Abstract
Thymic stromal lymphopoietin (TSLP) is an interleukin (IL)-7-like cytokine produced by epithelial cells and triggers dendritic cell-mediated Th2 type allergic inflammatory responses. This study investigated whether Toll-like receptor (TLR) ligands, lipopolysaccharide (LPS) and poly-IC affect TSLP production in synovial fibroblasts. Enzyme-linked immunosorbent assay showed that LPS and poly-IC upregulated TSLP production in synovial fibroblasts obtained from patients with rheumatoid arthritis (RA) and osteoarthritis (OA). In addition, we found that nuclear factor (NF)-κB inhibitor IMD-0354, dexamethasone, and interferon (IFN)-γ inhibited the LPS- and poly-IC-induced TSLP production in RA and OA synovial fibroblasts. Thus, LPS and poly-IC can upregulate TSLP via a NF-κB pathway in synovial fibroblasts, which is downregulated by dexamethasone and interferon (IFN)-γ. The current findings suggest that TSLP may be involved in the pathophysiology of inflammatory arthritis as well as allergic disease.

Key words
Nuclear factor-κB · Rheumatoid arthritis · Synovial fibroblasts · Thymic stromal lymphopoietin (TSLP) · Toll-like receptor ligands

Introduction
Thymic stromal lymphopoietin (TSLP) is an interleukin (IL)-7-like cytokine, which binds to the TSLP receptor (TSLPR) consisting of the IL-7 receptor α-chain (IL-7Rα) and a commonγ receptor-like chain (TSLPR-γ). TSLP is an interleukin (IL)-7-like cytokine produced by epithelial cells and triggers dendritic cell-mediated Th2 type allergic inflammatory responses. This study investigated whether Toll-like receptor (TLR) ligands, lipopolysaccharide (LPS) and poly-IC affect TSLP production in synovial fibroblasts. Enzyme-linked immunosorbent assay showed that LPS and poly-IC upregulated TSLP production in synovial fibroblasts obtained from patients with rheumatoid arthritis (RA) and osteoarthritis (OA). In addition, we found that nuclear factor (NF)-κB inhibitor IMD-0354, dexamethasone, and interferon (IFN)-γ inhibited the LPS- and poly-IC-induced TSLP production in RA and OA synovial fibroblasts. Thus, LPS and poly-IC can upregulate TSLP via a NF-κB pathway in synovial fibroblasts, which is downregulated by dexamethasone and interferon (IFN)-γ. The current findings suggest that TSLP may be involved in the pathophysiology of inflammatory arthritis as well as allergic disease.
gated the factors that downregulate TSLP expression in synovial fibroblasts.

Materials and methods

Reagents

Lipopolysaccharide, poly-IC, IMD-0354 (a selective inhibitor of IκB kinase), and dexamethasone were purchased from Sigma Aldrich (St. Louis, MO, USA). Recombinant human IFN-γ, human transforming growth factor (TGF)-β, human TNF-α, and human IL-6 were purchased from R&D (Minneapolis, MN, USA).

Patient profile

Synovial tissue samples of the knee joints were obtained from two RA patients (two males, age 79 and 65 years) and two OA patients (two females, age 84 and 85 years) diagnosed based on the revised criteria of the American College of Rheumatology for RA17 or OA.18 All RA and OA patients were receiving treatment at the time of the study; two RA patients were on disease-modifying anti-rheumatic drugs (DMARDs) and prednisolone, while the two OA patients were being treated with nonsteroidal anti-inflammatory drugs (NSAIDs).

Cell culture

Human synovial fibroblasts were obtained as previously described.19 In brief, after enzymatic digestion, human synovial cells were isolated from synovial tissues of the knee joints of RA and OA patients (see the Patient profile section) at the time of total knee arthroplasty operations. The investigation was approved by the Ethics Committee of the University of Yamanashi, Faculty of Medicine, and all subjects gave their written informed consent. The cells were suspended in Dulbecco’s Modified Eagle Medium (DMEM) (Gibco/Invitrogen, Carlsbad, CA) containing 10% fetal calf serum (FCS), 100µg/ml streptomycin, and 100 units/ml penicillin G solution and then were cultured in monolayers. After three to five passages, the subcultured cells were composed of morphologically uniform fibroblastic cells (synovial fibroblasts) that were free of macrophages.

Enzyme-linked immunosorbent assay (ELISA)

The amounts of TSLP in the culture supernatants were measured by ELISA using the Human TSLP sandwich ELISA Development Kit (R&D) according to the manufacturer’s instructions. The minimum detection level of ELISA was 31.25 pg/ml.

Western blot

Western blot analysis was performed as previously described20 with specific antibodies for IκB-α (Cell Signaling Tech., Beverly, MA, USA) and β-actin (Santa Cruz Biotech, Santa Cruz, CA, USA).

Cell viability assay

Cells (2.5 × 10⁴ cells/well) were cultured in DMEM containing 10% FCS with or without the indicated doses of IMD-0354 or dexamethasone for 24 h in a flat-bottomed 96-well microtiter plate. Cell viability was then determined by measuring the metabolic activity using 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl) [1H] tetrazolium monosodium salt (WST) using Tetra Color ONE kit (Seikagaku, Tokyo, Japan) according to the manufacturer’s instructions. In our preliminary studies, the metabolic activity measured in this assay was proportional to the cell number which was directly counted by a trypan blue exclusion assay.

Data analysis

The data are summarized as the mean ± SD. The unpaired Student’s t-test was used for the statistical analysis of the results. P < 0.05 was considered to be significant.

Results

Lipopolysaccharide and poly-IC upregulate TSLP production in RA and OA synovial fibroblasts

We first examined TSLP production in synovial fibroblasts obtained from patients with RA and OA by ELISA. As shown in Fig. 1, RA and OA synovial fibroblasts constitutively produced some amounts of TSLP. Synovial fibroblasts were then stimulated with TLR ligands, LPS, and poly-IC, and also with several cytokines such as IL-6, IFN-γ, and TGF-β. Among the stimuli that we examined, LPS and poly-IC significantly upregulated TSLP production in 2 RA and 2 OA synovial fibroblasts in a dose-dependent manner (Fig. 1). These results indicated that LPS and poly-IC upregulated TSLP production in RA and OA synovial fibroblasts.

Activation of NF-κB contributes to LPS- and poly-IC-induced TSLP production in synovial fibroblasts

Because TNF-α, LPS, and poly-IC share a NF-κB pathway as a common element for these signaling intermediates, we hypothesized that LPS- and poly-IC induced TSLP production in synovial fibroblasts via a NF-κB pathway. To test this hypothesis, we examined the effects of IMD-0354, a selective inhibitor of IκB kinase, and dexamethasone, a steroid hormone that inhibits NF-κB activation,21 on the TSLP production.

We first assessed the effective doses of IMD-0354 and dexamethasone. As shown in Fig. 2A, 10µM IMD-0354 significantly inhibited TNF-α-induced degradation of IκB-