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New insights in the pathogenesis of the human atherosclerotic plaque

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The morphology of the arterial wall is of special interest and a focus in the study of atherosclerosis-related cardiovascular diseases. Considering the importance of the atherogenic process, a number of *theories* based on morphological investigations have been developed in order to explain the pathogenesis of atherosclerosis. One of these hypotheses, the *filtration theory*, is interpreting atherosclerosis as a reaction of the arterial wall on invading blood-borne lipids. This has been suggested first by Marchand in 1904 (33) and by Anitschkov in 1913 (1). Similarly, in Doerr's *perfusion theory* of atherosclerotic alterations, the filtration mechanism is considered the major principle in atherogenesis (7). The *thrombogenic theory*, first introduced by Rokitansky (42) and later emphasized by the observations of Duguid (9, 10), considers the atheromatous plaque a result of incorporated thrombus material. This theory was refuted in later years because thrombi are rarely observed on normal arterial walls but generally occur on pre-existing plaques (6). The *"response to injury"* theory regards the injury of arterial endothelium as the primary lesion, concomitant with platelet aggregation and proliferation of smooth muscle cells (46, 47, 48). The *monoclonal theory* focuses on the myoproliferative component suggesting that the smooth muscle cells reveal neoplastic-like growth similar to benign smooth muscle cell tumors (2).

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These partly contradictory theories explain some phenomena of atherosclerosis. However, the precise pathogenetic mechanism of the atherosclerotic process is still poorly understood. Regression of atherosclerotic lesions is considered a question of high importance. Identification of early stages, however, would lead to new insights in functional pathogenesis (8). Therefore, interest today focuses on the inflammatory reaction within the intima of incipient atherosclerotic lesions (32). Attempts to further elucidate the pathogenetic mechanism should be based on the characterization of cellular components and their mórphological interactions (5). The analysis of chemical alterations in the arterial wall matrix alone only reflects the altered cellular reactions and must be combined with cellular and biochemical data.

Before discussing the recent cytomorphologic approaches in the research of atherosclerosis, it may be useful to give a brief description of the formal morphogenesis of the atherosclerotic plaque. Morphologic findings permit a tentative classification of several stages of atherosclerotic lesions. The early alterations are manifested macroscopically in yellow lipoidotic patches or streaks that may already occur in infants. Their origin is dependent on genetic factors or dietary habits (60). Histologically most of these patches are identified as intimal aggregations of macrophages, transformed into foam cells by increased lipid storage and characterized by electron lucent vacuoles at the ultrastructural level (11, 12, 15, 16, 17, 55). Lipid incorporation may also transform local smooth muscle cells into foam cells (13, 14). There is usually no

evidence of medial involvement at this stage.

Progression of these alterations is reflected by the formation of fibrous plaques. Besides foam cells, proliferating smooth muscles embedded in a matrix of collagen, elastic lamellae and glycoproteins, are predominant (21, 57). There are increasing necroses of xanthomatous cells, and deposits of phagocytized lipid substances occur in extracellular spaces. Fibrous plaques are first covered by endothelium and underlying smooth cells, but in due course the intracellular material, often in focal distribution, will disrupt the endothelial intimal lining and induce thrombotic aggregations and hemorrhages. This defines complicated atherosclerotic lesions.

Despite a relatively clear understanding of formal morphogenesis, the defined roles of different cell types involved in the development of atherosclerosis are not yet completely understood. According to the "response to injury" theory, it has been suggested that endothelial injury initiates this process and hence the further formal course (23, 43, 46, 47). So far, however, it could not be proven beyond doubt that structural endothelial injury in human arteries is a predisposing factor in development of atherosclerotic plaques. Animal experiments have shown that atherosclerotic changes are accelerated by mechanical endothelial lesions (3, 49). Ross et al. (50) suggested that "endothelial injury" might also include alterations of endothelial transport, and an impairment of the anti-thrombotic nature of the endothelium.

Instead of a primary structural endothelial injury, recent studies have shown an attachment of mononuclear cells to certain endothelial areas which transfer to the intima by diapedesis (16, 25, 26, 27). Investigations of Joris et al. (26). revealed the presence of about 90o/o monocytes and 10o/o lymphocytes in the intima of hypercholesterolemic rats. Mechanical endothelial lesions were not observed at the point of passage. It is still unclear, however, which factors (e.g. possibly functional changes within the endothelium) are supporting the endothelial passage or whether only certain endothelial areas are involved. This is probably a question of functional changes in the endothelial cell surface (24). Functional alterations of the endothelium might probably be caused by lipoproteins and hypercholesterolemia (22, 57, 59). The endothelial barrier can be passed not only by monocytes; from experiments with endothelial cells in culture it is known that LDL are transported through the endothelium by means of transcytosis, and it is presumed that this may cause accumulation of lipoproteins in the arterial wall (4, 36, 53).

In vitro studies have shown that macrophages play a major role in lipopotein metabolism. They incorporate various lipoproteins by receptor-mediated endocytosis (5, 20). The internalized lipoproteins are transported to lysosomes and the hydrolyzed cholesterol is released to the cytoplasm, re-esterified and stored in the form of cholesteryl droplets in the cytoplasmic compartment (5). The cholesteryl esters in the cytoplasm continuously undergo hydrolisation and reesterification. When the extracellular medium contains cholesterol acceptors such as high density lipoproteins (HDL), cellular unesterified cholesterol is released from these cells.

Along with increasing cholesterol ac-

cumulation, the macrophages also synthesize and secrete increasing amounts of apolipoprotein E which associates with cholesterol-enriched HDL to form HDL or HDL_C. This HDL_C particle targets the secreted cholesterol to hepatocytes (31), thereby mediating the "reverse cholesterol transport" to the liver.

Up to now the exact mechanism of Cholesterol secretion by macrophages and the origin of plasma HDL with regard to the "reversed cholesterol transport" was relatively unclear. The most recent observations by Schmitz et al. (52), using biochemical and electromicroscopical techniques lead to the assumption that HDL bind to specific receptors on the surface of macrophages. Subsequently, the bound HDL particles are internalized and transported in endosomes. The endosomes do not fuse with the lysosomal compartment but take up cytoplasmic cholesterol and are ultimately resecreted. Thus, macrophages do not degrade but rather re-secrete internalized HDL particles.

The importance of macrophages in the regulation of lipoprotein metabolism and cholesterol homeostasis is now beginning to focus on the secretory macrophage reactions in the atherosclerotic plaque in response to cholesterol accumulation. The monocytes which have reached the intima are transformed to macrophages and ingest cholesterol-carrying plasma lipoproteins which have penetrated into the intima through the endothelium, thereby acting as "scavenger"-cells. When they have incorporated more cholesterol than they can excrete under the conditions prevailing in the atherosclerotic plaque, the macrophages are transformed to foam cells (5).

The cytogenesis of these foam cells in the atherosclerotic plaque has been discussed already for quite some time. Recent observations revealed that the majority of the foam cells is indeed derived from macrophages (29, 51, 53, 56). However, the development of foam cells from transformed smooth muscle cells had also been postulated (25, 40). Goldstein and Brown (5, 19) recently have shown that fibroblasts and smooth muscle cells express LDL-receptors which allow these cells to accumulate cholesterol and cholesteryl esters. Consequently, there ought to be also transformed smooth muscle cells besides the macrophage-derived foam cells. However, at least in experimental atherosclerotic lesions the majority of foam cells is macrophage-derived (24), apparently due to the fact that cholesterol cannot be re-excreted under the conditions within the atherosclerotic plaque. So far, little is known about the fate of these macrophages. There is, however, a theory suggesting that some of them can pass through the endothelium and recirculate after transformation into foam cells (11, 15, 16).

From these findings we may infer a major impact of macrophages mainly on the initial stage of atherosclerotic plaque development. Mechanical injury to the endothelium with subsequent thrombocyte aggregation is hardly of primary importance. On the other hand, recent surveys have shown that an increased accumulation of foam cells in the early atherosclerotic plaque can lead to secondary endothelial changes in this area (12, 39) which might possibly be a mechanical extension. There is, however, another theory that macrophages are secreting certain substances into the plaque which are potentially toxic to endothelial cells (36).

To date, the monocyte/endothelial interactions resulting in the formation of lipid patches may certainly be considered as primary alterations, whereas mechanical endothelial injury should be considered secondary in most instances. This opinion is further supported by the fact that exfoliating endothelial cells are undermined by neighbouring cells, mainly in the vicinity of the ostia, which means that the sub-endothelium does not get in contact with the blood (41, 53).

Secondary mechanical endothelial injuries lead to the formation of thrombocyte aggregations above the atherosclerotic plaque. Thrombocytes can excrete the "platelet-derived growth factor" which has a stimulating effect on the smooth muscle cells in the arterial wall (44). On the other hand, recent surveys have shown that - besides this mechanism for stimulating the proliferation of smooth muscle cells - macrophages are also capable of secreting a factor with mitogenic effect on smooth muscle cells (18, 30). Here, too, recent results have underlined the impact of macrophage reaction. As early as 1948 MacMillan and Duff (35) had demonstrated that smooth muscle cells in the proliferating atherosclerotic plaque show increased activity. As is known from experimental atherosclerosis in pigs, hyperlipidemic diet leads to a significant increase in the number of smooth muscle cells in the intima (28).

Since cell culture studies revealed that smooth muscle cells can synthesize the main components of the extracellular matrix in the arterial wall, the main interest over the last years has been focused on the proliferation of smooth muscle cells in the arterial wall (46). Recent investigations on the distribution of different collagen types and glycoproteins in the atherosclerotic plaque revealed, that fibrous plaques contain considerably more collagen than the lipid patches. The atherosclerotic lesions contain similar amounts of collagen types I and III (61). Stimulation of the smooth muscle cells with increased synthesis of matrix components may cause further extension of the atherosclerotic plaque with secondary ulceration, thrombosis and regressive calcification, resulting finally in a complicated lesion. This justifies the rising interest in the behaviour of smooth muscle cells.

Mechanical injury to the endothelium with subsequent thrombosis and the release of the platelet-derived growth factor result in the proliferation of smooth muscle cells and their crucial consequences. This might be the second step in the course of atherosclerosis leading to clinically manifested lesions of the arterial wall, a process that has been extensively studied over the last years. The initial lesion which is pathogenetically decisive for the development of the atherosclerotic plaque obviously occurs at an earlier stage. It is effected mainly by transcytosis of lipoproteins through the endothelial cells into the intima and by the interactions of endothelia and blood monocytes, facilitating their passage into the intima. The monocytes which are of outstanding importance to the lipoprotein metabolism apparently also play a major role in the development of the early atherosclerotic plaque.

While smooth muscle cells and the analysis of matrix components in the arterial wall have occupied the center of

interest in recent investigations, current morphological studies will have to concentrate on the cellular interactions of the endothelium and the monocytes and mainly on the heterogeneity of the involved cells.

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