The role of \(\alpha\)-defensin-1 and related signal transduction mechanisms in the production of IL-6, IL-8 and MMPs in rheumatoid fibroblast-like synoviocytes

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Abstract

Objectives. To investigate the effect of \(\alpha\)-defensin-1 on the expression of IL-6, IL-8 and MMPs as well as the signal transduction mechanisms responsible for their expression in RA fibroblast-like synoviocytes (FLS).

Methods. The concentrations of \(\alpha\)-defensin-1 in SF were measured by ELISA. In RA FLS, mRNA expression of IL-6, IL-8 and MMPs and activation of signalling molecules were examined by real-time PCR, western blotting and electrophoretic mobility shift assay.

Results. Concentrations of SF \(\alpha\)-defensin-1 were significantly increased in RA patients compared with OA patients. The levels of mRNA expression of IL-6, IL-8, MMP-1 and MMP-3 were significantly increased in RA FLS treated with \(\alpha\)-defensin-1 compared with controls. Furthermore, \(\alpha\)-defensin-1 activated JNK and ERK in RA FLS, respectively. Treatment of RA FLS with ERK or JNK inhibitors prior to \(\alpha\)-defensin-1 treatment resulted in reduced expression of IL-6, IL-8, MMP-1, and MMP-3 compared with controls. Remarkably, treatment of RA FLS with an ERK inhibitor prior to \(\alpha\)-defensin-1 stimulation significantly reduced production of IL-6 and MMP-1 by approximately 71% and 98% compared with controls, respectively. The JNK inhibitor significantly suppressed \(\alpha\)-defensin-1-induced MMP-1 production by approximately 73% compared with controls. Finally, there was a significant induction of NF-\(\kappa\)B DNA binding activity in response to \(\alpha\)-defensin-1.

Conclusion. Our results suggest that \(\alpha\)-defensin-1 may play a role in RA pathogenesis by regulating the production of MMPs as well as IL-6 and IL-8. These processes were dependent on the regulation of the JNK and/or ERK and NF-\(\kappa\)B pathways.

Key words: RA, fibroblast-like synoviocytes, neutrophil, \(\alpha\)-defensin-1, IL-6, MMP-1.

Introduction

RA is a chronic inflammatory disease characterized by synovial inflammation, hyperplasia of synovial tissues and destruction of bone and cartilage, all of which contribute to joint disability and decreased quality of life. Fibroblast-like synoviocytes (FLS) play an essential role in the pathophysiology of RA [1]. Neutrophils are the most abundant cell type present in SF aspirated from RA patients experiencing flare-up and found at the rheumatoid pannus-cartilage junction [2–4]. In many studies there is evidence demonstrating that neutrophils play a critical role in the pathophysiology of RA [5–7].

Neutrophils secrete a range of potent proteinases and antimicrobial peptides and also have the ability to release bioactive protease into joint cavities [7]. Among the known antimicrobial peptides within neutrophils, defensins are...
small, cationic and cysteine-rich antimicrobial peptides with a molecular mass of 3–5 kDa. Defensins play an important role in the innate immunity response against bacteria, fungi, protozoa and viruses. Mammalian defensins are classified into α-, β- and γ-defensins based on structural difference [8]. Specifically, α-defensins, also known as human neutrophil peptides, are highly concentrated in the azurophilic granules of neutrophils and constitute approximately 50–70% of the overall abundance of azurophilic granule proteins [8, 9]. There are four α-defensins (α-defensins-1–4) in human neutrophils and two human Paneth cell α-defensins, namely α-defensin-5 and α-defensin-6. In particular, α-defensin-1 has the capacity to induce the synthesis of chemokines/cytokines such as IL-8 and IL-1β via lung epithelial cells and monocytes [10–13]. Administration of α-defensins with antigens to mice is associated with enhanced production of both Th1- and Th2-type cytokines by mice splenocytes [14, 15]. Furthermore, α-defensins-1–3 may induce macrophage-dependent release of TNF-α and IFN-γ [8].

In view of the enormous number of neutrophils present in active rheumatoid joints, the prominent presence of α-defensins in the primary granules of neutrophils and the ability of α-defensins to modulate the inflammation and immune response, we specifically looked at the role of α-defensins in the pathogenesis of RA. Together, these data led us to the hypothesis that α-defensin-1 is a potential direct inducer of inflammatory cytokines in rheumatoid joints. We assessed the concentration of α-defensin-1 in the SF of RA and OA patients. Also, to elucidate the exact role of α-defensin-1 in rheumatoid joints, we investigated the effect of α-defensin-1 on the expression of IL-6, IL-8, MMP-1 and MMP-3 and the signal transduction mechanisms responsible for α-defensin-1-induced IL-6, IL-8 and MMP expression in RA FLS.

Materials and methods

Patients and clinical assessment

The study was approved by the institutional review board of the Samsung Medical Center and a signed consent form was obtained from each patient who participated in this study. Patients with RA were diagnosed according to the standards of the ACR [16].

SFs were obtained by arthrocentesis from patients with joint effusion who attended the rheumatology clinic of the Samsung Medical Center. For all patients enrolled in the study, the following demographic and clinical data from medical records were obtained and analysed: sex, age, duration of disease and laboratory data on ESR, CRP and RF, respectively. Determination of bone erosion was based on radiographs of the hand and foot. The presence of bone erosion was defined as the loss or break of cortical linings at the joint.

Enzyme-linked immunosorbent assay

EDTA serum samples were centrifuged at 1500g for 10 min and the supernatants were stored at −20°C until assayed. Levels of α-defensin-1 in plasma were quantified using a commercially available ELISA kit according to the manufacturer’s instructions (R&D Systems, Minneapolis, MN, USA). Plates were read at 450 nm using a VERSAMAX automatic microplate reader (Molecular Devices, Sunnyvale, CA, USA). The concentration of α-defensins in plasma was calculated according to a standard curve.

Isolation and culture of RA FLS

Synovial tissue samples were either processed for cell culture or snap frozen and stored at −80°C. Synovial tissue was obtained from RA patients during joint replacement surgery or arthroscopic synovectomy, as previously described [17]. The cultures comprised a homogeneous population of fibroblastic cells between the third and fourth passages.

RNA extraction and real-time RT-PCR for production of MMPs and cytokines

Synthetic α-defensin-1 (Sigma, St Louis, MO, USA) was used to conduct experiments. Briefly, total RNAs were isolated from cultured FLS using Trizol reagent in a monophase solution following the manufacturer’s recommendations (Invitrogen, Carlsbad, CA, USA). The levels of IL-6, IL-8, MMPs and β-actin mRNA expression were measured by real-time RT-PCR using an ABI PRISM 7000 sequence detector system (Applied Biosystems, Foster City, CA, USA). Real-time RT-PCR amplification was performed using TaqMan Universal PCR Master Mix in combination with a pre-developed assay-on-demand gene expression set for IL-6 ( assay ID HS00174131_m1, GenBank accession number NM_000600, Applied Biosystems), IL-8 ( assay ID Hs00174103_m1, GenBank accession number NM_000584, Applied Biosystems), MMP-1 ( assay ID Hs00233958_m1, GenBank accession number NM_000600, Applied Biosystems), MMP-3 ( assay ID Hs00233962_m1, GenBank accession number NM_002422, Applied Biosystems) and human ACTB (β-actin), the latter of which was used as an endogenous control (VIC/MGB Probe, Applied Biosystems). Quantitation of IL-6, IL-8, MMP-1 and MMP-3 mRNA expression was performed using the method provided by the manufacturer (Applied Biosystems). The expression levels of selected genes in unknown samples were calculated as the ratio of the selected gene mRNA vs the respective level of β-actin. Quantitation of selected gene mRNA and β-actin levels was performed using a standard curve generated from a known serial dilution of rheumatoid FLS. A negative control without template was included in each experiment.

Western blot analysis

Whole cell lysates (WCLs) were prepared using radiomunoprecipitation assay buffer containing 50 mM Tris-HCl (pH 8.0), 150 mM NaCl, 1% NP40, 0.1% SDS and 10 mM sodium deoxycholate. Briefly, 30 μg of proteins from WCLs were separated by electrophoresis on a 10% acrylamide gel using a constant voltage of 80 V for 2 h. The separated proteins were then transferred
developed via exposure to Hyperfilm ECL.

the membrane was overlaid with working substrate solu-

streptavidin with 1

For EMSA analyses, the membrane was first blocked
for 45 min at 120 V and then transferred in 0.5% TBE onto

powder. The membranes were then incubated overnight
at 4

temperature in TTBS [20 mM Tris–HCl, pH 8.0, 500 mM NaCl,
0.05% (v/v) Tween-20] containing 5% skimmed milk
powder. The membranes were then blocked for 1 h with horseradish
peroxidase-conjugated anti-rabbit or anti-mouse IgG anti-
body (1:5000, Jackson Immunoresearch, West Grove,
PA, USA). Proteins were detected using an ECL Western
blot detection system (Amersham Life Science,
Buckinghamshire, UK).

Inhibitor of mitogen-activated protein kinases

Elucidation of the critical pathways responsible for
-α-defensin-1-induced cytokine expression was probed
using either a JNK inhibitor (SP600125, 10 μM) or ERK
inhibitor (U0126, 10 μM). RA FLS were pre-treated with
SP600125 and U0126 for 30 min prior to the addition of
-α-defensin-1, after which real-time PCR was used to
determine the mRNA expression levels of IL-6, IL-8 and
MMPs after -α-defensin-1 stimulation.

Nuclear extracts and electrophoretic mobility shift
assay

Nuclear extracts from treated cells were prepared with a
nuclear extract kit (Panomics Inc., Redwood City, CA,
USA) according to the user manual. Protein concentrations
determined by the Bradford assay (Bio-Rad,
Richmond, CA, USA). Electrophoretic mobility shift
assays (EMSAs) were performed using the EMSA Gel
Shift kit according to the user manual (Panomics Inc.).
Briefly, 5 μg nuclear extracts were incubated with 1×
binding buffer (20 mM HEPES, pH 7.6, 1 mM EDTA,
1 mM ammonium sulphate, 1 mM DTT, 30 mM KCl, 0.2%
TWEEN-20) and 1 μg poly(dI–dC) for 10 min on ice, after
which the following biotin-labelled probes were added:
NF-κB sequence (5’→3’), AGTTGAGGGGACTTTCCC
AGGC; activated protein (AP)-1 sequence (5’→3’), CGCT
TGATGACTCAGCCGGAA. All reactions were incubated at
15 C for 30 min, after which they were electrophoresed on
a 6% polyacrylamide gel in 0.5% Tris–borate–EDTA (TBE)
for 45 min at 120 V and then transferred in 0.5% TBE onto
a nylon membrane at 300 mA for 40 min. After transfer
the sample was fixed on the membrane by UV cross-linking.
For EMSA analyses, the membrane was first blocked
with 1× blocking reagent at room temperature for
15 min. The biotin-labelled probe was then detected with
streptavidin–HRP diluted 1:50. After washing three times,
the membrane was overlaid with working substrate solu-
tion for 5 min. The resulting membranes were promptly
developed via exposure to Hyperfilm ECL.

Statistical analysis

Data are expressed as the mean and S.E.M. and as per-
centages, unless otherwise indicated. To compare treatment
groups in terms of continuous variable distributions,
we utilized Student’s t-test. The Mann–Whitney rank sum
test was used for comparison. Values of P < 0.05 were
considered statistically significant. The Kruskal–Wallis
test was used to determine significant differences in the
case of multiple group comparisons. PASW Statistics 18.0
(Predictive Analytics Software, SPSS Inc., Chicago, IL,
USA) was used for all analyses.

Results

Comparison of synovial fluid -α-defensin-1
concentration between RA and OA patients

The concentrations of -α-defensin-1 in SF from 51 RA and
21 OA patients were measured by ELISA. The baseline
characteristics of patients with RA are summarized in
Table 1. The 21 OA patients included 19 females
(90.5%) with a mean age of 62.9 ± 1.9 years. The control
groups consisted of 21 healthy sex-matched volunteers
and were older than RA patients. In the RA patient
group, 33 patients (64.7%) had erosion in radiographic
imaging and 41 patients (80.4%) had positive RF with a
mean titer (±S.D.) of 161.6 (±24.2) UI/l. At the time of ac-
quisition of SF, the mean value (±S.D.) of ESR and CRP in
RA patients was 70.5 (±4.6) mm/h and 3.1 (±0.4) mg/dl,
respectively. As shown in Fig. 1, the SF -α-defensin-1 con-
centration was significantly increased in RA patients com-
pared with OA patients (39.3 ± 3.5 vs 18.0 ± 5.6 ng/ml,
P = 0.002). Specifically, the RA patients with RF positivity
were associated with significantly higher concentrations
of SF -α-defensin-1 compared with RF-negative patients
(43.1 ± 3.8 vs 20.8 ± 5.0 ng/ml, P = 0.006). The RA patients
with an ESR > 50 mm/h or CRP ≥ 2 mg/dl had a signifi-
cantly higher concentration of SF -α-defensin-1 compared
with patients with a lower level of ESR or CRP, respec-
tively (46.0 ± 3.9 vs 21.7 ± 5.0 ng/ml, P = 0.001; 48.2 ± 4.4 vs
29.2 ± 4.7 ng/ml, P = 0.005). The SF -α-defensin-1 concen-
tration was increased to a greater extent in RA patients
with erosive changes compared with non-erosive patients
(43.1 ± 4.2 vs 32.2 ± 5.8 ng/ml), although this difference did
not reach statistical significance.

Production of IL-6, IL-8 and MMPs by -α-defensin-1
in RA FLS

We investigated the effect of -α-defensin-1 on IL-6, IL-8,
MMP-1 and MMP-3 expression in RA FLS. As shown in
Fig. 2, while there was no significant difference in IL-6,
IL-8 and MMP concentrations at 4 h compared with con-
trols, mRNA expression of IL-6, IL-8, MMP-1 and MMP-3
in FLS from five RA patients was significantly increased
following treatment with -α-defensin-1 after 8 h com-
pared with controls (13.34 ± 22.89-fold, 2.89 ± 1.49-fold,
15.17 ± 7.15-fold and 4.10 ± 1.56-fold increase, respect-
ively; all P < 0.05).
Fig. 1 Comparison of SF α-defensin-1 concentration between RA and OA patients.

(A) SF α-defensin-1 concentrations were significantly increased in 51 RA patients compared with 21 OA patients.

(B) Seropositive RA patients (RF + RA) had significantly higher concentrations of SF α-defensin-1 compared with seronegative RA patients (RF-RA). (C) Further, SF α-defensin-1 levels were more increased in RA patients with erosive change than non-erosive patients, although this difference did not reach statistical significance. Among patients with radiographic erosion, 31 patients (93.9%) were female and the mean age (±S.D.) was 56.3 (±12.1) years. In non-erosive patients, 16 patients (88.9%) were female and the mean age (±S.D.) was 50.4 (±12.7) years. (D) RA patients with CRP > 2 mg/dl had significantly higher concentrations of SF α-defensin-1 compared with patients with lower levels of CRP. In the high CRP group (CRP > 2 mg/dl), 25 patients (92.6%) were female and the mean age (±S.D.) was 54.6 (±12.9) years. *P < 0.01.

Table 1 Baseline characteristics of RA patients

<table>
<thead>
<tr>
<th></th>
<th>RA (n = 51)</th>
<th>Seropositive (n = 41)</th>
<th>Seronegative (n = 10)</th>
<th>P-value</th>
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<tr>
<td>Female, n (%)</td>
<td>47 (92.2)</td>
<td>39 (95.1)</td>
<td>8 (80)</td>
<td>0.168</td>
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<td>Age, mean (s.o.), years</td>
<td>54.3 (1.8)</td>
<td>54.10 (13.79)</td>
<td>54.80 (10.51)</td>
<td>0.881</td>
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<tr>
<td>Disease duration, mean (s.o.), months</td>
<td>81.1 (9.9)</td>
<td>82.6 (70.9)</td>
<td>74.8 (73.1)</td>
<td>0.758</td>
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<tr>
<td>RF titre, mean (s.o.), IU/l</td>
<td>161.6 (24.2)</td>
<td>197.6 (174.4)</td>
<td>NA</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ESR, mean (s.o.), mm/h</td>
<td>70.5 (4.6)</td>
<td>72.9 (30.3)</td>
<td>60.6 (42.9)</td>
<td>0.296</td>
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<tr>
<td>CRP, mean (s.o.), mg/dl</td>
<td>3.1 (0.4)</td>
<td>3.3 (3.2)</td>
<td>2.4 (2.7)</td>
<td>0.431</td>
</tr>
<tr>
<td>Radiograph erosion, n (%)</td>
<td>33 (64.7)</td>
<td>31 (75.6)</td>
<td>2 (20)</td>
<td>0.002</td>
</tr>
<tr>
<td>α-Defensin-1 level, mean (s.o.), ng/ml</td>
<td>39.3 (24.6)</td>
<td>44.0 (24.0)</td>
<td>19.93 (17.4)</td>
<td>0.005</td>
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</table>

Medication, n (%)

<table>
<thead>
<tr>
<th></th>
<th>MTX</th>
<th>HCQ</th>
<th>SSZ</th>
<th>LEF</th>
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<tbody>
<tr>
<td>RA (n = 51)</td>
<td>40 (78.4)</td>
<td>43 (84.3)</td>
<td>23 (45.1)</td>
<td>20 (39.2)</td>
</tr>
<tr>
<td>Seropositive (n = 41)</td>
<td>37 (90.2)</td>
<td>35 (85.4)</td>
<td>17 (41.5)</td>
<td>17 (41.5)</td>
</tr>
<tr>
<td>Seronegative (n = 10)</td>
<td>3 (30.0)</td>
<td>8 (80.0)</td>
<td>3 (30.0)</td>
<td>3 (30.0)</td>
</tr>
</tbody>
</table>

NA: not applicable.
Inhibition of IL-6, IL-8 and MMP expression by treatment with mitogen-activated protein kinase inhibitors in RA FLS

We determined which signalling molecules were involved in the production of IL-6, IL-8 and MMPs by RA FLS treated with α-defensin-1. In our preliminary experiments we observed that α-defensin-1 induced phosphorylation of JNK and ERK in RA FLS, while no significant change was found in p38 activity (data not shown). Thus JNK inhibitor (SP600125) and ERK inhibitor (U0126) were tested in RA FLS to identify the relevant signalling pathway for α-defensin-1-induced cytokines and MMPs. Pre-treatment of SP600125 and U0126 reduced the expression of JNK and ERK protein, respectively, when stimulated with α-defensin-1 (Fig. 3A and B).

As shown in Fig. 3C and D, treatment of RA FLS with ERK or JNK inhibitors prior to α-defensin-1 treatment resulted in reduced production of IL-6, IL-8, MMP-1 and MMP-3 compared with controls. Remarkably, treatment of RA FLS with U0126 reduced the expression of JNK and ERK protein, respectively, when stimulated with α-defensin-1 (Fig. 3A and B).

Levels of IL-6, IL-8, MMP-1 and MMP-3 mRNA were significantly increased at 8 h in RA FLS (n = 5) treated with α-defensin-1 compared with controls. Data are mRNA level normalized to β-actin expression and shown as relative cell equivalents. Each real-time RT-PCR experiment was performed in triplicate. *P < 0.05.

Transcription factors associated with the α-defensin-1 activation pathway in RA FLS

We performed EMSA for NF-κB and AP-1 in order to detect any transcription factors associated with the α-defensin-1 activation pathway in RA FLS. There was a significant induction of NF-κB DNA binding activity in
FIG. 3 Effects of MAP kinase inhibitors on α-defensin-1-induced IL-6, IL-8 and MMP expression in RA FLS.

After treatment with α-defensin-1 and specific MAP kinase inhibitors, protein expression levels were determined by western blot analysis (A, B). Protein expression of JNK and ERK in RA FLS treated with α-defensin-1 were inhibited by JNK and ERK inhibitors. The effects of JNK and ERK inhibitors on α-defensin-1-induced IL-6, IL-8 and MMP expression (continued)
response to the stimulation of α-defensin-1 in RA FLS (Fig. 4A and B). Furthermore, stimulation of RA FLS with α-defensin-1 led to increased phosphorylation of IκB compared with the control group (Fig. 4C). However, α-defensin-1 had no effect on AP-1 binding in RA FLS (Fig. 4D and E). Together these results suggested that NF-κB is a key downstream transcription factor in the α-defensin-1-related signal transduction mechanism in RA FLS.

**Discussion**

Although researchers have been aggressively searching for an infectious aetiology for RA, it has not been possible to directly implicate infection as a causative factor in RA. The role of neutrophils in the pathogenesis of RA has gradually gained momentum. There is considerable evidence that neutrophils may be involved in the pathogenesis of joint inflammation and destruction in RA. Neutrophils express a range of potent proteinases and antimicrobial peptides as well as the ability to release bioactive protease, MMPs and inflammatory cytokines into joint cavities [7, 18]. In RA patients, synovial neutrophils show significantly lower levels of apoptosis compared with patients with resolving disease course [19]. Furthermore, neutrophils from RA SF exhibit activated osteoclastogenesis in co-culture systems [5, 20]. Neutrophils may have a critical role in the induction and progression of joint inflammation in the murine K/BxN mouse model of RA [2].

SF contains antimicrobial peptides that are thought to be derived specifically from neutrophil granules [21]. Among the antimicrobial peptides, α-defensin-1–3 was expressed in the synovial lining and adjacent sublining area and α-defensin-1–3 was detected in the superfusate of synovial tissue of RA [22]. Furthermore, there is a known correlation between joint erosion and α-defensin concentration [21]. In the present study, the SF concentration of α-defensin-1 was significantly increased in the joint fluids obtained from seropositive and more severe inflammatory patients compared with those from seronegative patients and those with a mild degree of synovitis. α-Defensins may modulate inflammatory responses via upregulation of IL-1β, IL-8, TNF-α or VEGF in human epithelial cells or monocytes [10–13, 23]. These results suggest that neutrophil-derived α-defensins have a potential role as an active participant in the regulation of the initiation or perpetuation of RA. By comparison, little is known about the direct response of RA FLS to α-defensins and the exact signalling pathway of the response in RA FLS. Because the activation of RA FLS induces the production of proinflammatory mediators such as IL-6, IL-8 and MMPs that act in an autocrine or paracrine fashion to further stimulate RA FLS as well as other cells [24], one hypothesis deduced from these findings is that neutrophil-derived α-defensins might induce inflammatory cytokines and MMP production in RA FLS, leading to chronic
destructive synovitis in RA. Thus we performed this study to determine whether increased levels of α-defensin-1 in the rheumatoid joint would affect RA FLS to produce IL-6, IL-8 and MMPs in RA FLS. Other studies have shown that α-defensins maximally induced the release of target cytokine (IL-8) in epithelial cell lines at 8 h and the monocyte line at 4 h [10, 13]. We thus assayed the release of IL-6, IL-8 and MMPs at 4 and 8 h, respectively. There were no significant differences in target cytokines at 4 h compared with controls. IL-8 production significantly increased at a concentration from 5 to 25 μg/ml α-defensin-1 at 8 h using human lung fibroblasts and endothelial cells [11, 13, 25, 26]. For these reasons we performed the assay using 15 μg/ml α-defensin-1 at 8 h for subsequent experiments. Interestingly, we demonstrated that α-defensin-1 induces the secretion of IL-6, IL-8, MMP-1 and MMP-3 in RA FLS. IL-6 is a pivotal cytokine that is implicated in many aspects of rheumatoid synovial inflammation, including T and B cell activation and neutrophil recruitment. The production of IL-8 contributes to the perpetuation of rheumatoid synovitis in a similar manner as IL-6 [1]. Intratracheal instillation of α-defensins induces IL-8 production, resulting in the infiltration of neutrophils and the perpetuation of an inflammatory response in mice [27]. From these findings, increased α-defensin-1 in RA joints may stimulate IL-6 and IL-8 production by RA FLS, which in turn may activate and recruit neutrophils to release a significantly larger amount of α-defensins, thus promoting inflammation and chemoattraction of inflammatory cells in a vicious cycle, resulting in persistent synovial inflammation. MMP-1 and MMP-3 have been demonstrated in rheumatoid synovial tissue and/or in pannus tissue invading cartilage, which are mainly synthesized and secreted by RA FLS [1, 24]. A correlation between MMP-1 expression and the degree of synovial inflammation and invasive-ness of RA FLS has been found [28, 29]. Taken together, our findings suggest that α-defensin-1 in the rheumatoid joint may contribute to the persistence of synovial inflammation and local cartilage destruction by regulation of IL-6, IL-8 and MMP production by RA FLS.

The signalling pathway of this production by α-defensin-1 is not well understood in any cell type, including RA FLS. The three major mitogen-activated protein (MAP) kinases—ERK, JNK and p38 kinase—as well as downstream transcription factors NF-κB and AP-1 were synovial tissue and cultured FLS. Signal transduction pathways involving MAP kinases and NF-κB are well known to play an important role in RA [30]. It was reported that α-defensin stimulation activated ERK, JNK and p38 in the lung epithelial cells and monocytes [13]. We thus investigated the role of these signalling mechanisms in α-defensin-mediated IL-6, IL-8 and MMP expression. Our study found that α-defensin-1-induced IL-6, IL-8, MMP-1 and MMP-3 production is regulated by JNK and ERK signalling pathways. In particular, the current results showed that ERK had an important role in the regulation of IL-6 and MMP-1 production and JNK was closely associated with MMP-1 production in RA FLS stimulated with α-defensin-1. On the other hand, α-defensin-1-induced IL-8 and MMP-3 expression appears to have salvage pathways other than the MAP kinase pathway. There is evidence that ERK, JNK or p38 enhances the activity of AP-1 or NF-κB, which control the regulation of proinflammatory cytokines and MMP genes in RA FLS [31, 32]. We thus performed EMSA for NF-κB and AP-1 to find the transcription factors associated with α-defensin-1 in RA FLS. Nuclear translocation of NF-κB and the degradation of IκB occurred in cultured FLS after α-defensin-1 stimulation, suggesting that NF-κB is a key downstream transcription factor in the α-defensin-1 activation pathway in RA FLS. In the present study, we demonstrated for the first time the signalling pathway of IL-6, IL-8 and MMP production by α-defensin-1 in RA FLS. Further studies on the role of defensins in transcription factor activation are needed to better understand the role of α-defensins in RA.

Herein we performed our experiments using commercially available synthetic α-defensin-1. Previously it was reported that the potency of synthetic α-defensin-1 to IL-6 production was somewhat lower compared with that of the combined purified α-defensins mixture [10]. This may be explained by the fact that some of these results did not produce a significant difference.

In conclusion, this is the first study to evaluate the role of α-defensin-1 on IL-6, IL-8 and MMP expression as well as the first to attempt to elucidate the signal mechanisms to participate in this process in RA FLS. The novel finding of our study was that increased α-defensin-1 in SF from RA induces the expression of IL-6 and MMP-1 and these processes were dependent on the regulation of JNK or ERK and the NF-κB pathway in RA FLS. These results suggest that neutrophil-derived α-defensin-1 has the potential to modulate inflammatory, degradative and invasive behaviour in RA FLS, resulting in facilitation of synovitis and bone destruction through regulation of IL-6, IL-8 and MMP production. The current study provides new insight regarding the mechanism by which α-defensin-1 participates in chronic destructive synovitis and provides a new rationale for considering α-defensin-1 as a therapeutic target aimed at reducing both inflammation and destruction in RA.

**Rheumatology key messages**

- Neutrophil-derived α-defensins may play a role in the expression of MMPs, IL-6 and IL-8 in FLS.
- Understanding the role of α-defensins could provide new insights into the pathogenesis of RA.

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References


