombotic mechanisms. The effective modulation of atherosclerotic diseases is therefore likely to require modification of multiple components of the disease.

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