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EFFECTS OF INFLAMMATORY MEDIATORS ON MATRIX METALLOPROTEINASE-2 EXPRESSION BY RHEUMATOID ARTHRITIS SYNOVIAL FIBROBLASTS

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1. INTRODUCTION

1.1 Rheumatoid arthritis synovial fibroblasts

Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by synovial hyperplasia and invasion and destruction of bone and cartilage. Synovial fibroblasts are involved in continuous matrix remodeling by producing matrix components such as collagen and hyaluronan as well as a variety of matrix-degrading enzymes.

1.2 Matrix metalloproteinases

Matrix metalloproteinases (MMPs) are a large family of proteolytic enzymes responsible for degradation of extracellular matrix (ECM) components and are thought to play a crucial role in the RA joint destruction. In RA, angiogenesis is thought to be a key event in the expansion of the synovial lining of the joints. Angiogenesis requires proteolysis of the extracellular matrix, proliferation, and migration of endothelial cells (EC), as well as synthesis of new matrix components. It has been suggested previously that MMP-2 plays an important role in RA. Elevated MMP-2 levels are detected in RA compared to osteoarthritic synovial fluid. RA patients with radiographic erosions have significantly higher levels of active MMP-2 in their synovial tissues than patients with no erosions.

1.3 Macrophage migration inhibitory factor

Macrophage migration inhibitory factor (MIF) is a proinflammatory cytokine produced by macrophages in response to inflammatory stimuli. Recently, MIF has been implicated in angiogenesis as well as in the pathogenesis of RA. Several independent studies described MIF enhancing angiogenesis and playing a role in tumor neovascularization. In type II collagen-induced arthritis, a murine model of RA, neutralizing anti-MIF antibody

delayed the onset and decreased the frequency of arthritis. Moreover, MIF gene deficient (MIF -/-) mice exhibited significantly less synovial inflammation than wild type (WT) mice after arthritis induction with type II collagen.

1.4. Epigallocatechin-3-gallate

A majority of pharmacologic properties of green tea are mimicked by its active constituent, epigallocatechin-3-gallate (EGCG). Previous studies using human chondrocytes derived from osteoarthritic cartilage showed EGCG to be an effective inhibitor of the production of catabolic mediators, such as nitric oxide and prostaglandin E_2. In addition, EGCG significantly inhibits the expression and activities of MMP-1 and MMP-13 in human chondrocytes.

1.5 Chemokines

In RA, chemokines mediate the migration of inflammatory leukocytes into the synovium. Among the four known chemokine families, CXC, CC chemokines and fractalkine/CX_3CL1 seem to be of outstanding importance in this process. Angiogenesis is also important during the perpetuation of inflammation underlying rheumatoid arthritis. CXC chemokines containing the ELR amino acid motif such as epithelial-neutrophil activating protein-78 (ENA-78)/CXCL5 and growth-regulated oncogene product alpha (gro-α)/CXCL1 promote angiogenesis.

2. AIMS

In the present study our goal was to further evaluate MMP-2 expression in the RA synovium. Hence, we aimed:

1. To determine the effect of MIF on RA synovial fibroblast MMP-2 expression
2. To define signal transduction pathways playing a role in MIF-induced RA synovial fibroblast MMP-2 expression
3. To investigate MMP-2 expression in inflammatory arthritis in MIF -/- mice *in vivo*
4. To elucidate the effect of chemokines on RA synovial fibroblast MMP-2 expression
5. To investigate whether EGCG inhibits cytokine and chemokine-induced RA synovial fibroblast MMP-2 expression.

3. MATERIALS AND METHODS

3.1 Synovial fibroblast culture

Human RA synovial fibroblasts were isolated from synovial tissue obtained from RA patients who had undergone synovectomy or total joint replacement surgery. Fresh synovial tissues were minced and digested in a solution of dispase, collagenase, and DNase. The cells were cultured in RPMI 1640 supplemented with 10% FBS in 175-mm tissue culture flasks at 37°C in a humidified atmo sphere with 5% CO_2.

3.2 Animals

MIF -/- mice were generated as described previously, SV129/J WT mice served as controls. Mice were maintained and bred in a specific pathogen-free facility at the University of Michigan. Experiments were in concordance with federal law and were

performed after approval by the University Committee in Use and Care of Animals.

3.3 Induction of arthritis

3.3.1 Zymosan-induced arthritis

Zymosan-induced arthritis (ZIA) was induced by intraarticular injection of zymosan (*Saccharomyces cerevisiae*). Zymosan was prepared by dissolving 30 mg of zymosan in 1 ml of PBS. Mice were injected with zymosan (10 µl) into each knee joint. After 24 hours, mice were euthanized and ZIA knees were harvested, one knee was homogenized in PBS containing protease inhibitors, while the other knee was stored in frozen tissue matrix.

3.3.2 Antigen induced arthritis

The AgIA model involves both cellular and humoral immune responses and shows histological similarities to human RA. Briefly, mice were immunized at day 0 with 200 µg of methylated bovine serum albumin (mBSA) emulsified in 0.2 ml of complete Freund's adjuvant injected subcutaneously in the flank skin. At day 7, mice received 100 µg mBSA/0.1 ml Freund's adjuvant by intradermal injection at the base of the tail. At day 21, arthritis was induced by intra-articular injection of mBSA (30 µg in 10 µl of sterile PBS) in the knee. On day 28, mice were euthanized and AgIA knees were harvested and homogenized in PBS containing protease inhibitors.

3.4 Quantitation of MMPs by enzyme immunoassay

MMP-1, MMP-2, MMP-3 and MMP-13 concentrations in cell culture supernatants and the MMP-2 concentration of mouse knee homogenates were measured using Quantikine Immunoassay kits. The ELISA assays detect both the pro-form and active form of MMP-2, MMP-3, and solely the pro-form of MMP-1 and MMP-13.

3.5 Cell lysis and Western blotting

After treatment with MIF, cells were lysed with lysis buffer containing protease inhibitors. The concentration of protein in each extract was determined using a bicinchoninic acid (BCA) protein assay. SDS-polyacrylamide gel electrophoresis was performed with cell lysates after equal protein loading, and proteins were transferred onto a nitrocellulose membrane using a semi-dry transblotting apparatus. After blocking, blots were incubated with phospho-specific antibodies. Then, blots were incubated with anti-rabbit horseradish peroxidase-conjugated antibodies. Finally, blots were incubated with enhanced chemiluminescence (ECL) reagents. The immunoblots were stripped and reprobed with rabbit anti-β-actin to verify equal loading.

3.6 Gelatinase Assay

Gelatinase activity of RA synovial fibroblast culture media was measured using EnzChek Gelatinase Assay Kits. Cell culture supernatants were incubated with fluorescein conjugated gelatin for 3 hours and fluorescence was measured using a Synergie HT Microplate Reader at 495 nm.

3.7 Gelatin zymography

MMP-2 enzyme secreted by RA synovial fibroblasts was analyzed on gelatin containing gels. Additionally, gelatin degradation was visualized in AgIA joint homogenates after equalizing protein concentration. Samples were mixed with 2x sample buffer, and then added to 7.5% SDS-polyacrylamide gels, for 2 hours. Following electrophoresis, gels were renatured in 2.5% Triton X-100 for 1 hour at room temperature. The gels were then incubated at 37^{o} C overnight in developing buffer. Gels were stained for 3 hours with Coomassie Brilliant Blue R-250, and then destained with destaining solution. Gelatinase activities were visualized as white bands on the blue background of the gels.

3.8 Immunohistology

Frozen tissue (WT and MIF -/- mouse ZIA joints) were cut (approximately 7 μm) and stained using alkaline-phosphatase and fast red substrate visualization. Staining was evaluated under blinded conditions and graded by a pathologist. Slides were examined for cellular immunoreactivity and cell types were distinguished based on their characteristic morphology. The percentage of cells expressing MMP-2 was analyzed in the synovial lining cells (fibroblast-like and macrophage-like synoviocytes), the subsynovial non-lymphoid mononuclear cells (monocytes, macrophages, mast cells) and on ECs.

3.9 Immunofluorescence staining

RA synovial fibroblasts were stimulated with MIF (50 nM) for 20 minutes. Cells were fixed with ice-cold methanol, and then stained with phospho-specific primary antibodies for JNK and c-jun, or with anti-MMP-2 antibody. Alexa Fluor-conjugated secondary antibodies were added then. 4',6-diaminido-2-phenylindole (DAPI) nuclear stain was added. Immunofluorescence staining was detected using an Olympus BX51 Fluorescence Microscope System with DP Manager imaging software.

3.10 Statistical Analysis

Data were analyzed using Student's *t*-test assuming equal variance. *P* values less than 0.05 were considered statistically significant. Data are represented as the mean ± standard error of the mean (SEM).

3. RESULTS

3.1 The effects of MIF on RA synovial fibroblast MMP-2 production

3.1.1 MIF induces the production of MMP-2 in RA synovial fibroblasts

MIF stimulation (50nM) showed no effect on MMP-1, MMP-3 and MMP-13 protein secretion by RA synovial fibroblasts, whereas MIF-stimulated RA synovial fibroblasts produced significantly higher amounts of MMP-2 protein compared to non-stimulated controls, measured by ELISA. This effect was seen after 6 hours stimulation (non-stimulated: 7.13 ± 0.86 ng/ml; MIF stimulated: 16.28 ± 1.71 ng/ml; $p<0.05$) and after 24 hours stimulation as well (non-stimulated: 23.88 ± 7.49 ng/ml; MIF stimulated: 51.36 ± 5.55 ng/ml; $p<0.05$).

3.1.2 MIF induces activation of RA synovial fibroblast MMP-2

To analyze enzymatic activity of RA synovial fibroblast supernatants, gelatinase assay was performed using fluorescein labeled gelatin as substrate. Gelatinase assay confirmed enhanced enzymatic activity of MIF-stimulated compared to non-stimulated RA synovial fibroblast supernatants after 24-hour incubation (649 ± 34 mean fluorescence, vs. 503 ± 19; $p<0.05$). Gelatin zymography was performed to visualize the gelatin degradation mediated by MIF in RA synovial fibroblast culture media. Zymography revealed a band of gelatin degradation at 72 kDa, representing pro-MMP-2 protein.

3.1.3 MIF-induced MMP-2 production is time- and concentration dependent

When RA synovial fibroblasts were stimulated with MIF for different time periods, we found that MIF-induced MMP-2 upregulation was time dependent, beginning at 1 hour and

increasing continuously through 24 hours. Using immunofluorescence staining, MMP-2 showed a strong perinuclear and discrete diffuse cytoplasmic pattern.

When RA synovial fibroblasts were stimulated with different concentrations of MIF; we observed no stimulatory effect at 1 nM, while increasing MMP-2 expression was seen in response to higher concentration of MIF by gelatin zymography. Similarly elevated MMP-2 levels were observed at concentrations of 25 and 50 nM MIF.

3.2 Signaling pathways involved in MIF-induced RA synovial fibroblast MMP-2 production

3.2.1 Signaling molecules involved in MIF-induced MMP-2 expression

To examine the signal transduction pathways induced by MIF, RA synovial fibroblasts were stimulated with MIF in the presence of different signaling inhibitors. MMP-2 concentrations in supernatants were measured by ELISA. MMP-2 upregulation by MIF was suppressed by inhibitors against pan-PKC, JNK and in part by Src, suggesting that these signaling pathways are involved in MMP-2 production by RA synovial fibroblasts.

In order to determine the role of different PKC isoforms in MMP-2 production we used a PKCα/β specific inhibitor Gö6976 and a PKCδ isoform specific inhibitor rottlerin. We found that rottlerin inhibited the upregulation of MMP-2 by MIF, while the α and β isoform-specific PKC inhibitor Gö6976 did not.

3.2.2 Signaling molecules activated by MIF

The phosphorylation of JNK and the JNK substrate c-jun by MIF was determined by Western blot using phospho-specific antibodies. MIF activated JNK phosphorylation at 1 minute and a maximum response was seen at 15 minutes. MIF-induced c-jun activation was observed after 30 minutes. Using immunofluorescence staining, we found diffuse

cytoplasmic staining of phospho-JNK in RA synovial fibroblasts stimulated by MIF. The intracellular localization of phospho-c-jun was primarily nuclear but there was also a small amount of cytoplasmic staining. Upon MIF stimulation, immunofluorescence staining of phospho-c-jun showed a stronger nuclear pattern, suggesting nuclear translocation of c-jun. In order to determine which PKC isoforms are phosphorylated upon MIF stimulation we used different anti-phospho-specific PKC antibodies. We did not find activation of pan-PKC, PKCα_I/β_{II} and PKCε isoforms upon MIF stimulation. Interestingly, MIF specifically induced phosphorylation of PKCδ at threonine 505. This effect was found after 1 minute with a maximum response between 30-45 minutes.

3.2.3 The signaling cascade

We wished to determine the downstream and upstream signaling mechanisms; therefore RA synovial fibroblasts were incubated with signaling inhibitors 1 hour prior to MIF stimulation. Phosphorylation of JNK was abrogated by the Src inhibitor PP2, and c-jun phosphorylation was abrogated by JNK and Src inhibitors. These data suggest that Src is upstream of JNK, and phosphorylation of JNK leads to the activation of the nuclear protein c-jun. Activation of PKCδ was inhibited by JNK and Src inhibitors, suggesting that Src and JNK are upstream of PKCδ.

3.3 The role of MIF in *in vivo* MMP-2 production

3.3.1 MMP-2 production in acute inflammatory arthritis

In order to evaluate the *in vivo* role of MIF in MMP-2 production, we induced acute arthritis by intraarticular injection of zymosan in MIF -/- and WT mice. ZIA is a model of acute inflammatory arthritis with early (day 1) and late phases. After 24 hours, mice were euthanized. MMP-2 concentrations of knee homogenates were measured by ELISA. We

found significantly elevated MMP-2 protein levels in knee homogenates of WT mice compared to MIF -/- mice (1.3 ± 0.08 ng/mg protein vs. 0.82 ± 0.08 ng/mg; mean ± SEM, $p<0.05$), pointing to an important role of MMP-2 in arthritis.

3.3.2 MMP-2 expression in AgIA

In addition, we measured MMP-2 expression in the knee joints of mice after induction of AgIA, a different murine model of RA. MMP-2 production of knee homogenates was measured on day 28 after AgIA induction, using gelatin zymography. In concordance with our previous results, zymography showed enhanced MMP-2 expression (both pro- and active forms of MMP-2) in WT mice compared to MIF -/- ones.

3.3.3 Immunohistological analysis of MMP-2 expression in inflamed joints

In order to evaluate the cell-type specific expression of MMP-2 in the arthritic synovium, we performed immunohistochemistry staining on ZIA joints. We found that MMP-2 was mainly expressed by synovial lining cells, sublining non-lymphoid mononuclear cells and by ECs. The immunostaining was quantified as the percentage of cells staining positively for MMP-2. Synovial expression of MMP-2 was enhanced on both lining cells and sublining non-lymphoid mononuclear cells of WT compared to MIF -/- mice (synovial lining cells: 74% ± 7 vs. 38% ± 7, sublining non-lymphoid mononuclear cells: 72% ± 4.9 vs. 22% ± 3.8; mean ± SEM, $p<0.05$). A similar trend was seen in case of ECs, however the difference was not significant (41% ± 13.5 vs. 14.4% ± 4.8; mean ± SEM).

3.4 Effect of EGCG on RA synovial fibroblast MMP-2 production

In order to study the effect of EGCG on MMP-2 production, RA synovial fibroblasts were incubated with EGCG for 12 hours, followed by stimulation with IL-1β (10 ng/ml) for 24 hours. Stimulation of RA synovial fibroblasts with IL-1β resulted in a 2.5-fold increase in

MMP-2 activity ($p<0.05$), measured using gelatin zymography. Treatment of RA synovial fibroblasts with EGCG at concentrations of 10 μM and 20 μM resulted in a 58 ± 14% and 75 ± 16% (mean ± SEM) inhibition of IL-1β-induced MMP-2 activity, respectively ($p<0.05$).

3.5 Effect of chemokines and EGCG on RA synovial fibroblast MMP-2 activity

3.5.1 Effects of chemokines on RA synovial fibroblast MMP-2 expression

RA synovial fibroblasts were stimulated with various chemokines (100 ng/ml) for 24 hours. ENA-78/CXCL5, gro-α/CXCL1, and RANTES/CCL5 increased RA synovial fibroblast MMP-2 activity by 3.9-, 5.4-, and 4.9-fold, respectively, as compared with untreated controls ($p<0.05$). MMP-2 activity induced by these three chemokines was 1.1-, 1.5-, and 1.3-fold higher, respectively, in comparison to IL-1β-stimulated samples.

3.5.2 Effects of EGCG on chemokine-induced RA synovial fibroblast MMP-2 expression

Treatment of RA synovial fibroblasts with EGCG markedly inhibited the ability of chemokines to stimulate MMP-2 activity, in a concentration-dependent manner. EGCG at 10 μM and 20 μM blocked MMP-2 activity induced by ENA-78 by 33% and 75%, by gro-α by 52% and 80%, and by RANTES by 55% and 84%, respectively ($p<0.05$).

4. DISCUSSION

Functional disability of the joints as a consequence of progressive cartilage and bone degradation is a hallmark of RA. Known effector molecules in the destruction of articular cartilage and bone are MMPs and cathepsins. These enzymes have been implicated in several pathological processes such as tumor invasion, angiogenesis, atherosclerosis,

and RA. In RA, angiogenesis is considered as a key event in the expansion of the synovial lining of the joints. Angiogenesis requires proteolysis of the extracellular matrix, proliferation, and migration of ECs, as well as synthesis of new matrix components.

RA synovial fibroblasts at sites of invasion or within the synovial lining layer are a major source of MMPs, and drive RA joint destruction as well as angiogenesis via these enzymes. MMPs have the ability to degrade ECM components including gelatin, collagens, fibronectin, and laminin. Among MMPs, MMP-2 plays a crucial role in the angiogenic process. Although the function of MMP-2 in RA has not yet been fully elucidated, several studies showed an important role of MMP-2 in RA. Increased levels of MMP-2 were detected in sera and synovial fluids of patients with RA, and increased MMP-2 production has been associated with enhanced RA synovial fibroblast invasion. Thus, in the present study, we wished to better understand the cellular and molecular basis of MMP-2 production by RA synovial fibroblasts *in vitro* and *in vivo*. Further, we also searched for a potent inhibitor of RA synovial fibroblast MMP-2 expression.

MIF is highly expressed in RA synovium and regulates the production of proinflammatory cytokines. MIF also induces the release of MMP-1 and MMP-3 by RA synovial fibroblasts. The angiogenic potential of MIF was previously demonstrated both *in vitro* and *in vivo*. MIF induces human dermal microvascular EC migration and tube formation, and induces angiogenesis in Matrigel plugs, as well as in the corneal bioassay *in vivo.* Two groups observed independently that in MIF -/- or in WT mice treated with neutralizing antibody against MIF, the onset of arthritis was delayed and synovial inflammation was decreased, although the specific role of MIF in tissue destruction has not yet been clarified. In the present study, a novel role of MIF, its ability to induce MMP-2 production by RA synovial fibroblasts and MIF-induced signaling events were analyzed.

First, we investigated the effect of MIF on RA synovial fibroblast MMP-2 production. Cell invasion and angiogenesis are crucial processes underlying diseases such as RA and cancer. Previously, positive correlation was reported between MIF and MMP-2 expression in prostate cancer cells. In this study, we showed that MIF induced MMP-2 production by RA synovial fibroblasts, which may lead to joint destruction in RA. Using *in vitro* methods, including gelatin zymography, ELISA, and immunofluorescence staining of RA synovial fibroblasts, we observed that MIF induced MMP-2 production by RA synovial fibroblasts. Stimulation of RA synovial fibroblasts by MIF resulted in a two-fold increase in MMP-2 production. In addition, MIF enhanced the gelatinase activity of RA synovial fibroblast-secreted proteins. Zymography analysis demonstrated the increase of pro-MMP-2 protein level by RA synovial fibroblasts stimulated by MIF.

In order to evaluate the *in vivo* role of MIF in MMP-2 production, we induced acute inflammatory arthritis in MIF -/- and WT mice by zymosan. The synovial inflammation mediated by zymosan is biphasic, with an initial peak at day 1, followed by a continuous decrease and a secondary increase at day 14, as previously described using quantification of joint inflammation *in vivo.* Our results confirmed the important role of MIF in MMP-2 production, as MIF -/- mice expressed less joint MMP-2 than WT mice. This observation may contribute to a less severe arthritis in MIF -/- mice compared to WT mice described previously. In parallel with these results, we measured MMP-2 levels in AgIA, a murine model of RA, using gelatin zymography. We found that both the pro- and active forms of MMP-2 were present in the joints and both forms were upregulated in WT compared to MIF -/- mice. Along with previous monocyte and fibroblast co-culture studies, our results also suggest that activation of MMP-2 produced by RA synovial fibroblasts requires the presence of other cell types, possibly monocytes or neutrophils.

Immunohistochemical analysis of ZIA joints revealed that MMP-2 was mainly expressed by synovial lining cells, non-lymphoid mononuclear cells and ECs in the synovium. In addition, we showed that MMP-2 expression by lining cells and non-lymphoid mononuclear cells was upregulated in WT compared to MIF -/- animals, suggesting an important role of MIF in MMP-2 induction by these cells. Regarding *in vivo* studies, it was previously shown that progressive joint destruction could be prevented by a novel synthetic MMP inhibitor in rat adjuvant induced arthritis and also in collagen-induced arthritis. In contrast, in the antibody-induced arthritis model, synovial inflammation was found to be more severe in MMP-2 -/- mice compared to WT animals.

We also assessed specific signaling pathways mediating MIF-induced MMP-2 production in RA synovial fibroblasts. We found that MIF-induced RA synovial fibroblast MMP-2 production was decreased in the presence of inhibitors of JNK, PKC and Src signaling pathways. Pretreatment of RA synovial fibroblasts with a PKCδ isoform specific inhibitor rottlerin suppressed MIF-induced MMP-2 upregulation. Interestingly, we also found that MIF induced the phosphorylation of JNK, c-jun and PKCδ in RA synovial fibroblasts in a time-dependent manner. We also found that activation of JNK and PKCδ by MIF required the interaction of Src. JNK and Src are upstream activators of PKCδ and phosphorylation of JNK leads to the activation of c-jun.

A number of molecules have been implicated in MIF-mediated signaling. Tyrosine kinases, PKC and NFκB signaling molecules are activated by MIF leading to IL-8 and IL-1β upregulation in RA synovial fibroblasts. MIF-induced MMP-1, MMP-3 and IL-1β mRNA upregulation in RA synovial fibroblasts was inhibited by staurosporine, a broad spectrum inhibitor of protein kinases such as PKC; a tyrosine kinase inhibitor, genistein; a PKC inhibitor, H-7; and a transcription factor AP-1 inhibitor, curcumin. In another study, two

tyrosine kinase inhibitors, genistein and herbimycin A, a selective MAPK kinase inhibitor, and curcumin inhibited MIF-induced MMP-13 mRNA upregulation in rat osteoblasts.

Other regulatory mechanisms, such as transcriptional and post-transcriptional control of MMP-2 mRNA levels by MIF may also be important, and are currently under investigaton. The transcription factors Sp1, Sp3 and AP-2 are functionally important in regulating the expression of the MMP-2 gene. Both Sp1 and AP-2 transcription factors have been implicated in tumor progression, as well as angiogenesis. Among these transcription factors, c-jun interacts with Sp1 and the expression of Sp1 is downregulated by the PKCδ inhibitor rottlerin, suggesting a possible interaction of Sp1 with PKCδ.

MMP-2 is involved in RA pathogenesis by assisting RA synovial fibroblasts in the invasion of microvascular basement membrane and the interstitium. Chemokine-activated RA synovial fibroblasts may mediate this process by their attachment to the cartilage surface and the release of MMPs. Interestingly, recent findings suggest an active involvement of selective chemokines in the destructive phase of RA, in which migration, proliferation, and MMP production by RA synovial fibroblasts are characteristic features. Therefore, in the present study, we wished to further characterize the role of chemokines in RA synovial fibroblast MMP-2 production. We showed that certain chemokines such as ENA-78/CXCL5, gro-α/CXCL1 and RANTES/CCL5 are potent inducers of RA synovial fibroblast MMP-2 production. Treatment of RA synovial fibroblast by any of these three chemokines resulted in a 3.9- to 5.4-fold increase in MMP-2 activity of these cells in comparison to untreated control cells.

Targeting chemokines and/or chemokine receptors appears to be an intriguing new approach to treating chronic inflammatory disorders like RA, inflammatory bowel diseases, multiple sclerosis, and transplant rejection. The involvement of chemokines and

chemokine receptors in inflammatory joint diseases, the *in vitro* and *in vivo* characteristics of the chemokine family in synovitis, and preliminary clinical data on chemokine blockade in patients with RA suggest that targeting the chemokine and chemokines receptor family might provide a new, promising antirheumatic strategy.

Green tea is rich in catechins, and several epidemiological and animal model studies have shown that its consumption is associated with some health benefits including inhibition of inflammation. Most of the beneficial health effects of green tea are mimicked by its most prevalent catechin, EGCG. EGCG influence a number of cellular mechanisms and has been shown to inhibit the activities of MMP-2 and MMP-9 in tumor cells. In addition, EGCG is also an inhibitor of metallo-elastase and serine-elastase activity and down-regulates the levels of several markers of oxidative stress. In our study, ECGC inhibited IL-1β- and chemokine-induced RA synovial fibroblast MMP-2 expression. Two different doses of EGCG inhibited MMP-2 activity induced by three chemokines by 33-84%.

In summary, we demonstrated an important role for MIF in RA synovial fibroblast MMP-2 production, which may contribute to tissue destruction in RA. *In vivo* MMP-2 upregulation by MIF was investigated in ZIA, an acute inflammatory arthritis model and in AgIA, a murine model for RA, using MIF -/- and WT mice. In addition, we described important pathways involved in MIF-dependent upregulation of MMP-2 production and activity. In our study, we showed that JNK, Src and PKCδ, a novel signaling intermediate, mediated MIF-induced RA synovial fibroblast MMP-2 expression. Furthermore, chemokines including ENA-78/CXCL5, gro-α/CXCL1 and RANTES/CCL5 all induced RA synovial fibroblast MMP-2 expression. These effects could be inhibited by EGCG, a constituent of green tea, suggesting that natural products, as well as pharmacological agents may interfere with inflammatory and destructive processes associated with arthritis.

5. JOURNAL PUBLICATIONS

5.1 JOURNAL PUBLICATIONS DIRECTLY RELATED TO THIS THESIS

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