Thrombin-induced IL-6 production in human synovial fibroblasts is mediated by PAR1, phospholipase C, protein kinase C, c-Src, NF-kappaB and p300 pathway

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INTRODUCTION:
Thrombin is a multifunctional protease that can activate hemostasis and coagulation through the cleavage of fibrinogen to form fibrin clots. Increasing fibrin deposition is a predominant feature of rheumatoid arthritis (RA) in synovial tissue, which contributes to chronic inflammation and progressive tissue abnormalities. Thrombin also acts as a mitogen to stimulate the abnormal proliferation of synovial cells during RA pathogenesis. In this regard, thrombin can elevate the expression of nuclear factor-xB, IL-6, and granulocyte colony-stimulating factor in fibroblast-like cells of the RA synovium. However, the signaling pathway for thrombin on IL-6 production in synovial fibroblast cells is mostly unknown. In the present study, we explored the intracellular signaling pathway involved in thrombin-induced IL-6 production in synovial fibroblast cells.

METHODS:
Cell culture: Human synovial fibroblasts were isolated using collagenase treatment from synovial tissue obtained from knee replacement surgeries of nine patients with RA, after approval by the local ethics committee. Patients with RA fulfilled the diagnostic criteria of American college of Rheumatology. Fresh synovial tissues were minced and digested in a solution of collagenase, and DNase. Isolated fibroblasts were filtered through 70 µM nylon filters. The cells were grown on plastic cell culture dishes in 95% air-5% CO2 with RPMI 1640 (Gibco, Grand Island, NY) which was supplemented with 20 mM HEPES and 10% heat-inactivated FBS, 2 mM-glutamine, penicillin (100 U/ml) and streptomycin (100 µg/ml) (pH adjusted to 7.6). Fibroblasts from passages four to nine were used for the experiments.

RT-PCR; Measurements of IL-6 production; Chromatin immunoprecipitation assay; Western blot analysis

RESULTS
It has been reported that clotting factors and fibrinolytic products, such as thrombin, are increased in synovial fluid of patients with RA. Human synovial fibroblast was chosen to investigate the signal pathways of thrombin in the production of IL-6, an inflammatory response gene. Treatment with thrombin (0.1–10 U/ml) for 24 hr induced IL-6 production in a concentration-dependent manner (Fig. 1A), induction occurred in a time-dependent manner (Fig. 1B).

DISCUSSION:
The signaling pathway involved in thrombin-induced IL-6 production in human synovial fibroblasts has been explored. Thrombin increases IL-6 production by binding to PAR1 and activating of protein kinase C (PKC). To study the intracellular signaling pathway involved in thrombin-induced IL-6 production, synovial fibroblasts were pretreated for 30 min with PI-PLC inhibitor, U73122 (1 and 3 µM). It was found that U73122 but not the inactive analogue of U73122, U73343 (10 µM) or PC-PLC inhibitor D609 (10 µM) antagonized the potentiating effect of thrombin. Furthermore, U73122, U73343 and D609 had no effect on the basal level of IL-6 release (Fig. 3). The results show that thrombin activates the PAR1 receptor and results in the activation of PI-PLC/PKC/c-Src/IKKβ/NF-xB and p300, leading to the upregulation of IL-6 expression.