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A Critical Role for Allograft Inflammatory Factor-1 in the Pathogenesis of Rheumatoid Arthritis

Mizuho Kimura,* Yutaka Kawahito,† Hiroshi Obayashi,‡ Mitsuhiro Ohta,§ Hirokazu Hara,¶ Tetsuo Adachi,§ Daisaku Tokunaga,¶ Tatsuya Hojo,¶ Masahide Hamaguchi,* Atsushi Omoto,* Hidetaka Ishino,* Makoto Wada,* Masatake Kohno,* Yasunori Tsubouchi,* and Toshikazu Yoshikawa*

Rheumatoid arthritis (RA) is characterized by massive synovial proliferation, angiogenesis, subintimal infiltration of inflammatory cells and the production of cytokines such as TNF-α and IL-6. Allograft inflammatory factor-1 (AIF-1) has been identified in chronic rejection of rat cardiac allografts as well as tissue inflammation in various autoimmune diseases. AIF-1 is thought to play an important role in chronic immune inflammatory processes, especially those involving macrophages. In the current work, we examined the expression of AIF-1 in synovial tissues and measured AIF-1 in synovial fluid (SF) derived from patients with either RA or osteoarthritis (OA). We also examined the proliferation of synovial cells and induction of IL-6 following AIF-1 stimulation. Immunohistochemical staining showed that AIF-1 was strongly expressed in infiltrating mononuclear cells and synovial fibroblasts in RA compared with OA. Western blot analysis and semiquantitative RT-PCR analysis demonstrated that synovial expression of AIF-1 in RA was significantly greater than the expression in OA. AIF-1 induced the proliferation of cultured synovial cells in a dose-dependent manner and increased the IL-6 production of synovial fibroblasts and PBMC. The levels of AIF-1 protein were higher in synovial fluid from patients with RA compared with patients with OA (p < 0.05). Furthermore, the concentration of AIF-1 significantly correlated with the IL-6 concentration (r = 0.618, p < 0.01). These findings suggest that AIF-1 is closely associated with the pathogenesis of RA and is a novel member of the cytokine network involved in the immunological processes underlying RA. The Journal of Immunology, 2007, 178: 3316–3322.

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1 Address correspondence and reprint requests to Dr. Yutaka Kawahito, Inflammation and Immunology, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, 465 Kajii-cho, Kamigyo-ku, Kyoto, Japan. E-mail address: kawahity@koto.kpu-m.ac.jp

2 Abbreviations used in this paper: RA, rheumatoid arthritis; AIF-1, allograft inflammatory factor-1; OA, osteoarthritis; SF, synovial fluid.

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both the glandular and stromal cells of endometrial tissues in endometriosis (17). A recent study also indicates that AIF-1 enhances the activation of lymphocytes (18). Therefore, AIF-1 is thought to play a fundamental role in several cell types involved in chronic immunological inflammatory processes but especially macrophages.

In the present study, we examined the potential role of AIF-1 in RA. We determined and compared AIF-1 expression in synovial tissues derived from patients with either RA or osteoarthritis (OA). We also examined the effect of AIF-1 on the proliferation of RA synoviocytes, and cytokines production by human synoviocytes and peripheral blood monocytes; both critical factors in the pathogenesis and progression of RA. Furthermore, we determined the concentration of AIF-1 and IL-6 in synovial fluid (SF).

Materials and Methods

Synovial tissues and preparation of synovial cells

This study protocol was approved by the Kyoto Prefectural University of Medicine institutional review board, and informed consent was obtained from each patient. Synovial tissues were obtained during joint replacement surgery from 10 patients with RA (3 males/7 females; mean age ± SE, 57.5 ± 3.9 years) and 10 patients with OA (4 males/6 females; mean age ± SE, 58.6 ± 4.0 years), according to the criteria of the American College of Rheumatology.

Synovial tissues were minced and stirred with 1 mg/ml collagenase (Sigma-Aldrich) in serum-free RPMI 1640 medium (Nissui Pharmaceutical) for 3 h, filtered through a nylon mesh and washed extensively. Cells were cultured in RPMI 1640 medium supplemented with 10% FCS and 1% antibiotics in a humidified incubator at 37°C in the presence of 5% CO₂. The synovial cells used in experiments were from passage 3–6 at which end point, rAIF-1 was treated with detoxi-gel endotoxin removing gel (Dojindo Chemical).

rAIF-1-coupled cyanogen bromide-activated Sepharose affinity column

rAIF-1 Ab IgG fraction was prepared by chromatography on a human peptide Ab by injecting 50 mg of human rAIF-1 into a rabbit. The human peptide was then emulsified with Freund's complete adjuvant (Difco Laboratories) and imidocaproyloxy)succinimide (Sigma-Aldrich). The carrier-conjugated fusion protein, the DNA fragment obtained was inserted in the Bam HI/Bam HI/Xho I sites of pGEX-4 (Amersham Biosciences) in frame. The fusion protein was purified by a GST fusion system (Amersham Biosciences) and affinity chromatography with anti-AIF-1 1/113–129 Ab.

To investigate the effect of AIF-1 on the cell proliferation and cytokines induction, rAIF-1 was treated with detoxi-gel endotoxin removing gel (Pierce). Endotoxin detection was performed using Limulus amoebocyte lysate analysis (Wako Pure Chemical), and treated AIF protein was confirmed to contain <0.1 ng/μg of endotoxin.

Anti-rAIF-1 antisera was raised in a rabbit using 50 mg of human rAIF-1 in a rabbit. The human rAIF-1 Ab IgG fraction was prepared by chromatography on a human rAIF-1-coupled cyanogen bromide-activated Sepharose affinity column and biotinylated with 5-(N-succinimidylidoxycarbonyl)pentyl ω-biotinamide (Dojindo Chemical).

Peptide synthesis and preparation of anti-human AIF-1 53–71 and AIF-1 113–129 Abs

Two synthetic peptides, which corresponded to residues 53–71 and 113–129 of human AIF-1 (AIF-1 53–71 and AIF-1 113–129, respectively) as deduced from the nucleotide sequence of the human AIF-1 gene, were obtained with an additional cysteine residue at the N terminus (Biologica).

Peptide synthesis and preparation of anti-human AIF-1 53–71 and AIF-1 113–129 Abs

Human AIF-1 cDNA was amplified from human peripheral lymphocyte PBMC that is identical to the molecular size of purified recombinant human AIF-1 (AIF-1 53–71 and AIF-1 113–129, respectively) as described previously (19). A 3+ grade implies unusually intense staining, whereas 0 implies no staining.

Western blot analysis

Total RNA was extracted from synovial tissues and SF cells using TRIzol (Invitrogen Life Technologies). The first cDNA synthesis from 1 μg of total RNA was catalyzed by the SuperScript First-Strand Synthesis System for RT-PCR using oligo(dT) 20 mers (BD Clontech). The PCR mixture comprised 1 μl of first strand cDNA, 0.8 μM of each of the primers indicated above and 1.25 U of Taq polymerase (Takara Premix Ex Taq; Takara Biochemicals) in a total volume of 50 μl of PCR buffer provided by the manufacturer. After an initial denaturation at 94°C for 5 min, PCR was conducted at 94°C for 45 s, at 58°C (for AIF-1) or 50°C (for G3PDH) for 1 min and at 72°C for 1 min, followed by a final extension step at 72°C for 6 min. The PCR products were electrophoresed in 2% agarose gel and stained with ethidium bromide. The intensity of each band was measured with an Image Master version 2.01 (Amersham Biosciences). Images were obtained and quantified using a Bio-Rad VersaDoc imaging system (model 5000) with Bio-Rad Quantity One software. The results were expressed as a ratio (protein of interest/β-actin) to correct for sample loading.

Immunohistochemical analysis of AIF-1 expression in synovial tissues

Immunohistochemical staining was performed using the avidin-biotin peroxidase complex system. Synovial tissue specimens were preserved in 10% formalin and embedded in paraffin.Specimens were serially sectioned onto microscope slides at a thickness of 4 μm and then deparaffinized. Tissue sections were immersed in 0.3% peroxide in 50% methanol solution for 5 min to inactivate endogenous peroxidase activity. Non-specific binding sites were saturated by exposure to 0.2% BSA and normal goat serum diluted 1/66.7 in PBS (pH 7.4) for 20 min. Primary Abs against human AIF-1 113–129 (1/800 dilution in PBS) and control normal rabbit serum were applied to tissue sections and incubated overnight at 4°C. The slides were then washed with PBS for 10 min. Biotinylated goat anti-rabbit IgG (Vector Laboratories) in 10% PBS was applied to the sections, and the slides were incubated at room temperature for 45 min. They were then washed with PBS for 10 min, followed by incubation with pre pared avidin DH-biotinylated peroxidase complex (Vector Laboratories) for 45 min. After washing with PBS for 10 min, color was developed by immersing the sections in peroxidase substrate solution containing 0.05% (w/v) 3,3'-diaminobenzidine tetrahydrochloride (Vector Laboratories) and 0.01% H₂O₂ in 0.05 mol/l Tris-buffered saline (pH 7.2) for 5 min. Positive staining was indicated by brownish black deposits, and counterstaining was performed with hematoxylin.

For each of the tissue specimens from RA and OA, the extent and intensity of staining with anti-AIF-1 113–129 Ab in synovial lining cells, macrophages, and fibroblasts were graded on a scale of 0 to 3+ by two blinded observers on two separate occasions using coded slides as previously described (19). A 3+ grade implies unusually intense staining, whereas 0 implies no staining.

RNA preparation and semiquantitative RT-PCR

Total RNA was extracted from synovial tissues and SF cells using TRIzol (Invitrogen Life Technologies). The first cDNA synthesis from 1 μg of total RNA was catalyzed by the SuperScript First-Strand Synthesis System for RT-PCR using oligo(dT) 20 mers (BD Clontech) in a total volume of 50 μl of PCR buffer provided by the manufacturer. After an initial denaturation at 94°C for 5 min, PCR was conducted at 94°C for 45 s, at 58°C (for AIF-1) or 50°C (for G3PDH) for 1 min and at 72°C for 1 min, followed by a final extension step at 72°C for 6 min. The PCR products were electrophoresed in 2% agarose gel and stained with ethidium bromide. The intensity of each band was measured with an Image Master version 2.01 (Amersham Biosciences). We calculated the intensity of the AIF-1 transcript at 26 cycles relative to that of G3PDH at 22 cycles to compare initial mRNA levels.

Cell growth assay

To evaluate the effect of AIF-1 on cell growth, RA synoviocytes were seeded on a 96-well cell culture plate (Nunc) at a concentration of 5 × 10⁴ cells/well in a volume of 100 μl. Twenty-four hours later, each well was treated with 1, 10, and 100 ng/ml recombinant AIF-1 and 10 ng/ml LPS (Escherichia coli LPS; Sigma-Aldrich) in RPMI 1640 medium containing 1% FCS and incubated at 37°C in 5% CO₂. Where indicated, cells were preincubated for 1 h with 5 mg/ml anti-AIF-1 113–129, before incubation with AIF-1 (100 ng/ml). Cell viability was measured at 96 h colorimetrically.
using the cell counting kit (Dojindo) and a microplate reader (model 550; Bio-Rad) at the test wavelength of 450 nm and the reference wavelength at 650 nm (21). This assay is based on the cleavage of the 2-(4-iodomophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium monosodium salt (WST-1) by mitochondrial dehydrogenase in viable cells. Each measurement was determined in six separate experiments, and the results are presented as a percentage of the value for the control cultures.

**Induction of cytokine production by cultured human synoviocytes by rAIF-1 stimulation**

For the assay of cytokines, RA synoviocytes were cultured in RPMI 1640 containing 10% FCS at 37°C in 5% CO2 in 6-well cell culture plates (Nunc). After reaching confluence, the cells were also incubated with or without 1–100 ng/ml rAIF-1 in a total volume of 500 μl of serum-free RPMI 1640 medium for 48 h. Where indicated, cells were preincubated for 1 h with 5 mg/ml anti-AIF-1113–129 before incubation with rAIF-1 (100 ng/ml). Each culture was performed in triplicate, and supernatants were separated by centrifugation and stored at −80°C until assay. IL-6 concentrations were measured using a commercial ELISA kit according to the manufacturer’s instructions. ELISA kits used were purchased from Biosource International for IL-6, and R&D Systems for IL-1β and TNF-α.

**Induction of IL-6 production by cultured human PBMCs by rAIF-1**

Human PBMCs were isolated from heparinized whole blood from normal healthy volunteers (n = 5) by Ficoll-Paque (Amersham Biosciences) density gradient centrifugation. The cells were washed three times with sterile PBS (pH 7.4) and resuspended in RPMI 1640 medium supplemented with 10% FCS and 1% antibiotics. The cells were seeded in 12-well flat-bottom plates at a density of 5 × 105 cells/well in a total volume of 500 μl of serum-free RPMI 1640 medium (Nissui Pharmaceutical). Cells were incubated with or without 1, 10, or 100 ng/ml rAIF-1 or 10 ng/ml LPS (Sigma-Aldrich). Where indicated, cells were preincubated for 1 h with 5 mg/ml anti-AIF-1113–129 before incubation with rAIF-1 (100 ng/ml). After incubation at 37°C in a humidified atmosphere of 5% CO2/95% air for 24 h, the culture supernatants were recovered and stored at −80°C until assay. IL-6 concentrations were measured using a commercial ELISA kit (Biosource International) according to the manufacturer’s instructions.

**Induction of AIF-1 production by cultured human synoviocytes by IL-6**

To investigate the effect of IL-6 on AIF-1 production, RA synoviocytes were cultured in RPMI 1640 containing 10% FCS at 37°C in 5% CO2 in 6-well cell culture plates (Nunc). After reaching confluence, the cells were also incubated with or without 0.1–100.0 ng/ml rIL-6 (R&D Systems) in a total volume of 500 μl of serum-free RPMI 1640 medium for 2000 rpm for 10 min) and concentrations of IL-6, IL-1β, and TNF-α were measured using the ELISA kits according to the manufacturer’s instructions. ELISA kits used were purchased from Biosource International for IL-6, and R&D Systems for IL-1β and TNF-α.

**Measurement of the concentrations of AIF-1 and IL-6 in SF**

SF samples were obtained from 12 patients with RA (5 males/7 females; mean age ± SE, 57.1 ± 4.1 years) and 8 patients with OA (3 males/5 females; mean age ± SE, 58.2 ± 4.8 years), according to the criteria of the American College of Rheumatology. Serum C-reactive protein (CRP) in the RA and OA patients was 1.3 ± 0.37 mg/dl (mean ± SE) and 0.53 ± 0.25 mg/dl (mean ± SE), respectively. Samples were centrifuged at 3000 rpm for 10 min and then treated with 1000 U/ml hyaluronidase (Sigma-Aldrich) for 60 min at 37°C.

The AIF-1 concentrations in SF were measured by ELISA as previously described (17) with a slight modification. In brief, microtiter plates were coated with affinity-purified anti-AIF-1113–129 IgG (2.0 mg/ml) diluted with 10 mM carbonate buffer (pH 9.3) for 2 h at room temperature. After washing, nonspecific binding sites in each well were blocked with 10 mM carbonate buffer containing 0.5% BSA. Standard solution (0–2500 pg/ml human rAIF-1) and SF samples treated with hyaluronidase diluted (1/2) with sample buffer (50 mM Tris-HCl buffer, pH 7.0, containing 200 mM NaCl, 10 mM CaCl2, 0.1% Triton X-100, and 1% BSA) were added to the wells, and the plate was incubated for 1 h at room temperature. After washing with BSA-free sample buffer, biotinylated anti-rAIF-1 IgG was added to each well. The plate was incubated for 1 h at room temperature, washed, and then incubated for an additional hour at room temperature with streptavidin-HRP diluted 1/10,000. After final washing, the plate was treated for 20 min with a substrate solution of 3,3’,5,5’-tetramethylbenzidine and H2O2 was added to each well and allowed to react for 20 min at room temperature. The reaction was stopped by the addition of 1 M phosphoric acid, after which OD values at 450 nm were read with an ELISA plate reader. The detection limit of the assay was 20 pg/ml and intraassay and interassay coefficients of variation were 6.2–9.2% at 115 pg/ml and 4.6–7.2% at 600 pg/ml. The IL-6 concentrations were measured using a commercial ELISA kit (Biosource International) according to the manufacturer’s instructions.

**Statistical analysis**

Differences in mRNA and protein levels of AIF-1, the graded scores of the extent and intensity of immunostaining with anti-AIF-1 and SF concentrations of AIF-1 and IL-6 between the two patient groups were analyzed by the Mann-Whitney U test. Difference in induction of IL-6 by AIF-1 was analyzed by the Kruskal-Wallis test followed by Dunn’s multiple comparison test. The results of cell proliferation studies were analyzed by two-way ANOVA followed by Scheffe’s multiple comparison test. Pearson’s correlation coefficient was used to examine the correlation between the concentrations of AIF and IL-6 in SF.

**Results**

**AIF-1 expression in synovial tissues**

Immunohistochemistry was performed to examine the expression and localization of AIF-1 in synovial tissues derived from patients with either RA or OA. Immunohistochemical staining showed strong expression of AIF-1 by infiltrating mononuclear cells and synovial fibroblasts in RA synovial tissue (Fig. 1, A–C) compared with tissue derived from joints affected by OA (Fig. 1, D). Immunostaining of tissue sections with normal rabbit serum was completely negative in all synovial tissue derived from patients with RA (Fig. 1E).

We found markedly enhanced expression of AIF-1 in infiltrating mononuclear cells (score; mean ± SE, 2.43 ± 0.12) and fibroblasts (2.33 ± 0.15) in RA synovial tissue (Fig. 2). In contrast, in OA tissues the immunohistochemical score of AIF-1 in infiltrating mononuclear cells (0.58 ± 0.12) and fibroblasts (0.55 ± 0.12) was lower.
significantly lower compared with patients with RA (both \( p < 0.001 \), Mann-Whitney \( U \) test) (Fig. 2).

As shown in Fig. 3A, AIF-1 protein was detected at 17 kDa as a single band in protein extracts from SF cells and synovial tissues of both patients with RA and OA. The intensity of the AIF-1 protein band was significantly elevated in both the SF cells (1.8-fold, \( p < 0.01 \)) and the synovial tissue from patients with RA (1.7-fold, \( p < 0.05 \)) compared with the levels in patients with OA.

RT-PCR analysis indicated AIF-1 mRNA expression in both SF cells and synovial tissues from all 13 patients with RA or OA (Fig. 4A). The levels of AIF-1 mRNA expression were significantly elevated in the SF cells (1.8-fold, \( p < 0.01 \)) and the synovial tissues (1.9-fold, \( p < 0.01 \)) from patients with RA compared with the expression levels in patients with OA.

**Effect of human rAIF-1 upon the proliferation of synoviocytes**

We examined the effect of AIF-1 upon the cell proliferation of synoviocytes isolated from RA patients. Fig. 5 illustrates the effect of several doses AIF-1 upon cell proliferation of synoviocytes. AIF-1 significantly increased cell growth in a dose-dependent manner. Incubation of synoviocytes with 100 ng/ml AIF-1 for 96 h resulted in a significant increase in cell growth compared with controls (0 ng/ml AIF-1; \( p < 0.0001 \)) or 1 ng/ml AIF (\( p < 0.001 \)). The AIF-induced cell proliferation was significantly inhibited by anti-AIF-1\(_{113-129}\) Ab (5 mg/ml).

**FIGURE 2.** Histopathological graded scores of the extent and intensity of immunostaining with anti-AIF-1 Ab in mononuclear cells (A) and fibroblasts (B) of patients with RA (n = 10) or patients with OA (n = 10). The box plot includes the median horizontal line and interquartile range (box), whereas the whiskers represent the 10–90th percentiles. The differences between two groups were analyzed by the Mann-Whitney \( U \) test. *\(, p < 0.001\).

**FIGURE 3.** Western blot analysis of AIF-1 protein extracts from SF cells and tissues of patients with RA (n = 8) or patients with OA (n = 5). A, lane 1, SF cells of RA; lane 2, SF cells of OA; lane 3, synovial tissue of RA; and lane 4, synovial tissue of OA. B, Comparison of the levels of AIF-1 protein expression by Western blot image analysis. Data are expressed as the mean ± SE. The differences between two groups were analyzed by the Mann-Whitney \( U \) test. *\(, p < 0.05\), **\(, p < 0.01\).

**FIGURE 4.** Semiquantitative RT-PCR analysis of AIF-1 mRNA in SF cells and tissues of patients with RA (n = 8) or patients with OA (n = 5). A, lane 1, SF cells of RA; lane 2, SF cells of OA; lane 3, synovial tissue of RA; and lane 4, synovial tissue of OA. B, Comparison of the levels of AIF-1 mRNA expression by image analysis. Data are expressed as the mean ± SE. The differences between two groups were analyzed by the Mann-Whitney \( U \) test. *\(, p < 0.05\), **\(, p < 0.01\).

**FIGURE 5.** Effect of AIF-1 on the cell growth of synoviocytes. The cells were treated with 0, 1, 10, or 100 ng/ml human rAIF-1 or 10 ng/ml LPS. Where indicated, cells were preincubated for 1 h with 5 mg/ml anti-AIF-1\(_{113-129}\) before incubation with rAIF-1 (100 ng/ml). Cell numbers were determined by WST-1 assay at 96 h. Each bar represents the mean ± SE of six experiments. The differences were evaluated by the one-way ANOVA followed by Scheffe’s multiple comparison test. *\(, p < 0.001\), **\(, p < 0.0001\).
Cytokines secretion from cultured synoviocytes following AIF-1 stimulation

As shown in Fig. 6A, the IL-6 concentration in the culture supernatant significantly increased after stimulation for 48 h by human rAIF-1 in a dose-dependent manner \( (p < 0.05, p < 0.01, p < 0.0001) \). The AIF-induced IL-6 secretion was completely inhibited by anti-AIF\textsubscript{1113–129} Ab (5 mg/ml). In contrast, human rAIF-1 had no effect on the secretion of both IL-1\( \beta \) (Fig. 6B) and TNF-\( \alpha \) (Fig. 6C).

IL-6 secretion from cultured PBMCs following AIF-1 stimulation

As shown in Fig. 7, the IL-6 concentration in the culture supernatant markedly increased after stimulation for 24 h by human rAIF-1 in a dose-dependent manner \( (p < 0.001, p < 0.0001) \). The AIF-induced IL-6 secretion was completely inhibited by anti-AIF\textsubscript{1113–129} Ab (5 mg/ml).

AIF-1 secretion from cultured synoviocytes following IL-6 stimulation

Next, we examined the effect of IL-6 on AIF-1 production from synoviocytes isolated from RA patients. As shown Fig. 8, AIF-1 secretion was not stimulated by the addition of human rIL-6, although slightly increase of AIF-1 secretion was seen by the addition of high-dose IL-6 (100 ng/ml).

Concentrations of AIF-1 and IL-6 in SF

The AIF concentration in SF was significantly elevated in patients with RA (mean \(+\) SE; 237.8 \(+\) 17.5 pg/ml) compared with patients with OA (162.6 \(+\) 17.6 pg/ml, \( p < 0.05 \)) (Fig. 9A). Similarly, the IL-6 concentration in SF was significantly elevated in
patients with RA (mean ± SEM; 14.05 ± 1.04 ng/ml) compared with patients with OA (7.80 ± 0.62 ng/ml, p < 0.001) (Fig. 9B). There was a positive correlation between the SF levels of AIF-1 and IL-6 (r = 0.618, p < 0.01) (Fig. 9C).

### Discussion

In this study, we have demonstrated for the first time that AIF-1 was more strongly expressed in superficial synovial cells, fibroblasts and infiltrating inflammatory mononuclear cells in the synovial tissue of RA patients compared with the synovial tissue of OA patients. The immunohistochemical findings were confirmed by semiquantitative Western blot and RT-PCR studies. Additional noteworthy findings of this study are the demonstration that AIF-1 increased the proliferation of synovial cells and enhanced IL-6 secretion from human RA synoviocytes and PBMCs in a dose-dependent manner.

Synovial cells proliferate like tumors and form a pannus within joints affected by RA. The pannus directly invades the cartilage and bone and activates osteoclasts which produce various proteases leading to severe cartilage and bone destruction. The proliferation of synovial cells is one of the most critical pathological changes in RA. Therefore, our findings suggest that the increased AIF-1 expression may play an important role in the development and progression of RA.

The exact mechanism(s) whereby increased AIF-1 expression influences synovial cell proliferation is unknown. A potential mechanism underlying the cell proliferation induced by AIF (22–27) may involve the chemical structure containing an EF-hand calcium-binding sequence. Calcium, the primary receptor protein calmodulin and calcium-dependent protein kinases are essential for entry into the cell cycle in response to mitogenic signals (28–31). The overexpression of calmodulin in mouse C127 cells enhances cell proliferation, predominantly through a reduction in the length of the G1 phase of cell cycle (32, 33). The overexpression of the calcium-binding protein sphingosine kinase in 3T3 fibroblasts enhances cell proliferation by promoting the G1 to S phase transition (34). Moreover, the transfection of rat vascular smooth muscle cells with AIF-1 that is able to bind calcium, increased cell proliferation with constitutive AIF-1 expression resulting in a shortening of the cell cycle and aberrant expression of cell cycle proteins (35). These findings suggest that AIF-1 may function as a cell cycle regulator affecting proliferation in several cell types. Thus, it is possible that AIF-1 promotes synovial cell proliferation by shortening the cell cycle of synovial cells in patients with RA.

Various cytokines are involved in the pathogenesis of RA, and TNF-α and IL-1 play central roles (36). TNF-α and IL-1 are key cytokines in inflammatory cytokine cascades that are directly involved in vascularization, outgrowth of cells of the synovial surface layer, inflammatory cell infiltration and destruction of bone and cartilage through activation of MAP kinase and NF-κB. TNF-α is believed to be upstream of IL-1 in the cytokine cascade of RA and is considered to be the most important cytokine underlying disease progression (36). IL-6 is believed to act downstream of TNF-α and IL-1 and is induced by these cytokines. IL-6 has various physiologic activities but also plays a critical role in the pathogenesis of many inflammatory diseases. IL-6 has been reported to activate B cells, T cells and osteoclasts in RA (37), suggesting its involvement in the pathology of RA. Recently, the effectiveness of biological drugs that target various cytokines has been emphasized. For example, infliximab is a mAb to TNF-α and has inhibitory effects on joint destruction (4, 38–41). Also, the anti-IL-6 receptor mAb tocilizumab has been reported to exert anti-proliferative effects on the synovial membrane, reduce inflammatory mononuclear cell infiltration, and inhibit the differentiation of osteoclasts in RA (5, 42–44). In vitro studies have demonstrated increased AIF-1 expression by macrophage cell lines in response to IFN-γ, whereas AIF-1 gene transfected mouse macrophage cell lines exhibit increased production of IL-6, IL-10, and IL-12 p40 following LPS stimulation (45). In this study, we have demonstrated increased IL-6 production by human PBMCs cells following AIF-1 stimulation. In contrast, there was no effect upon IL-1β expression by AIF-1 production (data not shown). Thus, AIF-1 may play a critical role in the chronic inflammation of RA synovial tissue by decreasing IL-6 production via IL-1β and TNF-α-independent pathways.

In this study, we also found for the first time that the level of AIF-1 in SF derived from RA patients is significantly higher than that in the SF of OA patients. Interestingly, SF AIF-1 levels significantly correlated with SF IL-6 levels. OA is a mild inflammatory disease with cartilage degradation. As noted above in the last paragraph, anti-IL-6 receptor mAb is very effective for RA and regulates various pathological mechanisms of RA. Furthermore, AIF-1 is more strongly expressed in synovial tissues in RA compared with OA. These things also suggest that synovial AIF-1 secreted from synovial cells and infiltrating inflammatory mononuclear cells in affected joints result in increased local IL-6 production in an autocrine manner and this may enhance the local immune inflammatory processes. IL-6 expression is activated by the binding of two transcription factors, NF-κB and NF-IL-6, to the regulatory region of the IL-6 gene (46). AIF-1 may potentially activate these transcription factors directly or through other intracellular signals. These findings indicate that AIF-1 is a novel member of the cytokine network involved in chronic synovial inflammation.

In conclusion, we have demonstrated that AIF-1 is expressed in synovial cells and mononuclear cells in RA synovial tissue and increases proliferation of cultured synoviocytes and production of IL-6 by these synoviocytes and PBMCs. AIF-1 plays an important
role in the pathogenesis of RA by affecting key processes such as the activation of synovial cell proliferation and the inflammatory cytokine cascade including IL-6 in joints. Recently, RA patients have achieved remission by anti-cytokine therapy, and the prognosis has improved dramatically. Although further work is needed to clarify the molecular mechanism of action of AIF-1 in RA, we suggest that AIF-1 may represent a new molecular target in RA therapy.

Disclosures

The authors have no financial conflict of interest.

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