

## HUMAN DETRUSOR SMOOTH MUSCLE CELLS PRODUCE MONOCYTE CHEMOATTRACTANT PROTEIN - 1

### Aims of Study

Interstitial cystitis is a chronic inflammatory bladder disease of unknown etiology characterized by detrusor mastocytosis, frequency, nocturia and pain. Recently, attention has turned to the "synthetic" properties of smooth muscle cells. Still, relatively little is known regarding the particular function of the detrusor smooth muscle. Chemokines are a large family of small proteins that play a crucial role in immune and inflammatory conditions. Monocyte chemoattractant protein-1 (MCP-1) is a small potent chemokine which causes mast cell recruitment and provokes mast cell activation in vitro. The aim of the study was to investigate whether MCP-1 is produced by human detrusor smooth muscle cells (HDSMC) cultured under inflammatory conditions.

### Methods

With ethical approval detrusor muscle biopsies were obtained from patients with benign non-invasive bladder diseases undergoing cystoscopy. HDSMC were isolated and cultured using explant technique. All experiments were carried out between passage 1 and 3. For experiments HDSMC were grown until confluence in 24-well plates. Before stimulation the culture medium was replaced with fresh medium without fetal calf serum (FCS). After 12 hours, the cells were incubated in fresh medium containing 2% FCS in the presence of interleukin-1 $\beta$  (IL-1 $\beta$ ), TNF- $\alpha$ , lipopolysaccharide (LPS), histamine, leukotriene D<sub>4</sub> (LTD<sub>4</sub>) and prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) for 24 h at 37 °C. The level of MCP-1 in cell supernatants was measured by enzyme linked immunoassay (ELISA). For each independent experiment the mean chemokine secretion was determined from two wells, each measured in duplicate.

### Results

MCP-1 was present in low levels in unstimulated cell cultures. Following 24-h of culture with IL-1 $\beta$  or TNF- $\alpha$  (1 pg/ml - 100 ng/ml) the level of MCP-1 increased in a dose-dependent manner. IL-1 $\beta$  was more potent than TNF- $\alpha$  as a stimulus. Histamine (100  $\mu$ M), LTD<sub>4</sub> (50 nM), PGE<sub>2</sub> (1  $\mu$ M) and LPS (10  $\mu$ g/ml) failed to induce MCP-1 production. When IL-1 $\beta$  (10 ng/ml) and TNF- $\alpha$  (10 ng/ml) were given in combination a highly synergistic effect on the MCP-1 production was obtained. The same synergistic effect was not seen when HDSMC cultures were stimulated with either IL-1 $\beta$  (10 ng/ml) or TNF- $\alpha$  (10 ng/ml) in combination with histamine (100  $\mu$ M), LTD<sub>4</sub> (50 nM), PGE<sub>2</sub> (1  $\mu$ M) or LPS (10  $\mu$ g/ml)

### Conclusions

This study, for the first time, shows that human detrusor smooth muscle cells cultured under inflammatory conditions produce significant amounts of MCP-1. In addition to its contractile function HDSMC have "synthetic" and secretory potential with the release of MCP-1. Thus, HDSMC might contribute to the local inflammatory process by producing proinflammatory mediators. Release of cytokines and chemokines by human detrusor muscle, even in small amounts, may have important functional consequences especially during inflammation. This may be the case in interstitial cystitis where MCP-1 may attract and activates mast cells. The role of chemokines and detrusor smooth muscle as a secretory tissue need to be investigated.