Regulation of proteoglycan synthesis by tumor necrosis factor-α in cultured vascular smooth muscle cells.

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Tumor necrosis factor-α (TNF-α) is a cytokine that is involved in the progression of atherosclerosis. Previously, we have shown that TNF-α suppresses the proliferation of and collagen synthesis in vascular smooth muscle cells in vitro. Proteoglycans are macromolecules that influence cellular behavior through interactions with the extracellular matrix and growth factors/cytokines. In the present study, we investigated the effects of TNF-α on the synthesis of proteoglycans using bovine aortic smooth muscle cells in a culture system. It was observed that TNF-α increased the accumulation of proteoglycan in the conditioned medium of the cells in dose- and time-dependent manners, particularly when the cell density was high. This increase was not observed in the presence of a TNF-α neutralizing antibody, indicating that the stimulation of proteoglycan synthesis requires TNF-α. The proteoglycans in the conditioned medium were separated into a high Mr subclass and a low Mr subclass by Sepharose CL-2B molecular sieve chromatography; TNF-α selectively increased the high Mr subclass with a decrease in the hydrodynamic size. After further purification by DEAE-Sepharose ion exchange chromatography, the glycosaminoglycan chains of the high Mr subclass were identified as chondroitin/dermatan sulfate by Sepharose CL-6B chromatography; the length of the chains was reduced by TNF-α from Mr ~ 45,000 to Mr ~ 31,000. The disaccharide composition of chondroitin/dermatan sulfate chains that accumulated in the conditioned medium was analyzed by fluorophore-assisted carbohydrate electrophoresis. The disaccharide units were detected as GlcA-GaINAc, GlcA-GaINAc(4S), GlcA-GaINAc(6S), GlcA-GaINAc(4S,6S), IdoA-GaINAc, IdoA-GaINAc(4S), and IdoA-GaINAc(6S); TNF-α particularly increased minor disaccharide units such as GlcA-GaINAc(4S,6S) and IdoA-GaINAc. The present results clearly indicate that vascular smooth muscle cells synthesize and secrete large chondroitin/dermatan sulfate proteoglycan molecules with shorter glycosaminoglycan side chains after exposure to TNF-α.

Anti-inflammatory effects of proteoglycan in cultured fibroblasts from the human uterine cervix

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Objective
Chorioamnionitis (CAM) is extremely important in the cause of preterm delivery. In addition, various inflammatory cytokine of amnion fluid plays an important roles for the onset of CAM and cervical ripening. On the other hand, proteoglycan (PG) was a main component of an extracellular matrix, but so far it was thought with the material which maintained merely tissue. However, it becomes know that PG had an important effect on cellular function manifestation by recent study, and the anti-inflammatory effects attract attention particularly. In the present study, The effect of PG on inflammatory cytokine was investigated in cultured fibroblast from the uterine cervix which was stimulated by lipopolysaccharide (LPS).

Methods
A specimen of normal human uterine cervix was collected after total hysterectomy for uterine myoma with the informed consent of the patient. LPS (1 μg/ml) was added in the fibroblastic culture media. The cells were incubated with or without PG for 48h. The amount of IL-1β, IL-6 and IL-8 in the medium was assayed by ELISA method.

Results
The amount of IL-1β in the medium produced by cultured fibroblasts from the uterine cervix was increased and reached a peak (40.69±3.61 ng/ml) 12 hours later after LPS addition, and decreased gradually afterwards. PG added to the medium of cultured cells reduced the amount of IL-1β in a dose-dependent manner. The amount of IL-1β incubated with 1mg/ml PG was suppressed by 58% compared with the controls (p=0.012). In addition, similar decrease was accepted about IL-6, IL-8 by PG addition.

Conclusions
These results indicate that preterm delivery may be suppressed using PG in the future, since PG inhibited the various kinds of inflammatory cytokine that mainly contributes to onset of CAM and cervical ripening remarkably.