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HETEROGENEITY IN SMOOTH MUSCLE CELL POPULATION ACCUMULATING IN THE NEOINTIMAS AND THE MEDIA OF POSTSTENOTIC DILATATION OF THE RABBIT CAROTID ARTERY

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SUMMARY: Rabbit smooth muscles contain at least three types of myosin heavy chain (MHC) isoforms; SM1, SM2 and SMemb (NMHC-B), the expression of which is developmentally regulated. We have recently reported that smooth muscles with the embryonic phenotype accumulate in the neointimas produced by endothelial denudation or high-cholesterol feeding. In this study, we examined MHC isoform expression in the neointimas and the media of poststenotic dilatation of the rabbit carotid artery, and determined the phenotype of the smooth muscle cell in the dilated segment. We report here that neointimal cells in the dilated segment are smooth muscle cells with the embryonic phenotype as previously reported in our ballooning-injury study. The medial smooth muscles, however, are composed of heterogeneous population of smooth muscles which differ in stage of differentiation as determined by the MHC isoform expression. These results indicate that MHC isoforms are useful molecular markers to identify abnormally proliferating smooth muscles in diseased arteries and to understand the process of atherogenesis occurring following vascular injury.

Arterial dilatation downstream the stenotic lesion has been known as poststenotic dilatation. Previously we demonstrated that the endothelial degeneration and fragmentation of elastic laminae occur in the dilated segment, which is possibly due to the turbulence-induced vibration of the arterial wall (1-10). However, phenotypic

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modulation of the intimal and medial smooth muscle cells in the poststenotic dilatation has not been well characterized (11).

We have recently reported that rabbit smooth muscles express at least three types of myosin heavy chain (MHC) isoforms; SM1, SM2 and SMemb (NMHC-B) (12-15). SM1 and SM2 are specific to the smooth muscle cell, but SMemb is one of nonmuscle-type MHCs. The expression of these isoforms are developmentally regulated at both levels of the gene transcription and alternative RNA splicing. SM1 is constitutively expressed from early development, but SM2 appears after birth. SMemb, on the other hand, is expressed predominantly at embryonic and neonatal stages, but almost disappears in adults. In experimental arterio- and atherosclerosis, we demonstrated proliferation of embryonic smooth muscles in the neointimas (15). Therefore, MHC profiles are useful to define the stage of smooth muscle cell differentiation during development as well as in diseased arteries. In this study, we investigated the phenotypes of smooth muscle cells which accumulate in neointimas and the media of the poststenotic dilatation by using immunofluorescence histology for MHC isoforms.

MATERIALS AND METHODS

Poststenotic dilatation was produced in six male white rabbits weighing between 1.8 and 2.2 kg by clipping the carotid aretery as previously described (4). Under sodium pentobarbital anesthesia (25 mg/kg intravenously), the carotid artery was exposed, gently dissected free from connective tissues and nerves, and constricted by a silver clip of 1.2 mm in internal diameter and 3.0 mm in length to produce 35 - 60 % stenosis. In sham-operated rabbits, the silver clip was placed around the carotid artery without constricting it

At one or two weeks after operation, the carotid artery was reexposed and external diameter was measured at two sites both proximal and distal to the clip. After measuring the diameter the animals were sacrificed, the artery 5 mm distal to the stenosis was excised and frozen in OCT compound for immunohistology. The expression of MHC isoforms was examined by using anti-SM1, anti-SM2, anti-SMemb antibodies as previously described (15). A part of sections were stained with hematoxylin-eosin and elastica Masson's stainings. This investigation conforms with *the guide for the care and use of laboratory animals* published by the National Institute of Health, U.S.A.

RESULTS

By macroscopic observation, the poststenotic dilatation was fusiform and the maximum dilatation occurred at 5 - 7 mm distal to the stenosis. The poststenotic

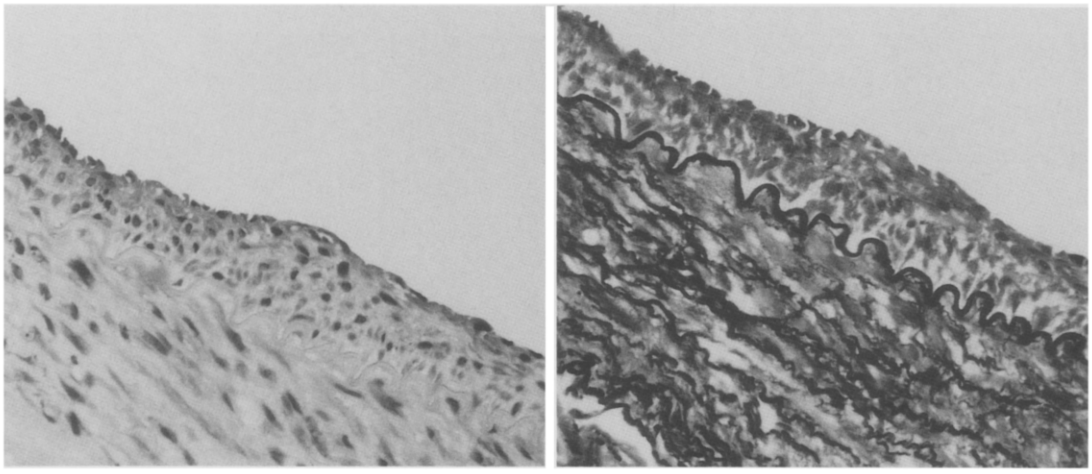

Figure 1. Hematoxylin-eosin (left) and elastica Masson's stainings (right) of the dilated segment of the rabbit carotid artery. Neointimal thickening and fragmentation of elastic fibers in the medial wall can be seen.

dilatation was about 1.2 times in diameter compared to the proximal portion at one week, and approximately 1.5 times at two weeks. Histological examination by light microscopy at one week showed the presence of mild intimal thickening and the disorganized arrangement of elastic fibers in the medial wall (Figure 1). The histological findings at two weeks were basically similar to those at one week.

At one week after operation, the neointimal cells were positive with both anti-SM1 and anti-SMemb antibodies, and almost negative against anti-SM2 antibody. On the other hand, the medial smooth muscles could be recognized by anti-SM1 and anti-SM2 antibodies, and most of cells were negative against anti-SMemb antibody. However, SMemb-positive smooth muscle cells were also present in the medial wall, which were more prominent at two weeks (Figures 2 and 3). This indicates that proliferating cells in the neointimas are smooth muscles with the embryonic phenotype. However, the medial smooth muscles are composed of mixed population of dedifferentiated cells which are heterogeneous in reactivity against anti-SM1 antibody. These findings were consistently observed in all rabbits used in this experiment.

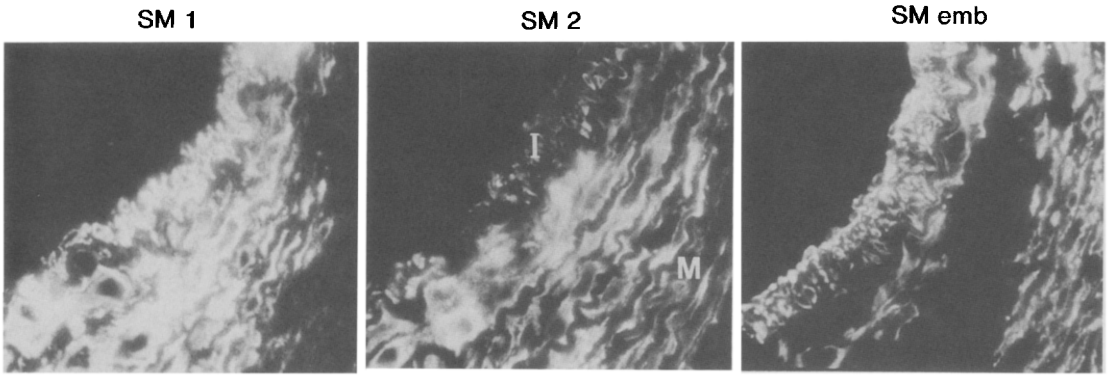

Figure 2. Neointimal smooth muscles in poststenotic dilatation of the rabbit carotid artery at one week after the carotid artery constriction. The neointimal cells are positive against anti-SM1 and anti-SMemb antibodies but negative against anti-SM2 antibody, indicating that these cells have the embryonic phenotype. In the medial wall, there are heterogeneous populations of SMemb-positive cells which are composed of SM1-positve and SM1-negative cells. I and M in the middle panel stand for neointima and media, respectively.

DISCUSSION

In this report, on the basis of three MHC isoform expression, we present evidence showing that poststenotic dilatation induces not only accumulation of neointimal cells with the phenotype of embryonic smooth muscles, but also dedifferentiation of the medial smooth muscles.

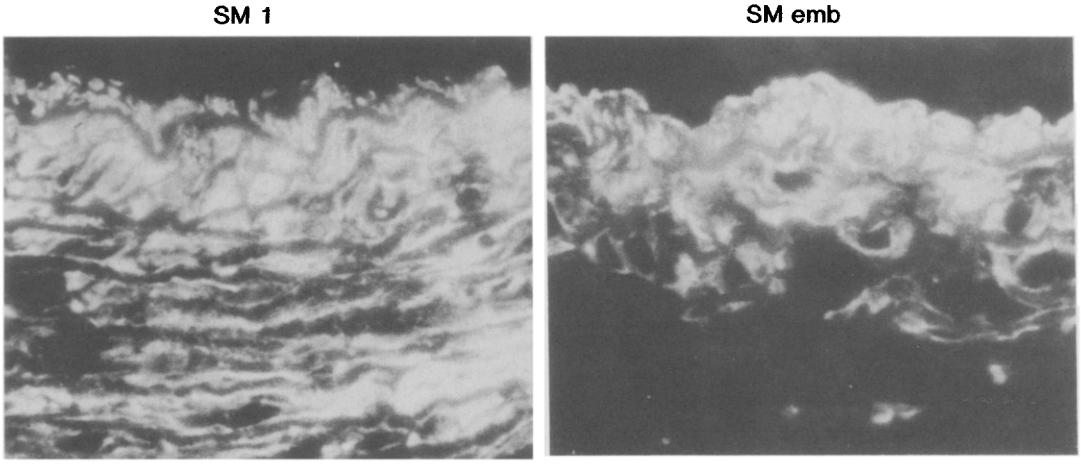

Figure 3. SM1-positive cells underneath the internal elastic lamina strongly express SMemb at two weeks of poststenotic dilatation, indicating the dedifferentiation of medial smooth muscles.

Previously we reported that 35 - 60 % stenosis of the rabbit carotid artery induced marked dilatation at 7 mm distal to the stenosis, dilating the artery by 50 % compared to the proximal segment (4). The vascular injury in this model is unique in that there is no direct mechanical injury to the endothelial cells. Atherosclerosis in human frequently develops in regions with relatively low wall shear stress (16, 17), indicating the hemodynamic effects on atherogenesis. The blood flow downstream the stenotic lesion is turbulent, causing the low shear stress against the endothelial cells (8-10). We suggest that endothelial dysfunction in the dilated region is responsible for smooth muscle cell proliferation and the neointimal formation, which can be analogous to those seen at the initial stage of atherosclerosis.

In the present study, we revealed that the expression of MHC isoforms in the neointima of poststenotic dilatation was quite different from those in the medial wall. In the neointimal cells, SM1 and SMemb were positive, and SM2 was negative. This clearly indicates that the neointimal cells are smooth muscles with the embryonic phenotype. Similar phenotypic changes in smooth muscles have been observed in the neointimas produced by endothelial denudation or high-cholesterol feeding (15). However, smooth muscles in the medial wall were also found to undergo phenotypic modulation because there are many SMemb-positive cells in the medial wall. Furthermore, these SMemb-positive cells are heterogeneous in the stage of differentiation because the reactivity against anti-SM1 is different among cell population. The SMemb-positive cells in the media have not been observed in a week after the ballooning-injury. It is likely that poststenotic dilatation induces more severe injury to the medial wall than endothelial denudation by ballooning, resulting in various degree of dedifferentiation of the smooth muscle cells in the media.

Based on these findings, we conclude that determination of the smooth muscle cell phenotypes by the expression of MHC isoforms is highly useful in understanding the process of atherogenesis occurring following vascular injury. We also suggest that

understanding on the regulatory mechanisms of smooth muscle cell differentiation is important to elucidate the role of smooth muscle proliferation in atherosclerosis.

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