THE EFFECT OF MACROPHAGE MIGRATION INHIBITORY FACTOR ON THE SYNOVIAL FIBROBLASTS OF RHEUMATOID ARTHRITIS AND ITS SIGNALING PATHWAYS

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. Matrix metalloproteinases (MMP), one of extracellular matrix degrading enzymes, are presently subclassified into 18 kinds. Among them, MMP-1 (collagenase) and MMP-3 (stromelysin), which are mainly synthesized by synovial lining cells via induction by IL-1 and TNF-alpha, are considered most important for joint destruction. 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 CD4+ T cells of rheumatoid synovium, and that the concentrations of MMP in the joint fluids are much higher in RA patients than in osteoarthritics (OA) patients or normal volunteers. 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 MMP 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 passage 1 and 3rd 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 MMP-1, MMP-3, their endogenous inhibitor TIMP-1, c-jun, c-fos, and IL-1 beta, 2) the effect of various signal transduction inhibitors on the MIF-induced up-regulation of MMP-1 and 3, 3) the effect of MIF on the AP-1 binding activity, and 4) the concentration of MMP-1 in the culture supernatants collected at 48 hr post-stimulation by MIF. As for the preparation of RNA samples, ISOGEN RNA extraction kit was used according to the manufacturer’s protocol. As for the gel mobility shift assay, nuclear extracts from cell samples treated with or without 1 microgram/ml of MIF were reacted with radiolabelled oligonucleotides containing AP-1 consensus sequence and subjected to 4% acrylamid gel electrophoresis. It is reported that MIF possesses isomerase activity, and this activity 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 MMP induction activity between rMIF and P1A mutant. The effect of heat-denaturing of rMIF at 65°C for 1 hr on the induction of MMP-1 and 3 was also investigated.

Fig. 1 Northern blot analysis of MMP-1, MMP-3, c-jun, c-fos, and IL-1beta mRNAs in response to 1 microgram/ml of MIF.

Fig. 2 AP-1 binding activity of RA synovial fibroblasts stimulated with or without 1 microgram/ml of MIF. a) lane 1, sample (+); 2, sample (+); 3, sample + excess amounts of nonlabelled specific competitor; 4, sample + excess amounts of nonlabelled nonspecific competitor. b) lane 1, control; 2, 5 min; 3, 15 min; 4, 30 min; 5, 1 hr; 6, 3 hr.

Results 1) MIF induced mRNA expression of MMP-1, MMP-3, and IL-1beta in a dose-dependent manner at 6 hr post-stimulation. Both the basal expression and induction levels of the mRNAs were higher in RA fibroblasts than in OA fibroblasts. TIMP-1 mRNA was slightly up-regulated by MIF. These events were preceded by transient up-regulation of c-jun and c-fos mRNAs, which was maximal at 30 min post-stimulation (Fig.1). 2) Pretreatment of the cells for 30 min with tyrosine kinase inhibitors (genestein and herbinycin A), protein kinase C (PKC) inhibitors (H-7 and staurosporine), or a c-jun/activator protein-1 (AP-1) inhibitor (curcumin) inhibited mRNA up-regulation of MMP-1 and 3 by MIF. A cyclic AMP dependent PKA inhibitor (H-8) and IL-1 receptor antagonist did not influence the induction of MMP-1 and 3. 3) The AP-1 binding activity was increased at 5 min post-stimulation by MIF, which was sustained at least up to 3 hr (Fig.2). 4) Addition of more than 1 microgram/ml of MIF significantly increased the concentration of MMP-1 in the culture supernatants (*p<0.0001, vs. cont. of RA; †p<0.005, vs. cont. of OA; ††p<0.0001, vs. cont. of OA.). The increase was significantly higher in RA fibroblasts than in OA fibroblasts (Fig.3). 5) P1A mutant did not induce mRNA of MMP-1 and 3. Heat-denatured rMIF showed much decreased induction of them.

Discussion This is the first report concerning the MMP-inductive activity of MIF. The coordinate induction of MMP-1 and 3 may be due to the similarity of their promoter structures, which possess TRE and PEA-3 in common. Our results showed that the induction of MMP-1 and 3 by MIF was regulated mainly by AP-1, via PKC/tyrosine kinase and the induction of c-jun/c-fos. On the other hand, the involvement of transcription factor Ets, which is also considered to be important for the transcription of MMP-1, is a further problem to be answered. Our results supported the reports that the biological activity of MIF is linked to its isomerase activity. To clarify the mechanism of the linkage between the two activities, it is essential to clone the receptor of MIF and analyze its structure. Mikulowska et al reported the effectiveness of anti-MIF antibody against type-II collagen induced mouse arthritis. 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 MMPs and IL-1beta from synovial fibroblasts. Taken together, MIF plays a pivotal role in the pathomechanism of RA, and can be a novel, promising target for the treatment of RA.