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The nature of atheroma

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Atherosclerosis represents a heterogenous group of pathological phenomena involving degenerative, inflammatory and proliferative processes which produce lesions containing mainly cellular material, lipid, collagen and calcium. Despite the varying pathological forms, the clinical sequelae of atherosclerosis are well defined and affect coronary, cerebrovascular and peripheral arteries.

The Office of Population Censuses and Surveys (1984) reported cardiovascular-related mortality of 42% of total mortality in England and Wales. Of these cardiological causes, 56% were due to ischaemic heart disease, i.e. 28% of total mortality (Table 1). Moreover, cerebrovascular disease represented 13% and peripheral vascular disease 4% of total mortality. With such a large proportion of our mortality therefore related to atherosclerotic disease there is a clear requirement for definition of pathology and pathogenesis in order to provide a framework for therapeutic intervention.

The onset of atherosclerosis is an early event beginning with diffuse, regular thickening of the arterial intima in childhood. The smooth appearance of the arterial tree may be lost during teenage years with the formation of nodular aggregates or cushions of fibroelastic tissue (Stout & Thorpe, 1980). However, the hallmark of established atherosclerosis is formation of the fibrous plaque. The clinical sequelae of this relate to the complications of the fibrous plaque giving rise to slow or sudden vascular occlusion. These include haemorrhage into the plaque, calcification, or mural thrombus on its surface. The present paper will therefore highlight some of the controversial areas of the nature and the theories of atheroma formation.

The fatty streak

The fatty streak probably represents the earliest lesion of atherosclerosis in terms of lipid deposition. However, the onset of lipid accumulation varies according to the anatomical location. For example, in coronary arteries only minimal lipid deposition occurs in early teenage years. In contrast, aortic lesions demonstrate significant lipid accumulation in childhood. Such fatty streaks are widely distributed in the thoracic aorta whereas the more-complex fibrous plaques, the characteristic lesions of established atherosclerosis, are found in the abdominal aorta. The precursor–product relation between the fatty streak and fibrous plaque is well established (Ross, 1986), and the different predominant location of plaques as compared with streaks suggests that they may even be reversible.

Table 1. *Mortality statistics for atherosclerosis-related disease in 1984 in England and Wales**

(Values in parentheses represent percentage of total deaths)

	Male	Female	Total
Total deaths	282357	284524	566881
Deaths from types of vascular disease:			
Cardiovascular	136559 (48)	142290 (50)	278849 (49)
Ischaemic heart	88923 (31)	68583 (24)	157506 (28)
Cerebrovascular	27143 (10)	44327 (16)	71470 (13)
Peripheral vessel	11760 (4)	11002 (4)	22762 (4)

*Values from the Office of Population Censuses and Surveys (1984).

The fibrous plaque

This consists of a superficial cap, with the bulk of the lesion underlying this (Fig. 1). The fibrous cap consists of flattened smooth muscle cells with connective tissue elements which are surrounded by a basement membrane. The combination of these elements produces lacunae of smooth muscle cells. Deep to this cap are foam cells combined with fibroelastic intimal thickening and calcification.

A key event in the formation of the fibrous plaque is smooth-muscle-cell proliferation in the intima, although these cells are originally derived from the media. This proliferation of modified smooth muscle or myointimal cells is a process which is central to the main theories of the formation of atheroma.

Another characteristic cellular component is the foam cell which contains a large quantity of lipid. Its origin is controversial but it appears to be related to the necrosis of smooth muscle cells or macrophages (Aqel *et al.* 1984). When these cells die they leave a necrotic mass of lipid which together with smooth-muscle-cell proliferation produces encroachment on the arterial lumen.

Lipid accumulation is an important component of atheroma and was first demonstrated by Anitschknow and Chalatow in 1913. In view of the hydrophobicity of lipid, it was thought that it entered the arterial wall as lipoproteins. Indeed both low-density lipoprotein (LDL) and apoprotein B of LDL have been found in atherosclerotic lesions
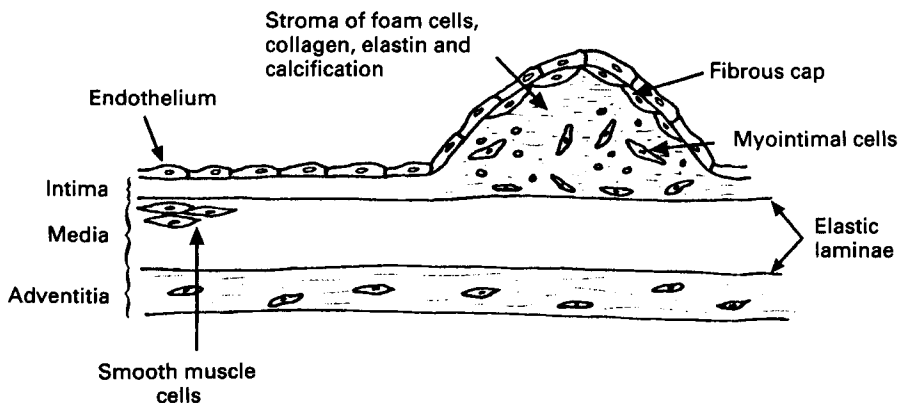

Fig. 1. Cross-section of an arterial wall demonstrating a fibrous plaque.

in concentrations directly proportional to serum levels. However, lipid accumulation may be found in variable amounts in the mature fibrous plaque and is not always a major feature.

Using more precise isotope methods, lipid components and fluxes have been studied in arterial walls. It has been shown that in plasma and normal arterial intima, cholesteryl linoleate is the major sterol ester whilst in the fibrous plaque and the fatty streak, cholesteryl oleate predominates (Day & Wahlquist, 1970). In fibrous plaques there is a shift in synthesis to cholesteryl linoleate (Smith *et al.* 1968) and concomitant accumulation of free cholesterol (Smith, 1974). Abnormalities in the activity of the enzyme responsible for sterol esterification (microsomal cholesteryl ester synthetase) have also been demonstrated. For example, the activity of this enzyme has been shown to be twenty-five times greater in atherosclerotic aortas compared with normal rabbit aorta walls (Hashimoto *et al.* 1974).

Another element in the formation of the plaque is that of calcification. This is classically representative of the 'complicated' lesion, in which the plaque may undergo haemorrhage, cell necrosis, mural thrombosis or calcification which may in turn lead to the common clinical sequelae of this occlusive vascular disease.

Aetiology of atherosclerosis

The theories of the formation of atheroma put forward by both Virchow and Rokitansky in the 1850s still remain to this day, but in modified versions. Virchow's imbibition theory suggested that transudation of serum lipids results in intimal proliferation, the 'filtration hypothesis'. Rokitansky proposed that formed elements in the blood, for example thrombi, were incorporated into the intima, the 'endothelial cell injury hypothesis'. It is the latter which has received most discussion over recent years. However, central to all theories is the excess stimulation of intimal smooth-muscle-cell proliferation.

The endothelial cell injury hypothesis

This model proposes that endothelial injury causes a sequence of events involving cellular dysfunction and separation which ultimately leads to exposure of the subendothelial connective tissue. Platelets and macrophages may then attach to this region and release their growth factors, including platelet- and macrophage-derived growth factor, which may then cause smooth muscle proliferation (Ross, 1984).

Evidence for this theory is compelling. Inducing injury to arterial walls in animal studies usually causes cellular proliferation. If these animals are then platelet depleted there is a reduction in the cellular proliferation (Moore *et al.* 1976; Friedman *et al.* 1977). Other animal experiments with the induction of hypercholesterolaemia to levels comparable with that of familial hypercholesterolaemia in man have demonstrated that the first event observed is monocyte attachment to random points on the endothelial surface with subsequent migration to a subendothelial position. Lipid ingestion occurs at these sites producing the beginning of a recognizable fatty streak. The remaining endothelium over these fatty streaks becomes thin and stretched and finally separates with continued growth of the lesion. This exposes the subendothelial foam cells which may detach into the circulation or which may become coated with adherent platelets to produce mural thrombi. Platelet attachment causes degranulation (Baumgartner, 1972) of their contents which includes platelet-derived and epidermal growth factor (Ross *et al.* 1974; Okya & Orth, 1983). This model also accommodates the concept of reversibility of the lesion since removal of the injurious mechanism would lead to repair and healing of the lesion, although it is not known how this might actually occur in vivo.

The types of injurious mechanisms initiating the process of smooth-muscle-cell proliferation include (Ross, 1986): hypercholesterolaemia, mechanical (e.g. hypertension), smoking, diabetes mellitus, hypoxia, immune complexes, toxins, viruses, free radicals. Some of these are of course well-known clinical risk factors for development of atherosclerosis.

Although this is the most widely considered hypothesis, it may be criticized because much of the evidence in favour of the initiating endothelial injury has accrued from animal studies and not in man. Furthermore it does not explain the process of arteriosclerosis associated with the ageing process.

Other theories, which again emphasize the central role of smooth-muscle-cell proliferation, have been put forward. These include the 'monoclonal' and the 'clonal senescence' hypotheses, the latter being the only theory to tackle the degenerative vascular changes associated with ageing.

The monoclonal and clonal senescence theories

Benditt & Benditt (1973) proposed that each atherosclerotic lesion develops from a single smooth muscle cell, suggesting that they therefore represent a benign neoplasm. Their theory was based on the Lyon phenomenon, whereby in any group of cells of a female, derived from a parent cell, the cells contained the same active X chromosome since the other was inactivated. In normal tissue there is a mixture of cells containing one or other X chromosome, which can be demonstrated by staining for an enzyme specifically coded for by the X chromosome, for example glucose-6-phosphate dehydrogenase (EC 1.1.1.49; G6Pase). In leiomyomas only one cell type of G6Pase exists, demonstrating an example of the monoclonal nature of such tumours. Benditt & Benditt (1973) found this to hold for atherosclerotic lesions also, suggesting that this process might indeed be monoclonal in origin. The transforming or mutagenic factors might be chemicals derived from cigarette smoke or cholesterol, even perhaps a virus. Indeed, these observations have been supported by other research groups (e.g. Pearson et al. 1977).

The clonal senescence theory is based on the studies of the dynamic relation between cell populations in the arterial wall (Martin & Sprague, 1973). The arterial media contains stem cells which are progenitors for smooth muscle cells. The latter are proposed to release regulating local hormones termed 'chalones' which negatively feedback on to the stem cells in both media and intima. However, with increasing age, the total number of stem cells is reduced thereby also reducing the smooth-muscle-cell population. This might result in a reduction in diffusion of the inhibitory 'chalones' into the intima. This would in turn allow increased intimal stem-cell proliferation into smooth muscle cells, providing the increase in intimal smooth-muscle-cell numbers seen with advancing age.

Neither of these theories has been tested experimentally to the degree of the response-to-injury hypothesis. Although this paucity of information is a drawback to their widespread acceptance, they do represent alternative and interesting theories warranting further investigation in man. Indeed it is possible that atheroma formation is encompassed by a combination of these theories. It is important to note that all three theories revolve around the key event of smooth-muscle-cell proliferation.

The smooth muscle cell

The centrality of the modified smooth muscle or myointimal cell has been stressed. Although these cells still possess, though to a reduced level, contractile properties, they also demonstrate secretory potential. This includes synthesis of collagen, elastin and

complex carbohydrates termed glycosaminoglycans. Furthermore, there is evidence to suggest that these modified smooth muscle cells actually undergo a change in phenotype during atherogenesis. For example, most smooth muscle cells do not express HLA-DR antigens, whilst most myointimal cells do express these class 11 transplantation antigens. This is a finding more compatible with macrophages and might reflect their change in functional role within the vascular wall (Jonasson *et al.* 1985). In addition, smooth muscle cells contain receptors for both LDL and platelet- and macrophage-derived growth factors (Moncada *et al.* 1977; Chait *et al.* 1980) and can thus respond to locally released chemotactic factors (Campbell & Campbell, 1985).

Calcification of the fibrous plaque

The importance of calcification in the development of the fibrous plaque has been a focus for discussion in recent years. Of potential significance is the long-known observation that medial calcification precedes intimal plaque formation (Blumenthal *et al.* 1944). Thus a model has been constructed that supports the concept that hypercholesterolaemia causes alterations in the components and relative proportions of membrane lipids, thereby altering their function which might lead to increased Ca uptake and subsequent deposition (Locher *et al.* 1984). Evidence from other human cells, for example erythrocytes, suggests that this may indeed be an important process. Moreover, the use of Ca-antagonist drugs has been shown to reduce the deposition of Ca and cholesterol in tissues (Rouleau *et al.* 1983; Henry, 1985) and might represent a new therapeutic approach to retard atheromatous plaque formation.

Conclusions

Although the exact mechanisms involved in the formation of atheroma are still not clear, several events are apparent. These include the probable initial abnormality of the fatty streak leading to the formation of the mature fibrous plaque with smooth-muscle-cell proliferation a key event. Triggering factors for this proliferation include hyperlipidaemia, hypertension and diabetes mellitus which may be modified by nutritional programmes or drug therapy. However, as the dynamic relation between the cellular and formed elements of both fatty streaks and fibrous plaques begins to unfold, so more logical treatment schedules may be designed in terms of dietary and therapeutic intervention, with the ultimate goal of primary prevention.

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