A potential role of TSLP in the recruitment of macrophages to the intervertebral disc via MCP-1 induction: Implications for herniated discs

INTRODUCTION:
Thymic stromal lymphopoietin (TSLP) is an IL-7-like cytokine, that binds to the TSLP receptor (TSLPR) consisting of the IL-7 receptor α-chain (IL-7Rα) and a common γ-receptor-like chain (TSLPR-γ). TSLP is expressed primarily by epithelial cells, whereas TSLPR is expressed by hematopoietic cells, including T cells, B cells, and dendritic cells (DCs). Recently, TSLP has been shown to be capable of activating DCs to up-regulate co-stimulatory molecules, leading to differentiation of CD4+ T cells into Th2 cells, and also playing a key role in the development of allergic diseases such as asthma or atopic dermatitis. However, the roles of TSLP in the pathophysiology of bone or cartilage remain largely unknown.

The current study provides significant evidence that TSLP may play an important role in the recruitment of macrophages by stimulating MCP-1 production in the intervertebral disc tissues and may therefore be involved in the pathophysiology of HD. To our knowledge, this is the first demonstration that TSLP may act as a key molecule for macrophage migration into intervertebral discs. TSLP may thus play a role, not only in allergic disease, but also in intervertebral disc disease.

METHODS:
Coccygeal intervertebral disc tissues were obtained from the mouse tail bone using a dissecting microscope after the skin and soft tissues were removed. Mouse intervertebral disc cultures were stimulated with TNF-α, IL-1β, and LPS and TSLP mRNA and protein expression was assessed by quantitative real-time PCR and ELISA and immunohistochemical analysis with an anti-TSLP antibody. The ability of mouse intervertebral disc cells to express TSLP receptor (TSLPR) and respond to TSLP stimulation was next examined by Western blot analysis with anti-TSLPR antibody and protein array analysis. To investigate the intracellular signaling pathways involved in TSLP-induced MCP-1 production in mouse intervertebral disc cells, the effects of several inhibitors on MCP-1 production were examined. Among the inhibitors tested, LY294002, a compound that can specifically inhibit phosphatidylinositol 3 kinase (PI3K) activity by targeting the ATP-binding site of the kinase, significantly inhibited TSLP-induced MCP-1 production in mouse intervertebral disc cells. A Western blot analysis demonstrated that TSLP induced phosphorylation of Akt, a downstream molecule of PI3K, in mouse intervertebral disc cells, which was inhibited by 10 µM LY294002. These results indicate that TSLP induces MCP-1 in mouse intervertebral disc cells primarily via the PI3K/Akt pathway.

TSLP and MCP-1 expression in human HD tissue
Immunohistochemical studies with anti-TSLP and anti-MCP-1 antibodies were performed using clinical specimens derived from patients who underwent a surgical resection for HD mass. Ten human HD specimens were examined for TSLP and MCP-1 expression and all the samples showed a high level of immunoreactivity for TSLP and MCP-1. These results suggest a potential role of TSLP in HD.

DISCUSSION:
We found that TNF-α, IL-1β, and LPS induced TSLP mRNA and protein expression in mouse intervertebral disc cells through the NF-kB pathway. These results are, overall, consistent with previous reports in bronchial epithelial cells, keratinocytes, and synovial fibroblasts. TSLP and MCP-1 expression were, in general, detected in both anulus fibrosus cells and nucleus pulposus cells. It is thus possible that TSLP released by these cells stimulates MCP-1 production in an autocrine or paracrine fashion. A previous study showed that STAT5, a member of the transcription factors from the family of signal transducers and activators of transcription [STAT], was activated in response to TSLP in myeloid cells, however, it remains unclear whether other intracellular signaling pathways are involved in TSLP-mediated signaling. The current results showed that the PI3K/Akt pathway is also involved in TSLP signaling in primary mouse intervertebral disc cells, suggesting diverse pathways for intracellular TSLP signaling in different cell types. In summary, we here-in demonstrated that TSLP, induced by NF-kB ligands such as TNF-α, stimulated the intervertebral disc cells to produce MCP-1, which was able to recruit macrophages. In HD tissues, both TSLP and MCP-1 were found to be co-expressed. Because MCP-1 is an important mediator for natural HD resorption by triggering macrophage infiltration into HD tissue, the current results suggest that TSLP plays a potential role in natural HD resorption and, possibly, re-organization post disc herniation via MCP-1 induction. TSLP that is considered to be a master switch for allergic inflammation may thus play a role, not only in allergic disease, but also in intervertebral disc disease.