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Expression of allograft inflammatory factor-1 in peripheral blood monocytes and synovial membranes in patients with rheumatoid arthritis

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ABSTRACT

Objective: Allograft inflammatory factor-1 (AIF-1) is a cytoplasmic protein expressed in various human cells such as monocyte/macrophages and activated T lymphocytes. A recent study showed that AIF-1 is strongly expressed in infiltrating mononuclear cells and synovial fibroblasts in rheumatoid arthritis and that AIF-1 induces the proliferation of cultured synovial cells. In this study we analysed the expression of AIF-1 in peripheral blood monocytes and synovial membranes from patients with rheumatoid arthritis (RA).
Methods: We examined 71 patients with rheumatoid arthritis and 25 control subjects.
Results: Using flow cytometry we found significantly increased numbers of circulating AIF-1$^+$ monocytes in peripheral blood from RA patients compared with controls. Moreover, there were statistically significant positive correlations between AIF-1$^+$ monocytes, DAS28 and the Sharp erosion score.
Immunofluorescence staining showed strong expression of AIF-1 by infiltrating mononuclear cells – predominantly macrophages in RA synovial tissues – compared with tissues derived from joints affected by osteoarthritis.
Conclusion: The results of this study suggest that AIF-1 may be associated with the pathogenesis of RA and may be a novel cytokine involved in the immunological process underlying RA.

1. Introduction

Allograft inflammatory factor-1 (AIF-1) is a 143 amino acid cytoplasmic protein primarily identified in human and rat allografts. Data from several studies suggest an important role for AIF-1 in inflammatory processes [1]. AIF-1 is produced by monocyte/macrophages and lymphocytes and its synthesis is mediated by several cytokines such as IFN-γ [2,3]. AIF-1 is a modulator of the immune response during macrophage activation. In addition, AIF-1 plays a role in the survival and proinflammatory activity of macrophages [4]. Thus, AIF-1 may play a pivotal role not only in immune responses to alloantigens but also in various host responses to inflammatory stimuli. AIF-1 overexpression modulates the capability of macrophages to produce various cytokines, which might influence subsequent immune responses [4].

In addition, AIF-1 is known to play an important role in T-cell activation and proliferation [5]. Moreover, AIF-1 mediates the proliferation and enhances the migration of T lymphocytes. Interestingly, conditioned media from AIF-1-expressing T lymphocytes enhances the proliferation of vascular smooth muscle cells (VSMCs) and induces endogenous AIF-1 expression [6]. Upregulation of AIF-1 was also observed in experimental rat autoimmune encephalomyelitis, neuritis, and uveitis [7]. AIF-1 protein is also expressed in tissues from patients with systemic sclerosis [8]. AIF-1 is expressed in infiltrating mononuclear cells and synovial fibroblasts in rheumatoid arthritis (RA) and induces the proliferation of cultured synovial cells [9]. Kimura et al. detected AIF-1 in

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synovial fluid from RA patients, suggesting an important role of this cytokine in RA pathogenesis [10].

In this study we analysed the expression of AIF-1 in peripheral blood monocytes and in synovial membranes from patients with rheumatoid arthritis.

2. Materials and methods

2.1. Subjects

We examined 71 patients (55 females, 16 males, mean age 58.0 ± 13.5 years) with rheumatoid arthritis (RA) diagnosed according to the criteria of the American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR).

The patients were recruited from the outpatient and inpatient populations of the Department of Rheumatology, County Hospital in Szczecin, Poland. All subjects were Caucasian from the Pomeranian (northwestern) region of Poland. The subjects enrolled in the study underwent routine biochemical blood analysis and, when required, assays for anticardiolipin antibodies, antinuclear antibodies, and immunological complexes. X-rays of the chest, hands, and feet were obtained from all patients and, when required, radiographs of other joints. These were interpreted by two expert radiologists. Radiographs were assessed using a modified version of the Sharp method and the erosion score was calculated on a scale from 0 to 230 [11,12]. The evaluation of the subjects included a physical examination, with particular focus on the pattern of joint involvement and the occurrence of extra-articular features such as vasculitis, anaemia, sicca syndrome, amyloidosis, and organ involvement. We also analysed laboratory features: blood morphology, rheumatoid factor (RF), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR) as well as disease activity scores for 28 joints (DAS28) were calculated. The patients were treated with low doses of methotrexate and glucocorticosteroids. The clinical characteristics of the RA patients are shown in Table 1.

The control group consisted of 25 healthy subjects without autoimmunological diseases (15 females, 10 males, mean age 54.0 ± 19.3 years).

For the immunofluorescence analysis synovial membranes from 10 rheumatoid arthritis patients and 10 patients with osteoarthritis (OA) obtained during knee arthroplasty were used.

The study was approved by the local ethics committee and written informed consent was obtained from all subjects.

Table 1
Characteristics of the study group.

	Mean ± SD	Median	Lower quartile	Upper quartile
Age (years)	58.0 ± 13.5	57.0	51.0	69.0
Duration of disease (years)	12.4 ± 9.7	9.5	7.0	15.0
DAS28	3.1 ± 1.4	2.4	2.1	3.8
ESR (mm/h)	30 ± 27	20	13	36
CRP (mg/L)	24.8 ± 30.2	13.9	5.8	34.5
WBC ($\times 10^9$/L)	7.95 ± 2.44	7.79	6.38	8.96
Lymphocytes ($\times 10^9$/L)	2.09 ± 0.68	1.91	1.77	2.49
Monocytes ($\times 10^9$/L)	0.58 ± 0.27	0.58	0.47	0.66
TNF-alpha (pg/mL)	2.58 ± 3.08	2.05	1.49	2.61
IL-6 (pg/mL)	31.6 ± 37.0	15.6	5.2	51.2
IL-18 (pg/mL)	377.2 ± 166.8	351.1	250.4	463.6
VEGF (pg/mL)	148.5 ± 161.0	92.3	56.7	150.9

DAS28 – disease activity score.
ESR – erythrocyte sedimentation rate.
CRP – C-reactive protein.
WBC – white blood cells.
VEGF – vascular endothelial growth factor.

2.2. ELISA

Plasma concentrations of TNF-α, IL-6, IL-18 and VEGF were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) Quantikine kit (R&D Systems, Minneapolis, MN, USA) and MBL's human IL-18 immunoassay (ELISA) (Medical & Biological Laboratories Co., LTD, Japan) according to the manufacturers' protocols. The concentrations of cytokines were expressed in pg/mL.

2.3. Flow cytometry

The full population of peripheral blood (PB) nucleated cells was obtained after lysis of red blood cells (RBCs) using 1× BD Pharm Lyse Buffer (BD Biosciences Pharmingen, San Diego, CA, USA). After lysis, the cells (1×10^6) were washed twice and resuspended in 100 μL phosphate-buffered saline (PBS) and stained with mouse anti-human monoclonal antibody for the marker expressed at high levels on monocytes: CD14 conjugated with phycoerythrin (PE) (BD Biosciences, San Jose, CA, USA). After incubation for 20 min at 4 °C, the cells were washed twice in PBS. To measure the expression of AIF-1 within the cells, cells were fixed in 3.5% paraformaldehyde for 20 min, permeabilized using 0.1% Triton X-100 for 5 min, washed twice in PBS, and subsequently stained with anti-human AIF-1 antibody conjugated with fluorescein isothiocyanate (FITC) (Santa Cruz Biotechnology, Santa Cruz, CA, USA). After incubation for 1 h at room temperature, the cells were washed twice in PBS. Samples stained with an appropriate isotype control (BD Biosciences, San Jose, CA, USA) were examined in parallel. Cell fluorescence was measured and the data were analyzed using a fluorescence-activated cell analyzer (LSRII, BD Biosciences, San Jose, CA, USA) and BD FACSDiva software. Typically, 20.000 events were acquired to determine the percentage of a population of $CD14^+$ cells expressing AIF-1 within the circulating PB nucleated cells. The number of AIF-1^+/$CD14^+$ cells was expressed as the absolute number of cells per 1 μL of whole blood. The absolute number of AIF-1^+/$CD14^+$ cells per 1 μL of PB was calculated on the basis of absolute leukocyte count multiplied by the percentage of specific positive cells/100 [13].

2.4. Immunofluorescence analysis of synovial samples

The synovial samples were fixed in 4% formaldehyde (24 h), and then were washed with water (2 h). Next, the samples were washed with 50% ethanol (2 h), 70% ethanol (2 times within 10 h), 96% ethanol (2 h), absolute ethanol (2 times within 1 h), absolute ethanol with xylene (1:1) (2 h), xylene (2 times within 2 h) and then after a 3-h saturation of tissues with liquid paraffin, the samples were embedded in paraffin blocks. Using a microtome (LEICA SM 2000R), 3–5 μm serial sections were made and placed on silane histological slides (3-aminopropyl-trietoxy-silane, Thermo Scientific, UK). Preparations were deparaffinized in xylene and ethanol with decreasing concentration and were used for further immunofluorescence staining.

For immunofluorescence analysis, the sections were deparaffinized in xylene (2 × 15 min) followed by hydration in decreasing ethanol concentration solutions (100%, 95%, 85%, 70%, and 50%) and antigen retrieval (20 min of boiling in citrate buffer, pH 6.0). After blocking in 10% normal donkey serum for 30 min in room temperature, the sections were incubated with the primary antibody rabbit anti-AIF-1 (1:100) (Proteintech Group, Chicago, USA) diluted in PBS complemented with 1% BSA, at 4 °C overnight. Next, incubation with the secondary antibody donkey anti-rabbit Alexa Fluor 647 (1:100) (Life Technologies, Paisley, UK) was performed, for 1 h, at room temperature, in the dark. For coexpression analysis slides were incubated with additional antibodies: mouse