INTERLEUKIN-4 AND INTERLEUKIN-13 MODULATE THE PRODUCTION OF EOTAXIN, MCP-1 AND INTERLEUKIN-6 BY HUMAN DETRUSOR SMOOTH MUSCLE CELLS

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Hypothesis / aims of study

Mast cells are important effector cells, which have been implicated in the pathogenesis of several diseases, including interstitial cystitis (IC). Mast cells infiltrate the detrusor muscle layer of the urinary bladder wall in patients with IC. Activated Th_2-lymphocytes are also important cells in inflammation. Mast cell activation and degranulation leads to the release of proinflammatory mediators, including histamine, tryptase, leukotrienes and cytokines (IL-1â and TNF-á etc.). It is known that Th_2-type cytokines IL-4 and IL-13 are primarily produced by T-cells, basophils and mast cells. The Th_2 cytokines are potent modulators of immune and inflammatory functions and they may have the potential to exert important effects on human detrusor smooth muscle cells (HDSMC). It was believed traditionally that HDSMC played a passive role during inflammation in the bladder wall and its function was limited to contraction. However accumulating evidence indicates that HDSMC are directly involved in bladder inflammation. Previously, we have shown that IL-1â and TNF-á induce chemokines (IL-8, RANTES, MCP-1and eotaxin) and cytokines (IL-6) production in HDSMC. In the present study, we extend these studies in order to investigate the capacity of IL-4 and IL-13 to promote the release of eotaxin and IL-6 and whether these Th_2 cytokines alter IL-1â and TNF-á induced MCP-1, eotaxin and IL-6 production in HDSMC.

Study design, materials and methods

With ethical approval detrusor muscle biopsies were obtained from patients with benign noninvasive bladder diseases undergoing cystoscopy. HDSMC were isolated and cultured using explant technique. All experiments were carried out between passage 1 and 3. HDSMC were grown in 24-well plates until confluency. HDSMC were starved for 12 hrs. Following this period HDSMC were stimulated in fresh medium containing 2% foetal calf serum in the presence of either IL-4, IL-13, TNF-á or IL1â or IL-4/IL-13 in combination with IL-1â or TNF-á for 24 hours. The levels of MCP-1, eotaxin and IL-6 in cell supernatants were measured with a commercially available enzyme linked immunoassay (ELISA) kit according to the assay procedure. For each independent experiment the mean chemokine or cytokine secretion was determined from 2 wells of culture supernatant, each measured in duplicate.

Results

HDSMC were incubated at 37C for 24 hrs with IL-4 or IL-13 over a concentration range (0.01-100ng/ml) and secreted eotaxin and IL-6 in the supernatants were quantified. HDSMC released significant amount of eotaxin and IL-6 in a concentration dependent manner. IL-4 was more potent than IL-13 at all concentration tested. Eotaxin production was detected after 6 hours of culture and the maximum concentration was achieved after 24 hours. To investigate whether there was a synergistic effect of cytokines on eotaxin, MCP-1 and IL-6 production, HDSMC were pre-treated with 10ng/ml IL-4 or IL-13 for two hours before additing 10ng/ml IL-1â or TNF-á. A highly significant effect on eotaxin production was observed when the cytokines were given together compared with IL-1â and TNF-á given separately. However, only the combination of IL-13 and TNF-á significantly enhanced the production of MCP-1 and IL-6.

Interpretation of results

Mast cells are capable of producing different pro-inflammatory cytokine profiles, including TNF-á, IL-1-â, IL-13 and IL-4. In IC mast cells infiltrate detrusor smooth muscle layer. Activated detrusor muscle cells or other infiltrating inflammatory cells locally produce cytokines and chemokines. The later may attract and activate tissue mast cells with the subsequent release of cytokines, chemokines and other inflammatory mediators.In this study,