THE SUPPRESSIVE EFFECTS OF COLLAGEN TYPE V ON THE BEHAVIOR
OF VASCULAR SMOOTH MUSCLE CELLS IN VITRO

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It has been long recognized that the extracellular matrices influence various kinds of behavior of vascular smooth muscle cells (SMCs). Little is, however, known about the influence of type V collagen on the behavior of SMCs. We studied the effects of various types of collagen including I, III, IV and V on the attachment, spreading, proliferation and DNA synthesis of cultured SMCs.

MATERIALS AND METHODS

Types I, III, IV and V collagen(I, III, IV and V) were prepared from human placenta by the salt differentiation method. Fibronectin(FN) was obtained from bovine fibrinogen by the modified method of Gilchrest et al. The dishes and glass cover slips were coated with varying amounts of the collagens, fibronectin and bovine serum albumin(BSA, AL), and then washed 3 times in PBS and twice in DMEM. Culture cells derived from porcine aortic media were seeded onto the substrata-coated cover slips. After 0.5, 1, 3 and 6 hours, the slips were washed 3 times with PBS, fixed with 10% formalin and subjected to HE stain. Three random cell counts were done on duplicate samples with a light microscope and the mean value for attached cells calculated. At 3, 6 and 24 hours, the spreading of attached cells was assessed by finding the area and shape index( SI=4πS/L², S:area, L:perimeter) of the cells. 150 cells of triplicate samples were examined and the average number was calculated. On the basis of plating efficiency after 1 day of cultivation, the numbers of cells seeded on BSA, fibronectin and collagen types I, III, IV and V were adjusted to 1.0, 0.9, 1.1, 1.1, 1.1 and 1.5X10⁵/ dish, respectively. After the 6th
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day of culture, the cells detached by trypsinization were counted, and popula-
tion doublings were calculated. After the 1st and 6th days in culture, the
uptake of $^3$H-thymidine into cells was determined with a liquid scintillation
counter. After the 1st day, DNA synthesis was also evaluated in terms of the
ratio of the number of silver grain-positive nuclei to total number of nuclei
by microautoradiography.

RESULTS AND DISCUSSION

The cells attached most rapidly to FN, moderately to I, III and IV, and most
slowly to V. The number of cells attached to FN was the highest at any time
and that to V the lowest (Fig. 1). The largest area and fastest rate of cell
spreading were observed with cells on FN. Those of cells on I, III, IV and Al
were intermediate. In contrast, cells on V had the smallest area and the
slowest rate of spreading (Fig. 2). There was a significant decrease in the
shape index of cells spreading on FN at 6 and 24 hours. There was no differ-
ence in the number of cells on any substrate at the 1st day. In the 6th day,
however, the number and population doubling of cells grown on collagen type V
were significantly lower than those on other substrates (Table 1). DNA syn-
thetic activity of cells on type V collagen was remarkably suppressed on the
1st day of culture, while there was no difference between substrates on the
6th day. These results suggest that type V collagen suppresses the attachment,
spreading and proliferation of cultured SMCs. 1:Fig. 1, 2:Fig. 2, 3:Table 1.