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Identification of macrophages and smooth muscle cells with monoclonal antibodies in the human atherosclerotic plaque*

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Summary. Sections of human atherosclerotic plaques, obtained from 21 autopsy cases with various degrees of atherosclerosis, were stained with the indirect immunoperoxidase technique using specific monoclonal antibodies against macrophages and smooth muscle cells. Distinctive results were found in differing stages: Single blood monocytes were observed in diffuse intimal thickening and the foam cells seen in fatty streaks were mostly identified as mature tissue macrophages, while only very few blood monocytes were present. The spindle cells observed in fibroelastic plaques showed positive reactions to antibodies against desmin, which points to their derivation from smooth muscle cells, whereas only a few macrophage-derived foam cells were seen in these lesions. In the complicated lesions the majority of foam cells were macrophage-derived, but there was also a small number of foam cells positive to antibodies against desmin, suggesting a smooth muscle cell derivation. – Our results confirm that in human atherosclerotic plaques the majority of the foam cells are obviously macrophage-derived, which emphasizes the important role of macrophages in the morphogenesis of these lesions.

Key words: Human atherosclerotic plaque – Phenotypic characterization of cell types – Monoclonal antibodies

Introduction

New morphological investigations on the pathogenesis of atherosclerosis have refocused attention on the role of monocytes and macrophages, especially in the early phases of the disease. This is in contrast to recent concentration of interest on smooth muscle cells (Fagiotto and Ross 1984; Fagiotto et al. 1984; Schaefer 1981; Schwartz et al. 1985; Stary 1983; Ross 1986). Various factors have prompted this reorientation: Macrophages were found to be essentially involved in intimal lipoprotein metabolism (Brown and Goldstein 1983, 1986). They incorporate cholesterol-binding lipoproteins entering the intima but if they have taken up more than they can digest the excess cholesterol will be stored as drop-like cytoplasmic deposits. Recent experimental data suggest that the majority of "foam cells" observed in atherosclerotic plaques are of this origin. Foam cell transformation of smooth muscle cells was rather less common (Schwartz et al. 1985). Although the functional importance of macrophages in lipoprotein metabolism was thus verified in vitro, our notions about their behavior, especially in the human atherosclerotic plaque, are still fairly vague, since most morphological studies in this field have been based on experimental models.

Monoclonal antibodies reveal a heterogeneity in human aortic intimal cells (Orekhov et al. 1986). Further phenotypic characterization of macrophages and their differential identification from other cell types of the human atherosclerotic plaque is facilitated by the use of this technique (Radzun 1985; Zwadlo et al. 1985, 1986) and recent studies have shown that the majority of antibodies against both macrophages and smooth muscle cells may be applied to fresh autopsy material (Klurfeld 1985; Aquel et al. 1985). The present study reports the immunohistological identification of different cell types in atherosclerotic plaques found in arteries taken from human autopsies. Recently developed monoclonal antibodies permit the differentiation of blood monocytes and

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Table 1. Monoclonal antibodies for phenotypic characterization of cells in the atherotic plaque

Name	Source	References
Exclusively monocytes		
27-E-10	Dept. Dermatology Mstr[a]	Zwadlo et al. (1986)
Exclusively macrophages		
25-F-9	Dept. Dermatology Mstr[a]	Zwadlo et al. (1985)
Monocytes and macrophages		
Ki-M-1	Behring/Marburg	Radzun + Parwaresch (1983)
Ki-M-2	Behring/Marburg	Radzun + Parwaresch (1983)
OKM-5	Ortho/Heidelberg	Knowles et al. (1984)
Anti-leu M-3	Becton-Dickinson Heidelbg.	Dimitriou-Bona et al. (1983)
Smooth muscle cells		
HM-19/2	Dept. Dermatology Mstr.	Sorg (1986)
desmin	Dakopatts/Hamburg	Schmid et al. (1982)

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mature macrophages (Zwadlo et al. 1985, 1986). Therefore, special emphasis was put on the differentiation of the mononuclear phagocytes involved in the formation of atherosclerotic plaque.

Materials and methods

Specimens from human aortas and coronary arteries were obtained from autopsies performed within 6 h after death. The youngest patient was 15, the oldest 77 years.

Comparative studies on fresh surgical specimens having proved the applicability of most antibodies against macrophages and smooth muscle cells to paraffin-embedded material, the autopsy specimens were also processed by fixation in 4% neutral formol, and carefully embedded in paraffin. Reactions were performed on thin paraffin sections, which offered an improved preservation of structures.

Immunohistochemical studies were performed with single and double indirect peroxidase and alkaline phosphatase methods using the substrates 3.3 diaminobenzidine-tetrahydrochloride (DAB) or 3-amino-9-ethylcarbazole (AEC) and naphthol salts as coupling reagent with hexazotic neofuchsin (SIGMA/Munich and MERCK/Darmstadt). Endogenous peroxidase was suppressed by $NaNO_3$, endogenous alkaline phosphatase by levamisole. For secondary and tertiary antibodies (enzyme-coupled) we used products of SIGMA/Munich, DAKOPATTS/Hamburg, and DIANOVA/Hamburg. Nuclear counterstaining was performed with acid haemalum. For negative controls we omitted the primary antibody, or incubated normal serum of the respective species. For positive controls we used the remaining slides of each set.

Light microscopical controls were stained with haematoxilin-eosin. The specific monoclonal antibodies for phenotypical characterization of cell types are listed in Table 1.

Results

Atherosclerotic lesions were classified according to the usual histologic criteria into fatty streaks, fibrous plaques, and complicated lesions. A first orientating survey verified analogous behavior in all of these stages, independent of their localization in coronary arteries or aorta, respectively. In Table 2, the semiquantified data obtained with the different antibodies in various lesions are compiled.

Vessels with incipient fatty streaks showed isolated subendothelial blood monocytes staining positively with antibody 27-E-10 directed against blood monocytes (Fig. 1). Foam cells never stained with this antibody. Developing fatty streaks were characterized by a considerable infiltration of macrophages changing to foam cells which stained negatively with antibody 27-E-10 against blood monocytes but strongly positive with antibody 25-F-9 against mature tissue macrophages. Subendothelial nests of foamy macrophages were early manifestations (Fig. 2). During the progression of lipid streaks, we observed extended subendothelial clusters of foam cells thickening the intima; these could be identified as mature tissue macrophages by their positive reaction to antibody 25-F-9, too (Fig. 3). Only some isolated smooth muscle cells were observed within the lipid streaks.

The antibodies Ki-M1 and Ki-M2 showed moderate reactions with macrophage-derived foam cells. 25-F-9 achieved the most significant staining, while the reactions to OKM5 and anti-leu-M3 were generally rather faint. Those lesions that had been identified as fibrous plaques in HE-stained control sections were characterized immunohistologically by a strong positivity of spindle cells to antibodies against HM-19/2 and desmin (Fig. 4). It was a striking feature that smooth muscle cells from the media of aorta and coronary arteries showed only poor or absent staining with antibody against desmin (Fig. 4). Staining with antibody 25-F-9 against mature macrophages revealed only a few positive cells in the fibrous plaques.

In complicated lesions, a few blood monocytes were observed when stained with antibody 27-E-10. Foam cells were predominantly positive to antibody 25-F-9, indicating their macrophage nature (Fig. 5). Thus the majority of foam cells in complicated lesions were identified as macrophages. Apart from these cells, however, there were also some spindle cells whose distinctly vacuolated, foamy degeneration was clearly verifiable by light microscopy. These cells showed strong positivity to desmin staining (Fig. 6). These findings can be

Table 2. Reaction of different cell types in the atherosclerotic plaque with monoclonal antibodies, whose specificity is plotted in Table 1

Cell types	27-E-10	25-F-9	Ki-M-1	Ki-M-2	OKM-5	Anti-leu	HM-19/2	Desmin
Fatty streak								
Monocytes	+	−	+	+	+	+	−	−
Macrophages	−	+++	++	++	+	+	−	−
Smooth muscle cells	−	−					+	+
Fibrous plaque								
Monocytes	−	−	−	−	−	−	−	
Macrophages	−	+	+	+	+	+	−	
Smooth muscle cells	−	−	−	−	−	−	+++	++
Complicated lesion								
Monocytes	+	−	+	+	+	+	−	−
Macrophages	−	+++	+	+	+	+	−	−
Smooth muscle cells	−	−	−	−	−	−	+++	++

+++ strong reaction; ++ moderate reaction: + faint reaction; − negative

taken as a demonstration of the coexistence of foam cells derived from macrophages, with those derived from smooth muscle cells in complicated lesions. Quantitation of both foam cell types, however, revealed a distinct predominance of those that had been identified as macrophage-derived by labeling with antibody 25-F-9.

Discussion

The development and commercial availability of specific antibodies against macrophages or smooth muscle cells has offered a new methodological approach to the quantitative analysis of cell components in various stages of atherosclerotic plaques in human atherosclerosis. No definite notion has been formed, so far, about the exact percent proportion of macrophage-derived and smooth muscle cell-derived foam cells in the advanced lesion. Moreover, the application of monoclonal antibodies provides a tool for differentitating in detail the heterogenous nature of monocytes and macrophages (Radzun and Parwaresch 1983; Zwadlo et al. 1983, 1985, 1986).

Distinct differences could be shown in our findings from incipient fatty streaks. Blood monocytes reacting specifically with antibody 27-E-10 (Zwadlo et al. 1986) were identified mainly in the earliest lesions, mostly in immediately subendothelial localization. In lipid streaks, however, foam cell aggregations would show positive reactions only to antibodies against mature tissue macrophages (Zwadlo et al. 1985). In vitro experiments have shown that monocytes after two days in culture express 27-E-10 antigens; from 6 days in culture, 25-F-9 antigens are expressed continually, whereas the expression of 27-E-10 antigens is decreasing (Zwadlo et al. 1985, 1986). This suggests that blood monocytes entering the intima are soon changing their phenotype in the direction of mature tissue macrophages. Similar findings were also recorded in atherosclerotic arteries obtained at autopsy by Klurfeld (1985) and Aqel et al. (1985). These authors, however, had no antibodies at their disposal which would allow for differentiating blood monocytes from mature macrophages proper. Antibodies against desmin (Schmid et al. 1982) and HM-19/2 (Sorg 1986) were equally unable to verify an increase of transformed smooth muscle cells within the lipid streaks.

Different data were gathered from the fibrous plaques localized in the aorta or coronary arteries. Here, spindle cell formations react positively with antibodies against desmin and HM-19/2. In striking contrast, the smooth muscle cells of the aortic media show only minor positivity, if any, when stained with these antibodies. The percentage of positive media cells was slightly higher in coronary arteries than in the aorta. Similar findings were published recently by Kocher and Gabbiani (1986). According to their data, however, transformed smooth muscle cells in fibrous plaques will react poorly with antibodies against desmin, whereas their reaction is strongly positive in complicated lesions, in agreement with our own findings.

In addition to spindle cells of smooth muscle

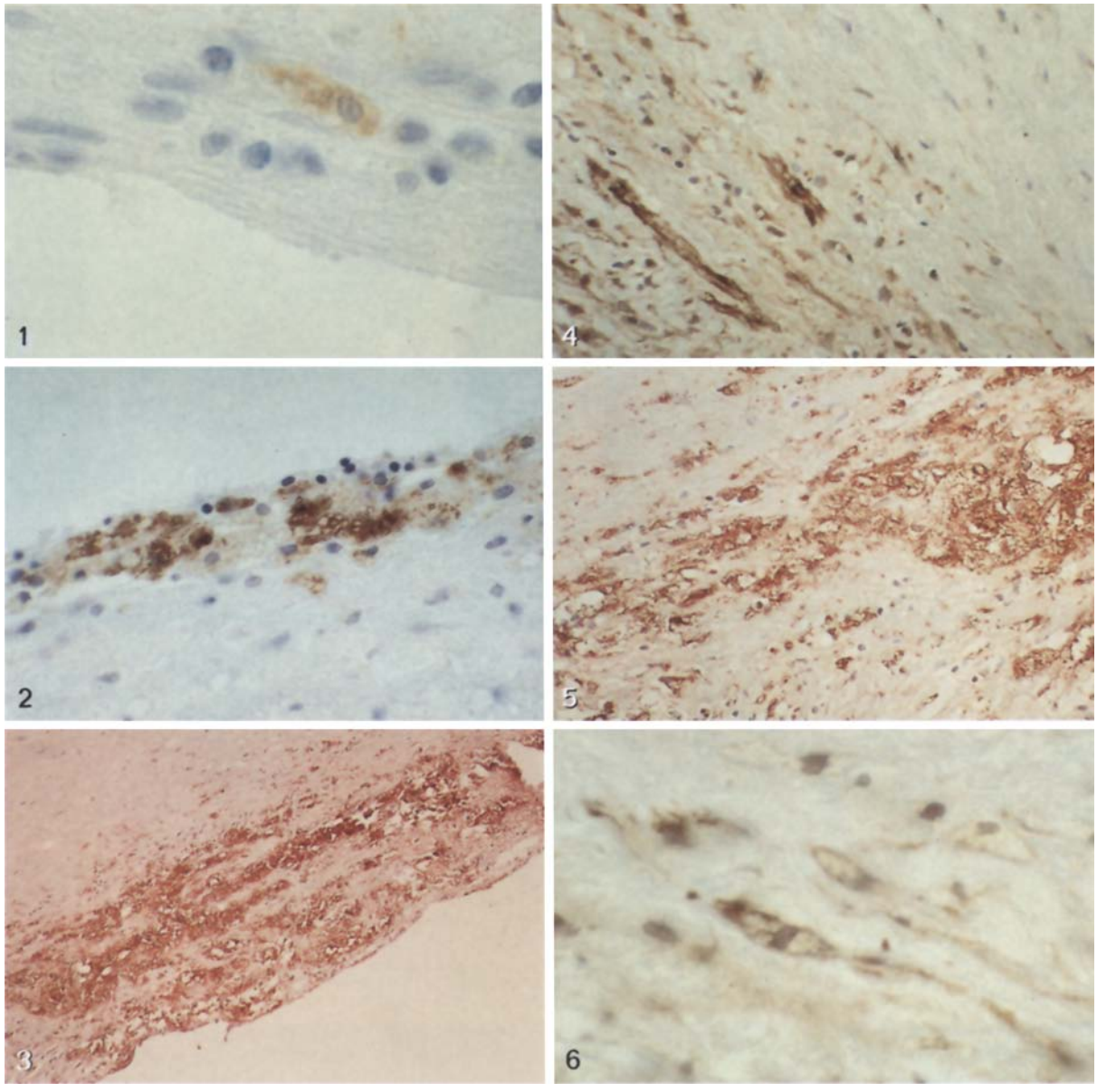

Fig. 1. Incipient fatty streak of human aorta, stained with monoclonal antibody 27-E-10 directed exclusively against blood monocytes: single positive monocytes are observed in the subendothelial space. (× 550)

Fig. 2. Incipient fatty streak of human aorta, stained with antibody 25-F-9 directed against mature tissue macrophages. Clusters of positive foam cells are observed in the subendothelial space. (× 330)

Fig. 3. Low power micrograph of a fully developed fatty streak in the human aorta, stained with 25-F-9 and showing abundant clusters of macrophage-derived foam cells. (× 136)

Fig. 4. Fibrous plaque in human coronary artery, stained with antibody against muscle cell-specific intermediate filaments of desmin type. Transformed smooth muscle cells in the intima reveal abundant desmin filaments, while smooth muscle cells in the media are but weakly positive. (× 550)

Fig. 5. Complicated lesion, stained with antibody 25-F-9 against mature tissue macrophages; evidence of abundant macrophage-derived foam cells. (× 330)

Fig. 6. Aortic intima of a complicated lesion, stained with antibody against desmin. Spindle-shaped cells in foamy degeneration contain intermediate filaments of desmin type, indicating their muscle cell nature. (× 550)

type reacting strongly positive with antibodies against desmin, there is always evidence of foam cells in the complicated lesions. Differentiation with the aid of monoclonal antibodies enabled us to define the quantitative composition of foam cell populations in the complicated lesion: It is obvious that macrophage-derived foam cells are always the majority, positive with antibodies against mature tissue macrophages, but not with those against monocytes. The role of macrophages consists in the uptake of cholesterol-carrying plasma lipoproteins that have entered the intima via endothelial pathways by receptor-mediated endocytosis, thereby acting a scavenger cells. Storing excess cholesterol esters leads to the transformation of macrophages into foam cells (Brown and Goldstein 1983). The macrophages can also secrete apolipoprotein E which associates with resecreted cholesterol and HDL in the surrounding medium to form the HDL_c particle which targets the secreted cholesterol to hepatocytes in what is called the "reverse cholesterol transport" (Mahley 1981). Recently, apolipoprotein E secretion was also demonstrated immunohistologically in biopsy tissue from human atherosclerotic plaques (Murase et al. 1986; Roessner et al. 1986).

There was, however, also some evidence of spindle-shaped foam cells showing strong positivity to muscle-specific antibodies against desmin and HM-19/2. These immunohistologic findings agree well with previous electron microscopical results wherein some foam cells were characterized as smooth muscle cells by the presence of numerous filaments with dense detachments in the cytoplasm (Newman et al. 1971). The development of smooth muscle cell-derived foam cells may be explained by the occurrence of LDL-receptors on their cytoplasmic membrane (Chait et al. 1980; Witte and Cornicelli 1980) which facilitate the uptake of cholesterol-carrying lipoproteins.

As a result of this study, we conclude that the predominance of macrophage-derived foam cells in the atherosclerotic lesions of different grades emphasizes the importance of macrophage reactions in the functional morphogenesis of the human atherosclerotic plaque.

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