PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 173, 1-13 (1983)

MINIREVIEW

Cellular Proliferation in Atherosclerosis and Hypertension (41601)

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Cell proliferation is now recognized as a common feature of the vascular response to injury characteristic of both hypertension and atherosclerosis. Our concepts of the role of proliferation in the underlying mechanisms for each disease, however, have been entirely different.

Current concepts about atherosclerosis began with the work of Virchow. He recognized the presence of cell proliferation in atherosclerosis over a century ago but viewed the cellular events as reactive rather than etiologic (1). This view has changed over the last two decades. With the development of the electron microscope, pathologists were able to identify the smooth muscle cell as the characteristic cell of the atherosclerotic plaque. A number of manipulations, including removal of the endothelium and exposure of the animals to hyperlipemia were shown to result in smooth muscle proliferation. These studies in animals and in humans support the idea that the initial step in lesion formation, prior to increase in intimal lipids, is the formation of focal masses of smooth cells in the intima (2–4). For at least some investigators, smooth muscle proliferation is now seen as the fundamental, etiologic event in atherosclerosis.

Our concepts of the role of smooth muscle cell proliferation in hypertension are more recent. Most research in hypertension has emphasized the role of physiologic controls of cell contractility or cell volume (5, 6). The pathology of the disease, much as with Virchow's early view of atherosclerosis, has been thought of as a reactive, rather than a causal part of the process. There is, however, general agreement that chronic hypertension is associated with vessel narrowing, presumably resulting from a thickening of the vessel wall (7–10). This "structural change" can account for a portion of the increased resistance to flow even when the vascular bed is chemically relaxed. These changes in vessel wall structure involve all elements of the vessel wall, including an increase in smooth muscle cell mass. We have suggested that the increase in vessel wall mass in essential hypertension may be secondary to an increase in DNA content following some acute hypertensive injury. The result is that "essential" hypertension may represent the common end stage form of hypertension responsible for maintenance of chronic hypertension as an apparently idiopathic, "essential" illness. This new idea also raises the possibility that abnormal proliferative responses underlie both of the major cardiovascular diseases affecting our species.

The role of endothelial denudation in smooth muscle proliferation. Our discussion of smooth muscle proliferation cannot begin until we consider the role of the other major cell type of the vessel wall, the endothelial cell. We can start this by considering current models of atherosclerosis. Ross has demonstrated that the platelet contains a critical growth factor that is able to control or initiate smooth muscle replication (4). This growth factor is contained in the alpha-granule (11). When the endothelium is removed by mechanical abrasion, platelets adhere, granules release, and smooth muscle cells proliferate. Immunocytochemical studies have shown the release of platelet alpha-granule contents at sites of endothelial denudation (12) and the abolition of smooth muscle proliferation when animals are depleted of platelets (13).

From the point of view of the cells of the vessel wall, this hypothesis posits that endothelial injury is the critical event in the pathogenesis of vascular proliferation. It is important to be precise about "endothelial injury." In this case, injury means denudation leading to thrombosis. We need to consider how large an area of denudation might be significant and how small an area of denudation is likely to be detected in microscopic examination of the vessel wall. Scanning electron microscopy (SEM) provides rapid and thorough examination of large areas of vascular surfaces in great detail. Many square meters of the endothelial surfaces of rabbits, rats, monkeys, and other species have been examined in the SEM. These studies, in both normal and hy-

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perlipemic animals, have shown a continuous layer of endothelial cells covering the surface, with no evidence of denudation or platelet deposition until after lesion formation (14–17). There is no convincing evidence that endothelial denudation and platelet aggregation occur prior to lesion formation. This is true even in hypertensive and hyperlipemic animals (18–20), both of which show an accelerated endothelial turnover as well as increased risk of atherosclerosis (21).

While morphologic data for denudation in the normal vessel are absent, it is possible that denudation involves areas that are too small to be detected by SEM. We can use available data to estimate the amount of denudation likely to be detectable. Our studies in the rat show that the endothelium can re-cover an area one cell wide within about 3 hr (22). Similar data have been published for regeneration in the rabbit with somewhat slower regeneration rates (23). Turnover studies in the rat imply a rate of cell loss of approximately 1×10^{-2} to 1×10^{-3} per day (24, 25). These values may be combined to estimate the total average area of denudation present at one time:

Denuded area = area per cell

$\times$ turnover per day $\times$ recovery time.

The assumptions used result in 0.12 μm^2/cell as a maximal estimate of the amount of denudation present. This amount of exposed surface might be difficult to detect at the usual level of resolution available by SEM. In an attempt to increase the extent of turnover, we reexamined the effects of endotoxin. Endotoxemia provides a rapid increase in the rate of endothelial desquamation (26, 27). We also found that turnover increases. Denuded areas were absent, but we could find cells loosely attached to the monolayer. On the basis of these data, we suggested that denudation occurs with a coordinated undermining of detaching cells by adjacent viable endothelial cells, a form of nondenuding desquamation (28).

The concept of nondenuding desquamation is important because there is substantial evidence for increases in the turnover of endothelial cells in both hypertension and atherosclerosis. Acute hypertension causes an increased uptake of thymidine in the rat aortic endothelium (29). Turnover returns to normal in chronic hypertension. It is possible, however, that endothelial injury occurs during periodic bouts of elevated pressure. Human hypertension is complicated by therapy. In our animal studies, withdrawal of therapy results in an "overshoot," with an elevation in endothelial turnover in either chronically hypertensive animals or normotensive animals released from antihypertensive medication (30). There is also increased thymidine labeling of the endothelium adjacent to atherosclerotic lesions in experimental hypercholesterolemia (19), and evidence that increases in DNA synthesis by endothelial cells may precede the development of the lesions (31).

The localization of normal cell turnover is also consistent with a possible etiologic role for endothelial turnover in atherosclerosis. Tritiated thymidine-labeled cells appear in clusters at branching points, both in normal and hypertensive animals (18, 29, 32, 33). This clustering may be related to increased cell turnover at sites of hemodynamic strain in these regions and corresponds to the distribution of atherosclerotic lesions (34). Under experimental conditions, endothelial cells can be detached from perfused vessel walls by shearing forces in excess of 200 dynes/cm^2 (34). Such forces can occur *in vivo* and if single cells or small patches of cells are removed, the endothelium has the capacity to reestablish continuity rapidly (22). In the normal rat such small areas of desquamation are covered by migration of surrounding cells, without proliferation, in a few hours. We simply do not know whether prolonged normal turnover at single sites, or changes in turnover seen in hypertension and hyperlipemia may ultimately lead to a loss of endothelial continuity.

In summary, these data allow the possibility that repeated, transient periods of denudation might occur. Multiple short periods of denudation might be expected to have a greater affect on the vessel wall than would be expected from a prolonged period of permanent denudation. Studies of endothelial denudation show that the exposed subendothelium remains thrombogenic for no more than a few hours after removal of the endothelium (12, 35). Thus, repeated brief periods of denudation could be particularly impor-

tant for platelet interactions and, therefore, for the proliferative responses of the underlying smooth muscle cells. Finally, we need to point out that there is no doubt that overt endothelial denudation does occur as lesions advance. We know this because of the obvious role of thrombus in the advanced lesion (36). Given our knowledge of the effects of platelet contents on smooth muscle proliferation, it seems likely that the endothelial denudation model is very important in lesion progression, regardless of the issues of initiation.

Smooth muscle proliferation without endothelial injury. Until now we have assumed that smooth muscle proliferation results from some causal event, an injury, occurring to an element of the vessel wall other than the smooth muscle itself, that is, the endothelial cell. Benditt has offered an alternative model for atherosclerosis, based on the changes in the replicative ability of the arterial smooth muscle itself. He observed that atherosclerotic lesions begin as singular focal masses, similar to benign tumors of other tissues. The comparison is strongly supported by evidence that human atherosclerotic lesions, like many tumors, have monoclonal phenotypes. That is, the cells of individual lesions appear to arise from single cells (37). Since the only other known monoclonal growths are neoplasms, Benditt posits that atherosclerotic lesions begin by neoplastic transformation.

Thomas and his colleagues (3) have explored similar data, but come to a somewhat different conclusion. They propose that smooth muscle proliferation may begin with focal masses of intimal cells subjected to mitogenic effect by cell injury or by entry of lipoproteins into the vessel wall. Either set of ideas raises the possibility that endothelial denudation may not be a crucial event in the initial stages of lesion formation. Neither Thomas' nor Benditt's hypothesis rules out the probability that progression of the lesion into a clinically significant mass, may depend on the thrombotic events described by Ross.

Smooth muscle proliferation secondary to endothelial dysfunction. At this point we should consider the possibility that smooth muscle cell proliferation results from some abnormal function of endothelial cells, without denudation. For example, direct interaction of platelets could occur with intact endothelial cells. Normal endothelial cell functions include antithrombotic and anticoagulation functions likely to be important to the issue of interactions of the vessel wall with platelets. There is, however, no evidence that the intact endothelium ever becomes thrombogenic (38).

More positive evidence for a role of interaction between endothelial cells and blood cells in atherosclerosis or hypertension, comes from studies of the early stages of these diseases. Monocytosis of the endothelium is a prominent finding in the aortas of animals with hyperlipemia (39). This fact implies that either the monocyte alone, the endothelium alone, or both cells are responding to some as yet undefined injury. The presence of monocytes is particularly important because these cells, like the platelet, can release a growth factor (40, 41). Similarly, there are data, although less extensive, for an interaction of the endothelium with leukocytes in some forms of hypertension (42). The nature of the changes in leukocytes or endothelium responsible for these interactions have only begun to be explored (43–45).

A further possible nonthrombogenic mechanism comes from evidence that metabolic products of the endothelial cell itself may be important for the regulation of smooth muscle proliferation. There are three reports of this sort of phenomenon *in vitro.* Chamley-Campbell *et al.* (46) found that freshly isolated smooth muscle cells are not responsive to growth factors. This nonproliferative state of smooth muscle cells exposed to serum is prolonged by cocultivation with endothelial cells. Similarly, Castellot and his associates (47) reported that endothelial cell-conditioned medium could inhibit the normal ability of serum to stimulate replication of smooth muscle cells despite the presence of platelet-derived growth factor (PDGF). They suggest that production of this inhibitor by normal endothelium prevents the entry of smooth muscle cells *in vivo* into a proliferative state. In this view, smooth muscle proliferation only occurs when the inhibitory function of the endothelium is lost.

In contrast to these reports of a growth inhibitor, we have described a factor from endothelial cells that stimulates smooth muscle growth. The protocols for these studies differ from those described above in that the inhib-

itor was found in medium from endothelial cells grown in serum. In other words, an inhibitory effect depends on growth of endothelial cells in medium containing platelet-released materials, including the PDGF. Our experiments have explored the effects of endothelial cells on smooth muscle cells in the presence of plasma-derived serum (PDS). PDS is a platelet-free, plasma substitute lacking the platelet-derived growth factor (4). Under these conditions, endothelial cells synthesize a polypeptide mitogen, endothelial cell-derived growth factor (ECDGF). ECDGF stimulates smooth muscle cells in the absence of other mitogens. Available biochemical data implies that ECDGF is a distinct polypeptide from PDGF. The endothelial cell factor has a molecular weight of about 20,000 daltons and has an acidic, rather than a basic charge. Before deciding that ECDGF and PDGF are different, however, we need to note that the peptides have been studied from different species (bovine vs primate).

The existence of a smooth muscle cell growth factor derived from endothelial cells clearly presents a teleologic paradox. Why would one expect the vessel wall cell associated with homeostasis to stimulate smooth muscle growth? Perhaps ECDGF represents an abnormal function. We do not know whether production of ECDGF is modulated. For example, the differences in our data and the observations of inhibitors could well depend on the use of plasma-derived serum rather than serum containing growth factors. Since we do know that endothelial cells *can* produce ECDGF, we have tried to explore a possible role *in vivo*. We reasoned that all forms of angiogenesis begin with endothelial differentiation, and considered the possibility that an endothelial cell factor could play a role in vascular development. To explore this possibility, we put bovine endothelial cells on chick chorioallantoic membranes. Although there was no evidence that these foreign cells induced smooth muscle differentiation, there was a dramatic angiogenic response (see Fig. 1). This response could also be produced by partially purified ECDGF of a dose-dependent fashion (48). These data demonstrate that endothelial cells can make a growth factor. They do not tell us when, how, or if this function is expressed *in vivo,* but they do illustrate the interaction between the two cell types can occur *in vivo.*

Evidence for endothelial cell dysfunction in vivo. Before leaving the topic of possible nondenuding endothelial injuries, we need to consider the evidence that some endothelial injury does occur *in vivo* in hypertension or hyperlipemia. A wide range of biochemical functions have now been studied in endothelial cells. Some of these, particularly the synthesis of prostacyclin, might well be critical to interactions with platelets (38). Unfortunately, we have no way of assessing *in vivo,* the biochemical phenomena established in cultured endothelial cells. Morphologic studies can be done *in vivo* or *in vitro,* but evaluation is usually quite subjective. Various authors have described increased roughness of the endothelial surface, changes in cell thickness, alterations in the cell coat, presence of blebs or changes in electron density of the cytoplasm of endothelial cells (49). These morphologic changes are difficult to quantify. In contrast, we know rather precisely how frequently endothelial cells replicate and it seems reasonable to associate this value with an indirect measure of cell injury (50). Göran Hansson approached this problem by using immunocytochemical techniques for detection of endothelial cell injury (49). Autogenous IgG can be demonstrated free in the cytoplasm of some endothelial cells using an immunoperoxidase or immunofluorescent technique. Since IgG is free in the cytoplasm, we suggested that these were lethally injured cells and looked at other markers of cell death. Approximately 1% of the cells in an adult rat stained for IgG. More than 85% of these cells also contain Ca^{2+} deposits, as indicated by the accumulation of the calcium probe, chlortetracycline (49). This implies that substantial numbers of cells have passed the point of no return and are destined to undergo cell death and necrosis. Somewhat similar observations of dying smooth muscle cells have been made by Thomas *et al.* (51) in the atherosclerotic fat-fed swine.

The role of cell death in current models of vascular reactions to injury is not clear. The injuries are nondenuding and we do not see platelets adherent to dying cells. There is no clear evidence that dying cells can stimulate smooth muscle growth *in vivo.* We have, how-

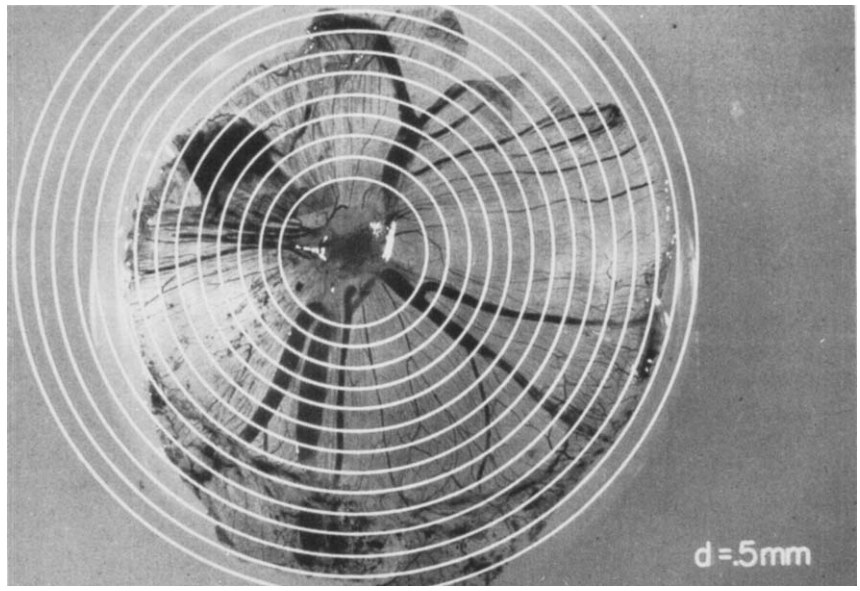

FIG. 1. Angiogenesis. Chick chorioallantoic membrane. An implant of bovine endothelial cells at the center has provoked a marked angiogenic response. Intercepts of new vessels with the circles allows us to do a dose-response assay and show that endothelial cell-conditioned medium is angiogenic over a range of concentrations.

ever, noted that cytoplasm released from lysed endothelial or smooth muscle cells is able to stimulate smooth muscle proliferation *in vitro* (52). It is also worth considering the interactions of materials released from dying cells with plasma protein, particularly the possible activation of the complement cascade. Finally, Hansson has suggested that the dying cells may attract monocytes which could, in turn, secrete their growth factor (53).

Characteristics of smooth muscle proliferation in hypertension. Up until this point we have concentrated on the control of smooth muscle growth in atherosclerosis with an emphasis on the stimulation of smooth muscle replication by PDGF. The stimulus for smooth muscle replication in hypertension is less clear. Some authors have speculated about "stretch" or injury, but these remain only as vague suggestions of specific mechanisms. We have suggested that the PDGF response may be important in hypertension (54). As noted already, there is increased endothelial replication in the early phases of the hypertensive response in the aorta as well as in small vessels. In contrast to the story about atherosclerosis, evidence for small vessel denudation early in hypertension is convincing. Bevan (57) has shown an increase in endothelial cell turnover. Goldby and Beilin (55), Reidy and Schwartz (77), and Giese (56) have shown denudation and increased permeation of plasma proteins. In contrast, changes in endothelial replication do occur in large vessels, but are not associated with any evidence for denudation (25, 28, 30, 55–60). The increased endothelial replication correlates in time with the increase in smooth muscle replication. Both endothelial and smooth muscle cells are quiescent in chronically hypertensive animals. These observations suggest that the smooth muscle replication in hypertension may depend on as yet undefined events occurring during the increase in blood pressure.

Comparison of cell proliferation in hypertension and atherosclerosis. The simplest aspect of this discussion is the distribution of cellular accumulation in the two diseases. In atherosclerosis, smooth muscle cells accumulate in the intima. In contrast, the bulk of the proliferative response in hypertension appears to be in the media.

We will return to the issue of distribution of cells in the two diseases below. More im-

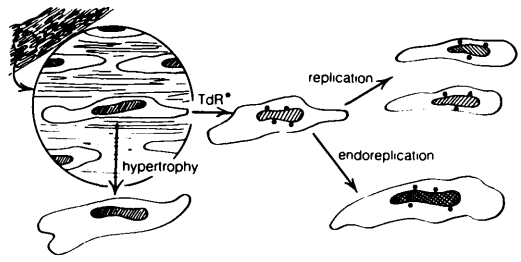

FIG. 2. Two forms of replication.

portantly, there is evidence that the type of proliferation seen in hypertension is quite different from that seen in atherosclerosis. Pathologists have frequently stated that hypertension is associated with proliferation of vessel wall cells in man. One laboratory has even claimed a highly significant quantitative correlation between morphological assessment of vessel change and level of blood pressure (8). The ability to quantitate this association is limited however, by the difficult morphometric problem of determining cell number as an absolute value. Since cells have complex sizes, shapes, and constitutions, the number of cell profiles or even nuclear profiles per unit area is a misleading estimate of the number of cells per unit volume. Alternative methods exist, including serial sectioning or step sectioning (3). These are, however, generally too laborious and are poorly applicable to complex vascular tissues. Olivetti *et al.* (61) used a simpler approach. These workers used the nuclei per unit area in sections of different thickness to determine nuclei per unit volume in a manner independent of variables of cell size, shape, or constitution. They found no increase in the number of cells in the aortas of hypertensive rats. The failure to find an increase in cell number presented a paradox, since Bevan and others had shown an increase in cell replication in the early phase of hypertension as well as an increase in DNA control in the late phases of hypertension (57, 62).

Our studies resolved this paradox, at least for the aorta, by showing that cells in the vessel wall can be tetraploid or have even higher levels of hyperploidy. The frequency of hyperploid cells was greatly increased in hypertensive rats, accounting for the apparent paradox. As noted above, this observation offers an intriguing and testable hypothesis for the etiology of some forms of essential hypertension. Increased DNA content of each cell could be expected to increase the cells' production of the contractile and structural proteins responsible for maintaining vascular resistance. More relevant to the present discussion these data imply that there are two forms of replication in the normal vessel wall: replication and endoreplication (Fig. 2). Their responses differ in the diseases they are associated with and in the distribution of the replicated cells:

Atherosclerosis	True cell replication Progeny accumulate in intima Stimuli: deendothelialization, platelet-derived growth factor, macrophage-derived growth factor
Hypertension	Endoreplication Progeny remain in media Stimuli: high blood pressure, specific mechanisms unknown

We should also point out that tetraploid smooth muscle cells are present in the normal vessel wall. Their frequency, in rats and in man, increases up to adulthood [approximately 20 years in man, 3 months in rat (63), see Fig. 3].

It is interesting to speculate about the similarity of this increase in frequency of tetraploid cells, to the change in replicative lifespan of smooth muscle cells in culture. Smooth muscle cells from man, like cultured fibroblasts, will only replicate a finite number of times. The total number of replications *in vitro,* called the replicative lifespan, declines greatly up to adulthood, with a slower decline at later stages of life. Martin and his colleagues (64) have suggested that a decline in the ability of most vessel wall cells to replicate may result in the escape of other cells from normal growth control, resulting in atherosclerotic lesions.

Distribution of cell proliferation in atherosclerosis. In neither experimental nor human atherosclerosis is there any evidence for the formation of smooth muscle cell accumulations in the media. This is startling, since in the normal wall the intima is relatively hypocellular, whereas the media is densely packed with smooth muscle cells. Thus, regardless of how or why proliferation occurs, any adequate hypothesis must explain the localization of lesions. One can go a step further and propose that localization itself, rather than the proliferative response, is the criticial initial step in lesion formation. This idea forms the basis for a hypothesis which is proposed here:

Migration/trapping. 1. The initial step in lesion formation is the trapping of small numbers of widely scattered smooth muscle cells in the intima. This may occur as a normal event during embryogenesis or during later life as a result of the migration of cells from the media.

2. Once in the intima, cells do not migrate back into the media, or do so only very slowly.

3. Smooth muscle cells located in the intima are freed from the normal contact inhibition growth controls found in the densely populated media. This results in focal proliferation. The ultimate extent of this proliferation is limited by the inherent replicative capacities of the trapped cells and by the same cell density controls which limit proliferation in the media.

4. Atherosclerosis risk factors act either by initiating migration of cells in the intima (chemotaxis) or by stimulation of proliferation of cells already located in the intima (mitogenic or neoplastic effect).

5. Since lesions begin with trapping of widely scattered cells in the intima, each individual lesion is likely to begin with one, or at the most, a small number of cells. Thus, lesions would be expected to be oligoclonal or monoclonal in origin, regardless of the mechanism of proliferation.

The first question to be asked is whether medial cells ever do migrate into the intima. The evidence that they do comes from experimental arterial injury. An extraordinary variety of forms of trauma have been used to injure the vessel wall (65). Regardless of the form of injury, the end result is accumulation of smooth muscle cells in the intima. For example, in our study of endothelial regeneration in the rat aorta, a balloon catheter was passed over the intima, removing the endothelium by abrasion (65). The normal aortic intima of the adult rat contains few, if any, smooth muscle cells, and none were observed in the immediate period following passage of the catheter. Despite this, lesions were found and arose only in the intima. Thus, it seems likely that an early step in lesion formation was migration of smooth muscle cells from the media. Similarly, quantitative, cell kinetic studies by Hassler (66) and Webster *et al.* (67) indicate that cells in intimal thickenings arrive there by migration from the tunica media rather than by simple replication of intimal cells. The same conclusion has been reached on the basis of morphologic evidence following a variety of forms of intimal injury. In some cases, smooth muscle cells have been seen, apparently migrating through fenestrae of the internal elastic lamella (68).

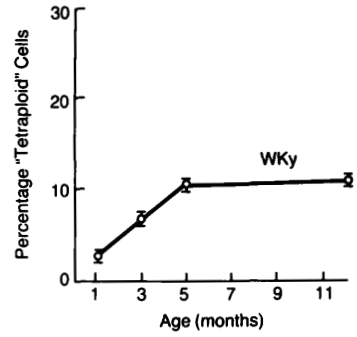

FIG. 3. Frequency of tetraploid aortic smooth muscle cells in normotensive Wistar–Kyoto (WKy) rats as determined by flow microfluorimetry. Nuclei were stained with diamidinophenylindole as described by Owens *et al.* (1981). Means ± SEM are shown.

It is important to note again that none of these manipulations produced masses of cells in the tunica media. Smooth muscle migration, therefore, appears to be an essential component of the type of lesion formation we call atherosclerosis. There is also evidence for migration in cholesterol-induced injury. Thomas *et al.* (69) studied the cell kinetics of lesion formation using [^{3}H]TdR and autoradiography, with estimation of thymidine index. Their studies demonstrated that lesion

formation following cholesterol feeding involves the recruitment of an increased number of intimal and medial smooth muscle cells into the growth fraction. Again, despite this increased mitotic activity, lesion formation occurs only in the intima. Thus any cells produced in the media must either die or migrate into the intima.

Thomas and his colleagues (3) have also suggested that *preformed* intimal accumulations may be the ground for development of lesions. The aorta of the newborn animal of all species contains few, scattered smooth muscle cells in the intima (70, 71). The fate of these cells is largely unknown. We do not know whether they die, whether they leave the intima by migrating deeper into the wall, whether they exit into the bloodstream, or whether they remain in the intima. What is known, however, is that the normal intima in many species shows an increase in numbers of intimal cells with age, as well as an increase in extracellular connective tissue (72). These are diffuse changes which occur in humans regardless of the presence of atherosclerosis, and are even found in animal species which do not develop atherosclerosis. The spontaneous accumulation of cells in the intima suggests either that embryologically derived intimal cells proliferate *in situ,* or that there is a spontaneous migration from the media into the intima as the animal ages. It remains to be seen whether these cells are irreversibly trapped within the internal elastic lamella. Thomas' data is also relevant to this issue. They have shown that intimal lesions produced in swine by cholesterol feeding require only a small number of generations of smooth muscle cell proliferation (51). They demonstrated this by labeling the aortic wall with [^{3}H]TdR before adding cholesterol to the diet. If lesions were formed by proliferation of single cells, then the number of generations required to make a lesion should be quite high. Thus, there should be a great dilution of the number of grains per nucleus of intimal cells in the lesions. This did not happen; the amount of grain-count dilution was consistent with only a small number of replications. They concluded that cholesterol-induced lesions originate by a few divisions of several cells rather than by a large number of divisions of single cells. In other words, cholesterol lesions in animals appear to be polyclonal. This would seem to contradict the known properties of atherosclerotic lesions in man, and might be interpreted as evidence that the experimental disease is a poor model for human atherosclerosis. However, the observation is consistent with monoclonality, if one posits that monoclonal accumulations exist in the intima prior to the effects of cholesterol. Thomas and his colleagues (3), moreover, have noted an impressive correlation of the distribution of lesions in swine aorta to the cells present in the intima prior to the onset of a high-fat diet. It is possible that cholesterol insudation or endothelial injury acts, not by initiating proliferation of cells to form lesions, but by accelerating the proliferation of cells which are already present prior to cholesterol feeding. This sequence seems reasonable since, as stated above, accumulation of cells in the intima of all animals is a normal age-related change, and in man the distribution of lesions correlates with the location of diffuse intimal thickening (73).

In summary, the origin of plaques from relatively small numbers of cells trapped in the intima could provide an alternative, non-neoplastic interpretation of the apparent monoclonality of atherosclerotic lesions. It is important to note, however, that the question of how cells arrive in the intima is a central issue in the neoplasia hypothesis as well. Since lesions are found only in the intima, the neoplasia hypothesis implies that either all transformed cells in the media leave that layer or that smooth muscle cells are only susceptible to transformation when they are located in the intima. This raises the interesting possibility that atherosclerosis may involve two quite separate processes, with intimal thickening occurring by a quite different etiology from more advanced lesions and providing a necessary initial step before clinically significant lesions can occur.

The origin of plaques from cells trapped in the media suggests the possibility that atherosclerosis could be a developmental disorder. In this view we are all subject to the initial events of atherosclerosis. However, risk factors may act on some of us by accelerating various later stages of lesion formation. Finally, if the critical event in lesion formation is trapping of cells in the intima, then the fate

of intimal smooth muscle cells becomes very important. Regression or progression of early lesions may depend on whether or not these cells die, migrate out of the intima, or proliferate. Thus, in addition to considering the role of lipid in regression of lesions, investigators should also consider the fate of smooth muscle cells.

Distribution of cell proliferation in hypertension. The distribution of cells in hypertension may also be important to our understanding of this disease. The best available data, at least for aortic smooth muscle cells, shows that the major change here is accumulation of cells—or cell mass in the media. The proliferative response thus differs from that seen in atherosclerosis in two ways: cell migration is absent and replicating cells fail to complete a normal mitosis. It is intriguing to consider the possibility that these two differences are causally connected. Chamley-Campbell *et al.* (74) have proposed that a change of smooth muscle state, a modulation from a contractile to a noncontractile form, must occur prior to the cell proliferation seen in atherosclerosis. Perhaps the reason that "modulation" must occur is that the cell must change from a contractile to a motile state before migrating. In contrast, the proliferative response in hypertension occurs without migration. If we imagine that the replicating cells remain contractile, they may lack the ability to reorganize their cytoskeleton in the fashion necessary to produce a contractile ring and may, therefore, be unable to complete cytokinesis. This hypothesis is summarized below:

1. Vessel wall mass is controlled by two mechanisms: (a) reversible protein synthesis, and (b) irreversible DNA synthesis.
2. Certain stimuli are able to stimulate DNA synthesis. These cause a permanent resetting of the protein synthetic apparatus of the vessel wall.

During acute episodes of hypertension these stimuli include factors associated with acute vascular injury, i.e. platelet-derived growth factor and macrophage-derived growth factor. On a more chronic basis other humoral factors, possibly including the sympathetic nervous system, may play a critical role. This provides a form of peripheral "resetting" of the pressure regulatory mechanisms.

3. Unlike the cell proliferation associated with atherosclerosis, cell replication associated with hypertension does not involve migration, does not require a change from an immotile to a motile state. The result is an increase in the contractile vessel wall mass.

At least in large vessels, hypertensive replication does not go on to mitosis. The result is hyperploid cells. Hyperploid cells, because of abnormal ratios of surface area to contractile mass and abnormal ratio of both of these to DNA are likely to have many abnormal biochemical, pharmacologic, and mechanical properties.

4. The end result is an increase in vascular wall "structural" resistance.

We may also speculate about the reasons why cells undergo endoreplication in response to changes in blood pressure, instead of replicating as in atherosclerosis. One intriguing factor in the proliferation of smooth muscle cells in hypertension is the role of the sympathetic nervous system. Although the data are incomplete, it is reasonable to note that there are consistent reports that vessel wall mass, and in some reports, DNA content, is decreased either by sympathectomy or by treatment of animals with drugs that block the sympathetic nervous system. It may also be worth noting that ablation of the sympathetic nervous system prevents development of one form of hypertension, the spontaneous hypertension seen in SHR rats. It is possible that this is due in part to a requirement for sympathetic innervation during normal development of the peripheral vascular smooth muscle mass involved in regulation of vascular tone. Consistent with this hypothesis, studies of smooth muscle cells *in vitro* show that some as yet undefined factor, derived from sympathetic neurons, is able to maintain smooth muscle cells in a differentiated contractile state (74). It is possible that endoreplication represents an adaptive device that allows the vessel wall to increase its content of DNA without requiring the disruptions of cell structure, including the cells' contractile apparatus, associated with cell replication. In this view, the sympathetic nervous system may play a critical role in the control of cell differentiation during the process of increase in cell mass.

Questions Raised. *1. Relationships of endothelial injury to smooth muscle proliferation.* The best available evidence implies that endothelial denudation probably does not occur until atherosclerotic lesions have begun to develop. This does not, however, eliminate the possibility that extremely small areas of denudation, perhaps brief in time as well, might lead to interactions of the vessel wall with platelets. It is also important not to forget the substantial evidence linking atherosclerosis to thrombosis. Thrombosis plays a clear role in advancing lesions. It is likely, therefore, that denudation and platelet interaction are important factors in the way lesions progress.

More subtle forms of endothelial injury without denudation remain largely unexplored as possible sources of stimuli to smooth muscle cell proliferation. The presence of monocytes adherent to the endothelium of hyperlipemic animals implies some alteration, a form of injury, in one or both cells. The nature of this injury remains unexplored.

Perhaps the most important need in this area is to extend the methods used to determine the extent and role of abnormal activities of biochemical functions in cell culture to the *in vivo* system. The one injury that does appear to be definable in the endothelium is cell death *in situ*. We know very little of the body's response to dead cells and less about their possible role in the evaluation of vascular wall lesions.

2. Role of endoreplication in hypertension. Many open questions exist. We do not as yet know the distribution of hyerploid cells in the microvasculature. This is a critical issue if we are to consider the possibility that endoreplication is an etiologic event in increasing resistance to flow. If endoreplication does exist at the microvascular level, this could imply that the effects of hypertension are, in part, irreversible. Furthermore, it is likely that an endoreplicated cell, with dramatically changed proportions between DNA, cell mass and cell surface area, will have decidedly different properties than a diploid cell. Perhaps those properties are reflected in some of the abnormal vessel wall function seen in this disease.

3. Relationship of endoreplication to true replication. We know that the normal vessel wall contains a large subpopulation of non-diploid cells. These cells are, by definition, arrested in a portion of the cell cycle other than the usual "Go" diploid arrest status. We know nothing, however, of their proliferative potential. A wide range of possibilities exists. For example, the liver contains tetraploid cells that represent a reserve of cells arrested *in situ* prior to mitosis. Upon injury to the liver, these cells rapidly enter the cell cycle, dividing without requiring DNA synthesis (75). At the other extreme, differentiation in striped myoblasts is associated with a loss of the ability of cells to divide. Further increases in cellular mass, however, are associated with increases in DNA/cell occurring either by fusion of myoblasts or by endoreplication. Similar differences in proliferative capacity may be important to considering the origin of atherosclerotic lesions. In this regard it may be important to remember that tetraploidy increases normally with age in human aortic smooth muscle cells (63). We have already noted Martin's hypothesis (64) relating loss of the ability of some cells to grow, with the overgrowth of other cells.

4. Migration/trapping hypothesis of atherosclerosis. The migration/trapping hypothesis of atherosclerosis raises several intriguing questions which are amenable to experiment.

First, in the case of experimental atherosclerosis, it should be possible to determine how much proliferation, if any, occurs before cells arrive in the intima.

Second, it would be of great interest to know how well the distribution of intimal cells in infants correlates with the distribution of lesions in adult humans.

Third, it may be possible to identify chemotactic factors for smooth muscle cells. It would be reasonable to examine this in a chemotaxis assay system, using cultured smooth muscle cells and putative atherosclerosis risk factors or normal components or serum. This has already been done for one component of lesion formation. Platelet-derived growth factor is not only mitogenic, but is chemotaxic for smooth muscle cells in culture (76).

Finally, an important aspect of the hypothesis is that it presents a plausible interpretation of the apparent monoclonality of lesions without necessarily requiring that lesion cells arise by transformation of single cells. This interpretation, however, assumes that

large patches of monoclonal cells exist in the arterial intima prior to the development of overt lesions. This can be tested if an appropriate cytogenic marker can be found.

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Received October 26, 1982. P.S.E.B.M.. 1983, Vol. 173.