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DRUGS AFFECTING LIPID METABOLISM VIII

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PREFACE

The Proceedings of the Eight International Symposium on Drugs Affecting Lipid Metabolism (8th D.A.L.M.) is the subject of this volume. Since the first symposium in 1960, each successive meeting has broken new ground in the field of pharmacological control of lipid levels - offering new and stimulating insights and exposing the audience to the state of the art. The field has progressed sufficiently to permit discussion of the cellular biology of atherosclerosis. The opening session was devoted to pathology, macrophages, lipoproteins and their receptors and cholesterol ester metabolism. Because of the recent emergence of new apolipoprotein technology, a workshop devoted solely to apolipoprotein methodology was introduced followed by a plenary session devoted to their metabolism and structure.

Another rapidly developing area of atherosclerosis research is non-invasive assessment of this condition. Accordingly, a session was devoted to new techniques for this research modality. The final plenary sessions were devoted to the roles of drugs and diet in atherosclerosis - cause, treatment and mechanisms of action. The meeting was summarized by Dr. O.J. Pollak, one of the "founding fathers" of this field. There were nine sessions of proffered papers whose abstracts appear in this volume. In addition, special workshops (to be reported elsewhere) were devoted to several drugs including Oryzanol, Probucol and Etofibrate.

We are grateful for the interest of the organizations whose generous support contributed to the success of the meeting. The sponsorship of the Lorenzini Foundation was especially appreciated. The success of this meeting was due, in part, to the strong support of the local organizing committee. We are deeply indebted to Mrs. Frances Murray Nigro for her efficient and cheerful handling of

the duties of the secretariat. The smoothness of this critical operation was principally instrumental for the success of the symposium. We also wish to thank Mrs. Edith Basedow Pappas for her expert help in the preparation of this volume.

David Kritchevsky
William L. Holmes
Rudolfo Paoletti

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INTRODUCTION

The main components of the advanced atherosclerotic plaque are the necrotic cholesteryl ester-rich core from which the disease process gets part of its name (the Greek stem "athero" means gruel or porridge) and the fibrous (sclerotic) cap which contains predominantly smooth muscle cells which often become encased in their own synthesis products of collagen, elastin and proteoglycans. Typically, many of these cells and their surrounding intercellular matrix are associated with abundant lipids which are demonstrable both chemically and morphologically. Most of the signs and symptoms and most of the life-threatening effects of atherosclerosis are due to these major components. They result in its being the leading cause of death and morbidity in the urban-industrial countries of the world, especially in Europe and North America.

In this brief manuscript an effort will be made to summarize in more or less chronological order some of the major recent advances in atherosclerosis research. These have resulted in substantially improved understanding of the modern pathobiological principles of atherogenesis.[1-5]

THE RECENT DISCOVERIES AND THEIR IMPLICATIONS

About 25 years ago research in this field began to advance rapidly primarily because of two major discoveries. Electron microscopic studies of the plaque, along with immunohistochemical investigations, demonstrated that the main cell of the plaque is the smooth muscle cell and not the fibroblast as was generally believed prior to that

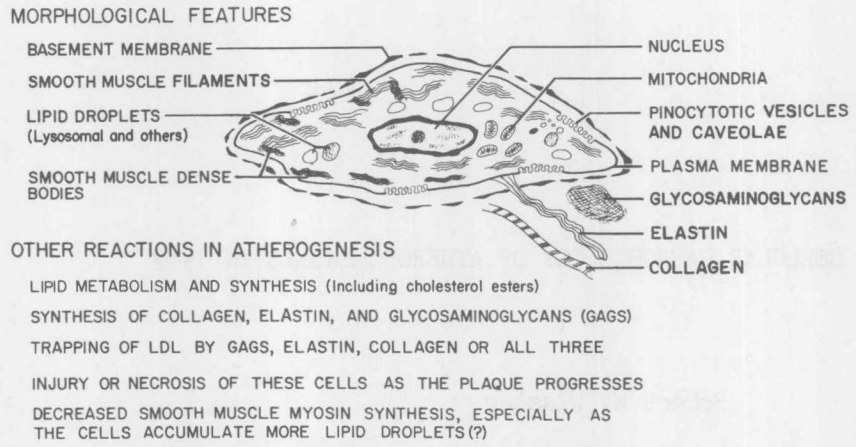

Fig. 1 The major morphological and functional features of the "multifunctional medial mesenchymal cell," the principal cell type involved in atherogenesis. This is the most prominent cell in the fibrous cap of the plaque, and probably makes most of the proteins and the other sclerotic parts of the disease process. (Modified from R. W. Wissler et al., Abnormalities of the arterial wall and its metabolism in atherogenesis, Prog. Cardiovasc. Dis. 18:5 (1976), by permission of Grune and Stratton.)

time (Fig. 1). Identification of the smooth muscle cell as the major cell of the atherosclerotic process helped to organize and unify knowledge about the plaque because this cell takes up lipid, which is an important part of the atherosclerotic process, it makes the fibrous components of the plaque, and it proliferates. It has become more and more evident that, just as in cancer, cell proliferation is an important part of the disease process in atherosclerosis.[1,2,3,6] Therefore, cell proliferation and the factors controlling the dividing of cells in the lesion are of special importance, as is the control of lipid accumulation, especially cholesteryl esters.

Additional work in the early sixties firmly established that the lipid and cholesterol in the necrotic core of the plaque come from the blood, mostly in the form of the lower density lipoproteins (LDL and VLDL). It is now well established that cholesterol-rich protein molecules, or particles, carry most of the cholesterol in the blood and supply most of the peripheral cells including the artery wall cells with cholesterol for cell membrane synthesis. The pathobiological mechanisms responsible for the buildup of cholesteryl ester deposits in and around these cells of the atherosclerotic lesion have become of increasing concern. Since almost all macromolecules in the blood get into the artery wall to some extent, it has become more and more apparent that there has to be some kind of mechanism that is

trapping them or preferentially localizing these low density lipoproteins in the wall.

Many forms of lipid-protein particles circulate in the blood, and these vary greatly in their composition, with varying proportions of protein, cholesterol, cholesteryl ester, phospholipid, and triglyceride (Table 1). Low density lipoproteins, very low density lipoproteins, and broad beta very low density lipoproteins carry the highest percentage of cholesterol and cholesteryl ester in the blood. They are the ones which are most closely associated with the development of atherosclerotic plaques in animals and humans. Some of the high density lipoprotein (HDL) molecules appear to have a protective function. They can carry cholesterol out of peripheral tissues, such as arteries, and transport it back to the liver where it can be secreted into the bile and out into the feces.

As we learned more about the functions of lipid in the plasma and the interaction of lipoproteins with arterial smooth muscle cells, we found that hyperlipidemic plasma with high blood cholesterol levels, relative to the levels we consider to be normal (i.e., 150-170 mg %),[7] does several things that plasma from normal lipidemic individuals does not do. In our laboratory in the late sixties we reported that hyperlipidemic serum from rhesus monkeys fed a high fat, high cholesterol ration for several weeks stimulated proliferation of arterial medial cells whereas normal serum did not.[8] We also found that most of this excess cell division was stimulated by the LDL fraction of the hyperlipidemic serum. Whether one measures the average diameter of cultured colonies of smooth muscle cells in tissue culture flasks, or whether one uses tritiated thymidine to measure the rate of proliferation, one finds that cells that are exposed to normal monkey serum or to LDL from normal monkey serum have very low rates of cell division, and that cells which are exposed to hyperlipidemic monkey serum or LDL from hyperlipidemic monkey serum have very high rates of increase.[9,10] If HDL from normal serum is added to a system like this, one can remarkably reduce the rate of proliferation as measured by the percentage of labelled cells, as compared to the system that only has LDL from hyperlipidemic serum.[11] So there is a protective effect of high density lipoprotein in tissue culture that is consistent with what we now find epidemiologically in populations of individuals.[12] We also found that LDL molecules from hyperlipidemic plasma induce excessive ingress and storage of cholesteryl esters in these arterial smooth muscle cells.

Subcultures of these smooth muscle cells can contain abundant excess cholesterol, much more than the excess amount of cholesterol in hyperlipidemic serum,[13] and if one adds HDL to that system after the cells are loaded, the decrease in cholesteryl ester is very evident.[14] So HDL works to interrupt these cholesterol accumulating processes and will counteract the effects of low density lipoprotein from hyperlipidemic serum.[15]

In these last few years we have had the opportunity to study *in vitro* the interaction of smooth muscle cells of the artery wall that we have sometimes referred to as multifunctional medial mesenchymal cells because they have so many functions and take part in atherogenesis in so many ways.[16] We can study their interaction with lipoproteins *in situ* and have learned that the lipoproteins contribute to the fibrous cap by stimulating migration, proliferation and collagen, elastin and proteoglycan synthesis by these cells. The lipoproteins, especially the LDL from hyperlipidemic sera, can also lead to cell injury and an increase in the rate of cell death, which may be one of the major contributions to the formation of the necrotic core.[17,18]

About 1970 Dr. Russell Ross and his colleagues at the University of Washington in Seattle introduced a new concept in atherosclerosis research: injury to the endothelium, the inner lining of the artery, with subsequent platelet sticking, spreading and liberation of the platelet growth factor, may be extremely important in relation to the development of atherosclerosis, especially in certain individuals. Platelets adhere to the surface of injured endothelium, then spread out and liberate platelet derived growth factor (PDGF) from their alpha granules. This stimulates smooth muscle cells to proliferate. At this point it is a reversible process, unless something happens to make that injury or that stimulus chronic. One of the types of sustaining factors in this injury, according to Dr. Ross and his colleagues,[6] is chronic hypercholesterolemia. This hypothesis is useful in understanding the pathogenesis of the atherosclerotic process. It contributes a great deal to those of us who have been studying atherosclerosis in relation to autopsy findings and pathology. It helps to explain the occasional paradoxical or exceptional case that develops severe atherosclerosis even though the blood levels of low density lipoprotein are low, or in those where there is no history of cigarette smoking or high blood pressure--the three major risk factors that are identified with atherosclerosis. Some of those cases probably represent individuals who do have chronic endothelial injury which is severe and continuous enough so that these can develop the classical atherosclerotic process in spite of the absence of elevated levels of lipid. We believe that this is a rather unusual occurrence in most environments and is probably the exceptional way that advanced atherosclerosis develops.[19] Under usual circumstances the deposit of lipid (and apo B) in the artery wall precedes the evidence of cell proliferation. In fact, in most individuals who have low levels of low density lipoproteins, the process of cell proliferation is intermittent or relatively infrequent, and there seems to be general agreement that at least in primates those kinds of lesions will be expected to reverse completely on their own because of the rapid healing of the endothelium (Fig. 2).

According to current concepts atherosclerosis usually develops in our environment because of relatively slight but continuous elevation of low density lipoproteins in the plasma. This leads to pro-

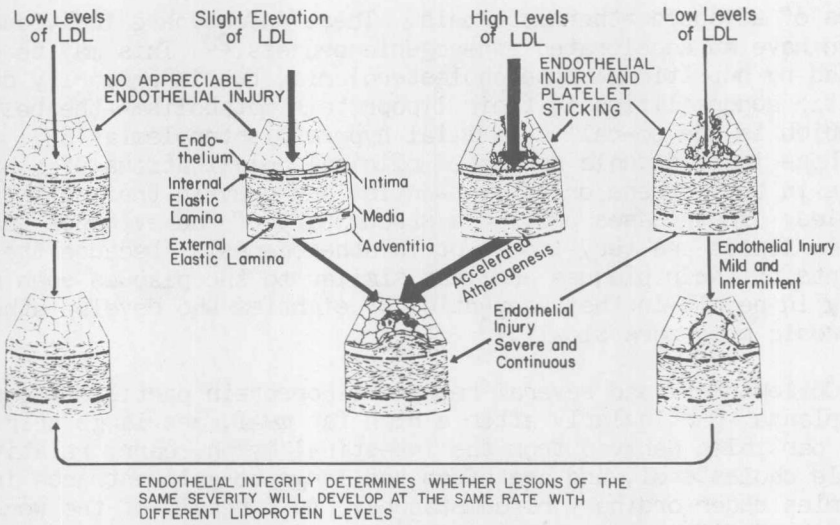

Fig. 2 Presumed pathways of interaction between elevated levels of low-density lipoprotein and arterial endothelial injury to produce a progressive atherosclerotic plaque. Although in extreme instances of continuous and severe endothelial damage, progressive plaque formation can occur even when the low-density lipoprotein levels are low (extreme right); this does not negate the protective effect of low levels of low-density lipoproteins if the endothelium is not being damaged severely (extreme left) or is damaged slightly and not very often. The accelerated atherogenesis frequently observed when familial hypercholesterolemia is present in its heterozygous or homozygous form is represented (second from right), while the usual slow pathogenesis resulting in the same kind of progressive atherosclerosis in individuals with moderate hyperlipoproteinemias over a period of decades is shown second from left. (Modified from R. Ross and J. A. Glomset, The pathogenesis of atherosclerosis, New Engl. J. Med. 295:420 (1976), reprinted by permission of the New England Journal of Medicine; see also R. W. Wissler, Principles of the pathogenesis of atherosclerosis, in: "Heart Disease: A Textbook of Cardiovascular Medicine," E. Braunwald, ed., W. B. Saunders, Philadelphia, p. 1183 (1984).)

gressive growth of the plaques due both to stimulation of smooth muscle cell proliferation and increased deposit of LDL and other low-density lipoproteins within the cells of the plaque and also between these cells.

There are also individuals with well defined genetic disorders in lipoprotein metabolism. They may account for more than 15% of the