MAST CELL TRYP maze EXACERBATES SYN ovitis in Osteoarthritis AND Rheumatoid Arthritis

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Introduction
Rheumatoid arthritis (RA) is usually associated with synovitis characterized by synovial fluid (SF), which frequently contains various chemical mediators, degradation products from articular structures, and exudation from the blood stream. Synovial fluid, therefore, is thought to reflect to some extent local inflammatory reactions of RA affected joints, and hyperplasia of synovial tissues producing SF is one of pathological bases of RA (1-5).

In this study, we measured mast cell tryptase activities at articular lesion, examined the effect of mast cell tryptase (MCT) on growth and the function of synovial fibroblast-like cells (SFC), and investigated the expression of PAR-2 (protease activated receptor-2) in cultured SFC, to clarify the pathophysiological significance of MCT in OA and RA.

Method
We measured tryptase activity in samples obtained from OA and RA patients. Synovial fluid was collected by puncture from the knee joints of each patient. Samples were then centrifuged for 10 min at 1500 rpm at 4 °C within an hour of collection. The resultant supernatants were immediately frozen at −20 °C until use. The precipitants obtained from the SF of each patient as described above, and synovial tissue collected during joint surgery, were washed with PBS, dissolved in PBS, homogenized, sonicated, and centrifuged at 3000 rpm for 10 min at 4 °C. The resultant supernatants were designated as SF cell and synovial tissue extracts, respectively. Tryptase activity was measured using a synthetic fluorogenic peptide (Boc-Gla-Ala-Arg-MCA). One unit of enzymatic activity was defined as the amount of enzyme required to release 1 μmol of aminomethylcoumarin per min.

Biochemical characterization of the partial purified mast cell tryptase obtained from synovial tissue extract was done to conform and correspond to purified lung MCT. To detect the presence of cells containing MCT, synovial tissue was immunohistochemically stained with MCT monoclonal antibody using standard procedures.

Synovial fibroblast-like cells were obtained from the fresh synoviums of patients during joint surgery. After adding MCT to a culture of SFC from each patient, the proliferation-promoting activity of MCT was evaluated by incorporation of H-thymidine, ELISA was used to measure the levels of IL-6 and IL-8. The expression of PAR-2 mRNA in cultured SFC was detected using RT-PCR.

Data are presented as means ± SD. Significant differences between groups were assessed by the non-parametric Wilcoxon-Mann-Whitney test, and any significant correlation of cathepsin B-like activity with other parameters was assessed by Pearson’s correlation. A p value of less than 0.05 was considered to indicate a statistically significant difference.

Results
There was no significant difference in the levels of PASE activity (mU/ml) of the SF supernatants between OA (n=25, 5.6±3.2) and RA patients (n=40, 5.8±3.5). The levels of tryptase activity in the SF cell extract were very low in both OA (n=7) and RA patients (n=10). There was no significant difference in the levels of tryptase activity (mU/ml) of the ST extract between OA (n=6, 16.9±21.7) and RA patients (n=8, 14.1±15.4). The tryptase activity in ST extracts was eluted in a fraction corresponding to purified lung MCT in gel filtration using a Sephadex G-200, and the substrate specificity of the fraction was similar to that of MCT. Synovial tissue was stained with MCT monoclonal antibody as in Fig. 1.

When MCT (0–100 ng/ml) was added to the culture medium for SFC, MCT tended to enhance SFC proliferation in a dose-dependent manner, and also the concentrations of IL-6 and IL-8 in the medium. RT-PCR of cultured SFC showed that PAR-2 mRNA was expressed (Fig. 2).

Discussion
The present study showed that tryptase-like activity was relatively high in both the SF supernatants and ST extracts from OA and RA patients, but almost undetectable in the SF cell extracts. These findings strongly suggest that the tryptase in SF of OA and RA patients is mainly released from synovial tissue. Moreover, mast cells have been reported present in synovial tissue, and we demonstrated that the molecular weight and substrate specificity of the tryptase-like protease purified partially by gel filtration from synovial tissue in the present study, were very similar to those of purified lung MCT. Taken together, it suggested that the tryptase-like protease detected in the SF from OA and RA patients is a mast cell tryptase derived mainly from mast cells in synovial tissue.

In addition, the present results indicated that MCT tended to enhance proliferation of SFC and to release/synthesise IL-6 and IL-8 by SF in a dose-dependent manner, and the levels of tryptase-like activity in SF were high in both OA and RA. So it was suggested that mast cells played some role in the pathogenesis of arthritis in both OA and RA, and that the tryptase-like protease are intimately related to pathogenesis of OA and RA (Fig. 3).

Fig. 1 Photomicrograph of immunohistochemical staining of human synovium using MCT monoclonal antibody.

Fig. 2 PAR-2 mRNA expression in cultured SFC.

Fig. 3 Mast cell tryptase in OA and RA.

References
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