Developmental Origins of Hypertension and Atherosclerosis*

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Hypertension and atherosclerosis seem to have as one of their major targets the vascular smooth muscle cell. In hypertension, it is obviously the vascular smooth muscle cells whose contractile activity accounts for the elevation of peripheral resistance and therefore the elevation of blood pressure. Observations in a number of laboratories suggest that this pathology may reflect an increase in the mass of the vessel wall, a concept called "structural hypertension", and pioneered by Folkow.¹⁾ Thus one might think of hypertension as a disease of abnormal growth of the *arterial media*. In contrast, atherosclerosis is a disease of the *arterial intima*.²⁾ While emphasis on apolipoprotein disorders has focused attention on the role of lipid accumulation, we should not forget that all significant atherosclerotic lesions contain a large component of smooth muscle proliferation. Perhaps more importantly, there is reasonable evidence that the atherosclerotic lesion begins in pre-existing focal accumulation of smooth muscle cells in the intima. This "intimal cell mass" may be the original lesion of atherosclerosis.³⁾ In this review we will discuss evidence that both the initial lesion of atherosclerosis and the structural changes underlying genetic hypertension are early, developmental events.

Origins of the Atherosclerotic Lesion

Figure 1 demonstrates the natural history of the atherosclerotic lesion. It is important to note that many of the most significant features of the disease, e.g., narrowing, thrombosis, and coagulation, are relatively late events. While most pathologists assume that the early events are associated with

lipid insudation, the experimental data implies that the earliest change may well be focal proliferation of smooth muscle cells, as the intimal cell mass (Fig. 2). Intimal cell masses probably occur in all people and can be found at birth. In this sense, then, the intimal cell mass may be a precursor of the atherosclerotic lesion.

Aside from morphologic studies, evidence that an early proliferative change underlies atherosclerosis begins with the work of Earl Benditt. As early as 1973, Benditt and his colleagues used cell lineage analysis to establish that the human atherosclerotic lesion was monoclonal.⁴⁾ The basis of their idea came from the first application of molecular genetic technique to analysis of this lesion.

Some human black females are heterozygotic for the x-linked marker: G6PD. When Benditt analyzed the mixture of G6PD allotypes in the vessel wall, however, he found that while both allotypes were equally well represented in the normal wall, plaques consisted predominantly of one or the other allotype. When this concept was considered against the background of the possible mechanisms for formation of the atherosclerotic lesion, three conclusions could be reached:

1. The atherosclerotic lesion must begin with smooth muscle proliferation. There is no other way to account for a lesion being monoclonal.
2. The lesion probably begins with some event intrinsic to the vessel wall. Most exogenous events would be expected to produce polyclonal proliferations.
3. The initial formation of the lesion as a smooth muscle proliferative event must involve only a relatively small part of the population of the vessel wall, possibly as few as one cell.

Benditt's data was experimentally confirmed by

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Fig. 1 Natural history of atherosclerosis.

Fig. 2 Intimal cell mass: Monoclonality must be an early event in atherogenesis.

two other laboratories.^{5,6)} The concept also led to predictions which seem now to be valid. Pathologic studies of human tissue by the Velicans⁷⁾ in Romania, as well as more quantitative studies of swine by Thomas in the United States, have shown that atherosclerotic lesions begin as intimal cell masses.^{5,8)} While other pathologists, primarily in the United States, have classified the intimal cell mass as a “normal change”, their argument has been based upon the fact that the intimal cell mass is a universal lesion found in all populations, even in populations where the incidence of atherosclerosis is very low.⁹⁾ It is implied, however, that as the precursor of atherosclerosis, a focal proliferation could exist without progressing to a fully developed lesion. Experiments by Lee et al. address this concern.¹⁰⁾ These investigators used a hybrid hare, a system resembling human black females in heterozygosity for G6PD. They found that the lesion of the fat-fed animal, unlike the lesion in the human, was polyclonal. While one might conclude from this experiment that the rabbit is a poor model for atherosclerosis, a more reasonable analysis

may just be that the rabbit, with its extensive invasion of the lesion by monocytes, is showing the allotype of the dividing cells. In support of this hypothesis, Thomas and his colleagues found that the number of cell replications required to make a lesion in fat-fed swine was very small, too small to account for monoclonality.⁵⁾ Again, this observation is consistent with monoclonality if we assume that the small number of divisions was superimposed on a pre-existing intimal cell mass.

Finally, there is data in humans to support the hypothesis that smooth muscle replication is a very early part of the atherosclerotic process. David Gordon has developed a new method for the analysis of cell proliferation in human lesions. This method uses a cell cycle specific antibody, “cyclin”, to detect replicating cells in human tissues. The method was first validated in rats undergoing angioplasty.¹¹⁾ The index of replication in human plaques was not significantly different from the index of replication of the underlying normal wall. Moreover, the plaques contained replicating macrophages in approximately the same frequency as replicating smooth muscle cells. While one may speculate about the importance of these replicating macrophages, the low frequency of replicating smooth muscle cells implies either that smooth muscle replication is a very early event in the ontogeny of the disease, an episodic event only occurring occasionally during the ontogeny of the lesion, or an indolent event, that is, one that occurs only at a very low rate over the many years required to form the lesion.

If the intimal cell mass hypothesis is correct, one might expect intimal smooth muscle cells to have properties distinct from smooth muscle. In collaboration with David Gordon of the Department of Pathology at the University of Washington, and Josiah Wilcox of Genentech, Inc., San Francisco, we have used *in situ* hybridization to discover that the intimal smooth muscle cell expresses two genes of special interest for atherosclerosis: PDGF A-chain is expressed by intimal smooth muscle cells, and tissue factor is expressed by intimal smooth muscle cells. The observation of tissue factor is particularly exciting because of the possibility that this is a critical initiating factor for coagulation and therefore may be critical to the final outcome of the atherosclerotic lesion.^{12,13)}

In summary, it is important to realize that the number of cell doublings required to make some-

Fig. 3 Structural hypothesis of hypertension; SHR vessel wall versus WKy rat vessel wall.

thing the size of an atherosclerotic lesion, even if one begins with only a single cell, is very small. Only 35 doublings are required to make an entire adult human being! Thus it seems likely that accumulation of smooth muscle cells to form intimal cell mass is an early step, perhaps one that is universal to many animals. It is upon this ground that the atherosclerotic lesion develops.

Origins of Hyperplasia in Genetic Hypertension

Somewhat the same issues arise when one begins to consider the origins of hypertension, at least the origins of hypertension in the SHR (spontaneously hypertensive rat). Many different hypotheses have been offered for the origins of the hypertension of the SHR. One simple fact, however, is obviously true: the vascular wall of the resistance vessels in the SHR is obviously thickened. This is most easily understood from functional studies. Figure 3, based on Folkow's work,¹⁾ schematically shows the effect of increased wall mass on the over-response of the hypertensive wall. A simple structural change can account for massive changes in peripheral resistance.

This fact led Folkow¹⁾ to propose that a change in the structural mass of the arterial wall was responsible for hypertension and led our laboratory to determine whether the increase in mass was due to hyperplasia or hypertrophy. Somewhat to our surprise, we found that aortic smooth muscle cells of the adult SHR are polyploid.¹⁴⁾ Polyploid replication contrasts with the usual mechanism of cell replication seen in atherosclerosis, i.e., with

true hyperplasia. In other words, increase in vessel wall mass in hypertension, at least in large arteries, is accompanied by an increase in the total amount of DNA without the cells undergoing normal mitotic replication. The obvious assumption was that the difference in DNA content represented something which happened in the *adult* animal. Recent experiments from our laboratory have thrown doubt on this assumption. In these experiments, we examined the replication of smooth muscle cells in adult SHR and adult WKy control strains. In both cases, tritiated thymidine was infused for two weeks by an Alza pump. We failed to see any difference in rates of replication.

Sarah Gray of the University of California at Davis, has looked at vessel wall mass in fetal and newborn SHRs compared to WKy rats.^{15,16)} She found that the vessel wall mass is increased in the SHR, even before birth! While Dr. Gray provides no direct data on cell number, she has also examined the number of lamelli in the aorta of the newborn SHR, and compared that value with the number in the newborn WKy. There is approximately one more lamella in the newborn SHR. It seems likely therefore that the SHR rat by birth already has an increase in cell number.

It appears that the increased DNA of the SHR represents a developmental event.

Development of Distinct Smooth Muscle Lineages

In summary, the data imply that the early, developmental differences may play a role in the pathogenesis of both hypertension and atherosclerosis. Recent studies from this lab have suggested that the molecular biology underlying atherosclerosis and hypertension may reflect the existence, in the vessel wall, of two distinct smooth muscle lineages.

The key experiment was comparing smooth muscle growth from the newborn aortas (pup cells) with cells grown from adult animals. Morphologically, cells derived from the newborn rat differ remarkably from cells derived from the adult animal. The cells of the newborn rat are epithelial in appearance. Moreover, these cells have a number of other peculiar properties.^{3,17)} Unlike adult smooth muscle cells, smooth muscle cells begin as diploid cells, but become tetraploid in culture. Since the normal animal develops polyploidy with age, and the hypertensive animal develops polyploidy with a greater frequency with age, these data

suggest that the cell *in vitro* is recapitulating an *in vivo* phenomenon.

Of greater interest to us, however, has been evidence that the pup cell produces very different gene products than does the normal adult cell. When we tried to arrest pup cells in culture, we found that their growth could not be arrested by the normal procedure of preparing serum depleted of platelet factors. In trying to find the mechanism underlying this proliferative response, we discovered that pup cells secreted PDGF.

These morphological and growth differences are also found at a genomic level. PDGF B-chain gene is not expressed in cultured adult cells but is constitutively expressed in pup cells. In contrast, adult cells, but not pup cells, express the PDGF α receptor. This difference is independent of medium or substrate and is stable for many generations *in vitro*.

The stable differences in behavior led us to consider the possibility that the intimal cells formed after angioplasty might arise from a similar population already present in the normal vessel wall. Intimal cells, like pup cells, show proliferation *in vivo* under conditions where there is no evidence for interaction with the vessel wall with exogenous sources of growth factor such as platelets or macrophages. When the intimal cells were put into culture, they showed a morphology very similar to pup cells, and also showed growth factor independent growth.¹⁸⁾ Again intimal cells showed the PDGF B-chain mRNA. Despite these observations, we have been unable to prove that growth of the pup intimal cell requires either the PDGF B-chain or the PDGF B-receptor. Thus, as of today, we know that pup and intimal cells have a mechanism for growth that does not require either PDGF or FGF, but we do not know what the mechanism is. Nevertheless, it is interesting to speculate that such a mechanism may be important in the normal developmental growth of the vessel wall.

Since pup cells and neointimal cells have very different properties in culture, we were intrigued with the possibility that the reappearance of this phenotype in cells derived from the vessel wall following a simple balloon injury might suggest that the vessel wall contains a normal population of cells similar to those seen in large numbers in a newborn animal. To test this hypothesis, we cultured cells from the vessel wall under conditions where no platelet-derived growth factors were

available. This cell, like the pup and intimal cell, has an epithelial morphology and grows readily under conditions free of serum mitogens.¹⁷⁾ In summary, at least based on behavior in culture, the normal rat aorta contains two distinct subpopulations of "smooth muscle cells". One of those subpopulations is enriched in the newborn aorta and in the neointima formed after angioplasty. We have gone on to use differential colony hybridization to identify genes that identify the subpopulations *in vitro* and also show enriched expression in fetal or intimal tissue. The first gene to show this pattern is elastin. We find it intriguing that only some of the vessel wall smooth muscle cells might be specialized for secretion of a molecule responsible for forming the structural core of the vessel wall. Finally, it is of interest to note that Gray and her colleagues¹⁵⁾ found an increase in the number of layers of elastin in the aortas of newborn SHR as compared with WKY rats.

Summary

At least *in vitro* the arterial smooth muscle cell population appears to include two cell types that have stable phenotypes *in vitro*. Existence of similar distinct lineage is suggested by the developmental pattern of expression of elastin, a gene whose expression is greatly enhanced in pup or intimal smooth muscle cells. Importantly for this hypothesis, elastin synthesis has been noted by others to be abnormal in the atherosclerotic plaque and we find enriched elastin synthesis in the neointima formed after balloon injury.

For atherosclerosis, the second cell type could explain the ontogeny of the intimal cell mass and might contribute to monoclonality.

For hypertension, the second cell type may represent a developmental stage that could be important in the ontogeny of early structural changes in hypertension.

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