INTRODUCTION
Rheumatoid arthritis (RA) is an autoimmune disease that is characterized by severe synovitis and cartilage destruction. Synovial cells play a major role in the pathogenesis of RA through the synthesis of various cytokines, proteases, superoxide, etc. Interleukin-8 (IL-8), one of CXC chemokines, is known to recruit neutrophils into inflammatory loci. Chemokines have been reported to be highly expressed within the RA inflammatory synovium, mainly produced by macrophages, fibroblasts, and endothelial cells within the synovium, induced by proinflammatory cytokines such as IL-1 or TNF-alpha, and considered to participate in the inflammatory process at an early stage of RA. We already reported that macrophage migration inhibitory factor (MIF), which has recently been re-evaluated as a mediator in various inflammatory diseases, is exclusively expressed in CD45+ T cells of rheumatoid synovium, and that the concentrations of MIF in the joint fluids are much higher in RA patients than in osteoarthritis (OA) patients or normal volunteers [1]. We also reported that MIF is a potent inducer of matrix metalloproteinases (MMPs) in rheumatoid synovial fibroblasts [2]. However, the role of MIF in RA still remains unelucidated. In this study, we investigated the effect of MIF on the rheumatoid synovial fibroblasts from the viewpoint of IL-8 induction.

MATERIALS AND METHODS
Synovial tissues from RA and OA patients retrieved at TKA surgery were minced, subjected to 0.2% collagenase digestion, and the cells obtained were cultured in MEM containing 10% FCS and NEAA. These synoviocytes, >95% fibroblast-like as confirmed by microscopic analysis, were used at 3rd passage as synovial fibroblasts. Following the addition of 1 microgram/ml of human recombinant (r) MIF under serum-free medium, the cell samples or culture supernatants were collected at indicated times and analyzed by Northern blot analysis, ELISA, and gel mobility shift assay concerning the follows; 1) the effect of MIF on the mRNA expression of IL-8 and IL-1beta, 2) the effect of various signal transduction inhibitors on the MIF-induced up-regulation of IL-8 and IL-1beta, 3) the concentration of IL-8 in the culture supernatants collected at 24h, 48h, and at indicated intervals post-stimulation by MIF, and 4) the effect of MIF on the NF-kB binding activity. As for the preparation of RNA samples, ISOGEN RNA extraction kit was used according to the manufacturer’s protocol. As for the gel shift mobility assay, nuclear extracts from cell samples treated with or without 1 microgram/ml of MIF were reacted with radiolabeled oligonucleotides containing NF-kB consensus sequence and subjected to 4% acrylamid gel electrophoresis. It is reported that MIF possesses isomerase activity, which is closely related to its N-terminal proline. Using site-directed mutagenesis technique, we constructed mutant MIF (P1A mutant), in which the N-terminal proline was substituted for alanine, and investigated the difference of IL-8 induction activity between rMIF and P1A mutant. Statistical analyses were made using the ANOVA with the Fisher’s PLSD test for post hoc multiple comparisons.

RESULTS
1) MIF induced mRNA expression of IL-8 and IL-1beta mRNAs in a dose-dependent manner, beginning at 1 hr post-stimulation (Fig. 1). Both the basal expression and induction levels of the mRNAs were higher in RA fibroblasts than in OA fibroblasts. 2) Pretreatment of the cells for 30 min with tyrosine kinase inhibitors (genistein and herbimycin A), protein kinase C (PKC) inhibitors (staurosporine), NF-kB inhibitor (PDTC), or a c-jun/activator protein-1 (AP-1) inhibitor (curcumin) inhibited mRNA up-regulation of IL-8 and IL-1beta by MIF. Addition of IL-1 receptor antagonist did not influence the inducition of IL-8 mRNA. 3) Addition of more than 1 microgram/ml of MIF significantly increased the concentration of IL-8 in the culture supernatants (P<0.05) (Fig. 2 a and b). The increase was significantly higher in RA fibroblasts than in OA fibroblasts (P<0.05). 4) The NF-kB binding activity was increased at 5 min post-stimulation by MIF, which was sustained at least up to 3 hr. 5) P1A mutant did not influence the levels of IL-8 and IL-1beta mRNAs.

DISCUSSION
This is the first report concerning the IL-8 inductive activity of MIF in the RA synovial fibroblasts. The coordinate induction of IL-1beta and IL-8 may be due to the similarity of their promoter structures, which possess TRE and NF-kB binding site in common. Our results showed that the induction of IL-8 by MIF was regulated mainly by AP-1 and NF-kB, via PKC/tyrosine kinase, independent of their endogenous IL-1beta production. IL-8 is known to cause the emigration of neutrophils into synovial fluid [3], resulting in the promotion of joint inflammation and neutrophil-mediated cartilage degradation [4]. Mikulowska et al. reported the effectiveness of anti-MIF antibody against type-II collagen induced mouse arthritis [5]. In RA, after receiving antigen presentation, T cells infiltrate into synovium and produce Th1/Th2 lymphokines. It is suggested that MIF may be one of them, playing a major role in the progression of synovitis and cartilage destruction via promoting the synthesis of IL-8 and IL-1beta from synovial fibroblasts, in addition to the synthesis of MMPs. Taken together, MIF plays a pivotal role in the pathomechanism of RA, and can be a novel, promising target for the treatment of RA.

**Macrophage Migration Inhibitory Factor Induces IL-8 Production in Synovial Fibroblasts of Rheumatoid Arthritis**

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**References:**

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**Fig. 1** Northern blot analysis of IL-8 and IL-1beta mRNAs in response to 1 microgram/ml of MIF. The samples were retrieved at indicated intervals.

**Fig. 2** ELISA of IL-8 concentrations in the culture supernatants of RA synovial fibroblasts that were, a. stimulated with or without MIF for 24 and 48 hr, and b. stimulated with MIF (1 microgram/ml) for the indicated time. *P<0.05 (vs. value at 0), **P<0.05 (vs. value at 0)