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Cell Proliferation in Human Arteries

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The development and progression of human atherosclerosis appears to be associated with low levels of cell proliferation and with proliferative activity seen in both smooth muscle cells and monocyte/macrophages. The time courses and patterns of cell proliferation in this disease are just beginning to be addressed.

During the development of atherosclerotic plaques, the progressive accumulation of intimal smooth muscle tissue, with its associated extracellular matrix components, has been assumed to be secondary to the proliferation of smooth muscle cells in the intima (1–3). In recent years this has prompted a search for "the responsible growth factor" in human arteries and in experimental animal model systems. What may not have been anticipated is the presence of several growth factors in the arterial wall, some of which—such as platelet-derived growth factor (PDGF) -A and -B isoforms, basic fibroblast growth factor (bFGF), and others—have also been demonstrated in human arterial tissues (4–10). In at least rat models of balloon catheter-induced arterial injury and subsequent intimal thickening, antibodies to bFGF and to PDGF have shown inhibitory effects on arterial smooth muscle replication and on intimal development, with the anti-bFGF antibodies seemingly having the more potent antiproliferative effect (9,11). Perhaps in the future, similar studies in humans will elucidate those growth factors, which appear to be responsible for at least the proliferative response that occurs after balloon angioplasty, often culminating in a restenotic lesion (12–14). In any case, the current important question for human atherogenesis might be: Which of several growth factors is most responsible for proliferation in atherosclerosis?

Although no direct data are available on this question in human atherosclerosis, an equally basic question remains: What is the extent of cell proliferation in human atherosclerosis? Some data do exist here (Table 1). Previous studies by Villaschi, and colleagues Spagnoli, (15,16) using ex vivo tritiated thymidine labeling on freshly obtained human arteries indicated very low rates of cell proliferation (0%–0.09% of cells), which approximates that seen in normal adult rat arteries (0.04%) (17). Recently, using an antibody to the proliferating cell nuclear antigen (PCNA), my colleagues and I found a similar low level of cell proliferation in human coronary arteries, normal and atherosclerotic (18), with most arteries displaying a 0% to 1% labeling index, but with occasional intimas displaying as much as a 5% labeling index. We have also seen similar levels of cell proliferation in advanced carotid plaques, in samples of restenotic coronary intimas obtained by atherectomy catheter, and in samples of human coronary transplant arteriosclerosis (unpublished observations). These levels are much lower than the 30% to 50% maximal levels seen after balloon catheter injury in the rat (17). The human artery measurements, however, are in concert with levels of cell proliferation seen with hypercholesterolemia models of atherosclerosis (19–25).

A comparison of these two animal models of intimal thickening reveals differing time courses and spatial patterns of cell proliferation. Thus balloon injury to the

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Table 1. Human Vascular, Intimal Proliferation Summary

Vessel	Approx. PCNA[a] Index	Max. PCNA Index
Int. Mammary Art.[b]	0%–0.3%	0.3%
Coronary DIT[c]	0%–1%	1.2%
Coronary Plaque	0%–1%	4.7%
Carotid Plaque	0%–1%	1.0%
Coronary Restenosis[d]	0%–1%	*
Cor. Transpl. Arterio.[e]	0%–1%	*
AV Dialysis Shunt[f]	5%–30%	37.8%*

[a]Proliferative activity is expressed as a percentage of cells displaying proliferating cell nuclear antigen (PCNA) immunoreactivity.

[b]Internal mammary artery segments. [c]Coronary artery diffuse intimal thickening. [d]Coronary restenosis tissue obtained via atherectomy catheter. [e]Coronary arteries obtained from transplanted hearts. [f]Intima from arteriovenous hemodialysis shunts, taken from the vein anastomosis site.

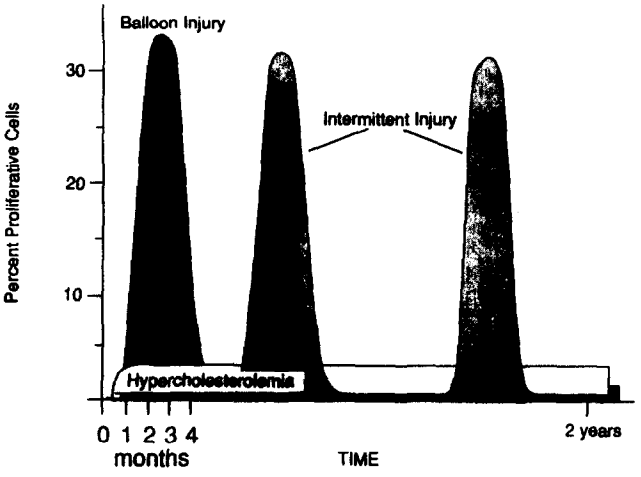
*Studies still in progress.

rat carotid artery causes an abrupt, large increase in proliferative index, from a control level on the order of 0.04%, to a maximum on the order of 30% to 50% in the media within 1 to 2 days of injury. The subsequently developing neointima has an even higher labeling index, and after 2 weeks the bulk of proliferative activity remains localized to the region adjacent to the lumen. This is then followed by a gradual decline in proliferative index such that, by 8 weeks after injury, this overall intimal proliferative index is less than 1%. In contrast, cell labeling appears to be more random in location in the hypercholesterolemia animal models. Although increased proliferative activity can be detected in pigs within days after the start of a cholesterol-rich diet (26), no proliferative indices above 5% have been described during the evolution of atherosclerotic lesions induced by hypercholesterolemia alone (19–25). Admittedly these hypercholesterolemia models may not have been sampled specifically for ongoing proliferative activity as frequently as were the balloon injury models. Nevertheless the data suggest a slow-growing, indolent proliferative response, which is able to produce significantly stenotic lesions in several months to a few years. Such models may thus be more representative of human atherosclerosis development, which takes several years before it can become prevalent at autopsy (27–30). Such one-time observation autopsy studies, however, do not address the actual rate of development of individual atherosclerotic plaques. Individual plaques may differ in their rates of growth, as has been suggested by some serial angiographic and Doppler ultrasound studies (31–34). Thus at least three patterns of human atherosclerotic plaque development and growth can be proposed, and these are diagrammed in Figure 1: (a) a single, brief proliferative burst associated with some injury at an unspecified time and followed by a long, minimal-to-absent proliferative phase (analogous to the balloon injury models); (b) several similar episodes of brief proliferative bursts caused by repeated injuries; and (c) a slow, indolent growth pattern similar to that in the hypercholesterolemia models. Perhaps in the future newer, noninvasive imaging modalities (such as magnetic resonance imaging), which are able to visualize the whole plaque and its constituents (35) rather than primarily lumen size, will be used to serially follow individual human lesions to allow discrimination among these possible modalities of plaque growth.

Of further interest is the detection of cell proliferation among monocyte/macrophage cells as well as among smooth muscle cells (18,36). Such macrophage proliferative activity has also been recently been seen in rabbit hypercholesterolemia models using either tritiated thymidine labeling (23) or colchicine mitotic arrest (37). Recently the presence of mRNA for the colony stimulating factors (m-CSF) and receptor (fms) in primate and human plaques (5,6,38,39) suggested an intricate network controlling inflammatory cell proliferation in human atherosclerosis as well. Lymphocyte proliferation is also a possibility, and whether these different cell types and smooth muscle cells have similar or different patterns of proliferation is currently unknown.

Preliminary data have indicated a particularly high proliferative index in certain arteriovenous shunts used for dialysis in renal failure patients. In at least those shunts with a piece of polytetrafluoroethylene (PTFE, Gore-Tex) graft placed between the artery and vein,

Figure 1. Graphic depiction of three possible patterns of cell proliferation in the development and progression of human atherosclerosis: (**A**) single injury (balloon injury) pattern with an initial burst of proliferative activity followed by a long latent period; (**B**) intermittent injury pattern, in which each injury has its own burst of proliferative activity followed by a latent period; and (**C**) an indolent, low-level proliferative pattern as seen in hypercholesterolemia models.



the most prominent intimal thickening occurs at the graft-vein anastomosis region and is composed of predominantly smooth muscle cells (40). In a sample of such grafts, my colleagues and I recently saw proliferative indices in the 5% to 30% range (unpublished observations). The growth factors responsible for such growth are not clear, but they could be related to the release of platelet factors on the graft or to growth factor production by cells around this graft material. Indeed PDGF gene expression has been reported to be associated with PTFE graft material (41). Alternatively growth could be driven by thrombin activation on the graft, considering that thrombin is a mitogen for smooth muscle cells (42). The time course of proliferation in this arteriovenous shunt lesion is not known.

Future studies will determine what correlations, if any, exist among the spatial presence of growth factors and cell proliferation in the development of human arterial and venous intimal lesions. These may be technically difficult from a pathological point of view, because the investigator will have to keep track of at least three tissue markers in the same tissue location: proliferative activity, specific growth factor, and the cell types appropriate for the specific growth factor. Other important variables, such as the types of growth factor receptors the presumed target cells possess, will also have to be considered.

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