Evidence for a Monoclonal Origin of Human Atherosclerotic Plaques and Some Implications

WELL-DEVELOPED LESIONS of atherosclerosis have substantial amounts of lipid underlying them and cholesterol is present in this fatty debris. These facts fastened attention of early investigators on this substance and led to the supposition that cholesterol is a major factor in the evolution of the disease process. This assumption was strengthened by the production, in animals, of superficially similar lesions by dietary manipulations causing increased levels of blood cholesterol. Taken together with the apparent relationship of coronary disease and dietary lipid intake in different geographical and ethnic groups, investigators have been encouraged in their belief in the "insudation" concept of the origin of atherosclerosis.\(^1\)

There is no serious question of the fact that the main lesion of human atherosclerosis is a cellular proliferation, the fibrous plaque, and the prime cell comprising the lesion is a form of smooth muscle cell. Recent proposals concerning the pathogenesis of atherosclerosis have modified the older hypothesis to include the proliferative component.\(^1\)\(^,\)\(^2\) One suggestion has been that lipoproteins, since they seem to be preferred nutrients for the arterial medial cells, by their delivery in excess of usual amounts through damaged endothelium, stimulate arterial wall cell proliferation. The assumption in this and similar formulations is that the medial cells, as such, are the proliferating cells.

Comparison of the naturally-occurring atherosclerotic plaques of human beings and birds with the reparative response of mammalian aorta to injury\(^3\)\(^,\)\(^4\) led us to consider an alternative to the injury-repair hypothesis currently in vogue. This was based upon the following considerations: Cells of spontaneous lesions differ from cells of normal arterial wall media and also from cells populating a repair site. They differ in size and arrangement of cells, in composition of associated extracellular material. In particular the cells of the plaque are smaller than those of the media and appear to be producing abundant collagen and little or no elastin. This contrasts with normal media where cells are surrounded by elastin and only moderate amounts of collagen. Moreover, intercellular junctions between cells of the plaque appear reduced in number or are absent.\(^5\) These apparent differences in the cell populations encouraged consideration of the possibility that the focal proliferative lesions of atherosclerosis either are derived from a different group of cells than those populating the media or are an altered cell population, the change being similar to that occurring in benign smooth muscle cell tumors.\(^6\)

It has been found that benign smooth muscle tumors, uterine leiomyomas,\(^6\) and other preneoplastic lesions\(^5\) appear to originate each from a single precursor cell, i.e., are monoclonal in origin. Recent developments in our understanding of somatic cell genetics and in our capacity to analyze these in human material made the question concerning the cell composition of plaques susceptible to an answer.

The method of analysis applicable to study of the origins of proliferated masses of cells from one or from several precursor cells requires organisms having a mixture of cells of two or more kinds. According to the concept of Mary Lyon, all human females are mosaics.\(^8\) That is, they are composed of two phenotypically distinct cell types. This is due to the fact that early in embryonic development there is random inactivation of one or the other of the two X-Chromosomes. Once inactivation has occurred, each cell population reproduces true to type throughout somatic growth. Given a means of distinguishing the two populations, it is possible to ask whether a pathologic new formation derives from one or from many cells.

About one-third of the black females in the population are heterozygous for glucose-6-phosphate dehydrogenase (G-6-PD) and exhibit mixtures, in tissue extracts, of the A and B forms of the enzyme as the expression of this cellular mosaicism. This property of females has been used to assess the origin of the

From the Department of Pathology, University of Washington, Seattle, Washington.

Supported in part by USPHS grants HL-03174, GM-13543, and GM-00100 and the Greenwald Fund.

Address for reprints: Earl P. Benditt, M.D., Department of Pathology, University of Washington, Seattle, Washington 98195.
cell populations in a number of tumors, including the benign uterine smooth muscle tumors, by electrophoretic assay of the pair of X-linked glucose-6-phosphate dehydrogenase. Analysis of the fibrous plaques by the method indicated is somewhat more complicated than the analysis of the leiomyomas because of the following: While many lesions of the arterial intima are clearly discrete, the presence of blood and of infiltrated blood cells provided some background "noise." Furthermore, as we have found, there is a tendency for the plaques to interdigitate or to overlap. Moreover, many small plaques are frequently present; this makes it essential to monitor the histology of the site. In spite of this "noise" a reasonably clear answer emerged.

In the original paper we reported the findings on 30 plaques from three cases and 50 samples of nonplaque intima from four cases. The data showed that fibrous caps, even of relatively large plaques, appear to be composed of cells that produce solely or predominately one enzyme type. On the other hand, the arterial media seems regularly to be composed of a mixture of cell types in samples as small as 0.1 cubic mm of tissue. Further experiments provide consistent support of the original data: Analysis of 12 more plaques from four more cases including one coronary plaque has yielded enzyme patterns consistent with a monoclonal character of the plaques against a background of mixtures of the two cell types for the intima and inner media.

The apparent monoclonal character of the atherosclerotic plaques invited further exploration of the idea that these proliferative lesions have properties resembling benign neoplasms. One feature of neoplasms expressed by some on culture of their cells in vitro is an apparently infinite capacity to divide. This is in contrast to a finite replicative life span for normal somatic cells. With this idea in mind, Dr. Ned Moss and I have compared in vitro the replicative behavior of smooth muscle cells from human atherosclerotic plaques and uterine leiomyomas. The cells of the atherosclerotic plaques and the cells of the leiomyomas behave in an identical manner under conditions of culture; both, somewhat to our surprise, exhibit a finite life span. We are searching further for features of plaque cells and of leiomyoma cells that may provide them with their selective advantage over normal adjacent cells.

If plaques were derived directly from the media as a response to an injury, a reaction to a generally acting growth stimulant, or an increased local availability of nutrients due to increased permeability of the overlying endothelium, one would expect proliferation of many cells, i.e., a polyclonal growth. Similarly, the organization of a mural thrombus would be expected to yield a proliferative response involving many cells.

The evidence indicating that the plaques are monoclonal proliferations and the implication that monoclonal growths are, at least in a general sense, neoplasms invites us to look anew at factors which could be involved in the pathogenesis of atherosclerosis. The pattern suggested for the atherosclerotic plaques is parallel to those that operate on other tissues to produce neoplasia. In using this approach it seems well to take into account the general and special features of arteries and their function as conduits for blood plasma and its constituents.

We can factor the pathogenesis of the fibrous plaque into three stages as follows: In the initial phase some factors acting on cells of the inner lining of the arteries cause a change (mutation). Such scattered, altered cells in a "subthreshold neoplastic state" can exist for years unexpressed, unless called upon to multiply. In order for such cells to express their special potential for multiplying they must be "encouraged." Thus, in the second stage, some adjuvant or promoting factors are required. The third stage, that of "complication," can be modulated or induced in several ways.

Thrombosis as an initiating cause of atherosclerosis does not seem an immediately tenable postulation. Drs. Steven Schwartz and I have looked at experimental lesions produced by injury of the aortic lining with a balloon catheter. In this injury model thrombosis does not appear as a significant feature. Platelet sticking is absent over much of the denuded area. While it occurs early in some areas, it is gone in a few days and no evidence of a thrombus with organization is found. On the other hand there is no doubt that thrombosis is a feature of the disease that, in its later stages, one sees with reasonable regularity. The mechanism of thrombosis must be left for discussion elsewhere.

Factors which need to be considered as acting in the initial stage or in the origination of atherosclerosis, as has been shown for neoplasms, are such things as mutagens, viral, chemical, or physical. These likely act on a background of genetic susceptibility of the whole individual or some particular portion of an individual's cells. Operating in the second stage may be any one of a series of factors accelerating or stimulating proliferation of the cell population containing the altered subset of cells. Moreover, some selective feature may be acting in this stage to permit the altered cell population to display a condition-dependent advantage over the "normal" population. In the third stage, that of complication, the proliferated cells may be expressing some of the disadvantages of the conditional neoplastic state. One expression of this may be the fatty changes seen in cells in the deeper layers of fibrous plaques. These cells are
separated a greater distance from the lumen and their nutrient supply and so may be nutritionally impoverished. This deprived group of cells can go on to die and thus contribute to the atheromatous debris.

The hypothesis that atherosclerotic plaques are conditional benign neoplasms suggests new ways to look at risk factors. Cigarette smoking seems to be one well-documented entity which increases the risk of coronary heart disease. Mutagenic hydrocarbons are known to be present in cigarette smoke. Other "promoting" substances may also be present. It seems reasonable to examine the possibility that plasma lipoproteins can take up, during passage through the lung, such mutagenic or stimulatory substances and by delivering them to the lining of various vessels expose cells of the artery to these chemicals. It is already known that low density lipoproteins can preferentially take up lipid soluble materials, including benzo (a) pyrene. With this line of thought one becomes aware of the possibility that a good many substances, besides cholesterol, may be carried by the lipoproteins.

The role of cholesterol in the pathogenesis of atherosclerosis is, for all the work done on it, still a moot question. That some relationship of cholesterol to induction of atherosclerosis exists seems likely. What accounts for the relationship is unclear. We can ask some questions in the light of our new concept: Is cholesterol a carrier of something else? Is there a metabolite derived from cholesterol which is the active ingredient causing cell alteration or cell proliferation? Or is the cholesterol merely a concomitant of other substances or features important in the origin of atherosclerosis?

Looking further into the suggested formulation it is evident that things that increase turnover or proliferation of cells can act to promote expression of the selective advantage over its normal neighbors enjoyed by the aberrant cell population. Hypertension, a major risk factor, or some feature associated with hypertension, may operate in this way. Stimulated by the concept outlined and as an offshoot of our work on endothelial cell turnover, we have examined the multiplication rate of endothelial cells in rats made hypertensive by the Goldblatt procedure. The data accumulated indicates that the proportion of cells undergoing mitosis is on the average ten times greater in the cells lining the aorta of hypertensive than in that of normotensive animals. The observed increase in multiplication rate of endothelial cells is compatible with the concept that increased cell turnover elicits the peculiar potential for useless growth present in conditional neoplasms. A similar explanation is tenable for the increased "risk" of atherosclerosis seen in diabetics, particularly of the familial and juvenile variety. We have found evidence that endothelial cells and pericytes of diabetics exhibit greater rates of cell death and cell proliferation than nondiabetics. This appears to be due to an innate susceptibility of diabetic's cells to injury.

Current investigations into the nature of atherosclerosis have been guided by some preconceptions. These have derived largely from nineteenth century investigations and hypotheses. An interesting feature of investigations in the nineteenth century was that new instruments, the light microscope in particular, provided our scientific forebears with an expanded view of life. With this they were freed from old dogmas for fresh speculation. No one can deny that we live in a very different scientific world from that of Virchow and Rokitansky. Our recently acquired insights into the structure and operation of organisms give us freedom once again to generate new questions that in turn guide us to more relevant facts and to more fruitful concepts.
Evidence for a Monoclonal Origin of Human Atherosclerotic Plaques and Some Implications

EARL P. BENDITT

Circulation. 1974;50:650-652
doi: 10.1161/01.CIR.50.4.650

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1974 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/50/4/650.citation

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org//subscriptions/