

Serum levels of macrophage migration inhibitory factor are associated with rheumatoid arthritis course

Mara Anaís Llamas-Covarrubias · Yeminia Valle · Rosa Elena Navarro-Hernández · Iris Paola Guzmán-Guzmán · María Guadalupe Ramírez-Dueñas · Héctor Rangel-Villalobos · Ciro Estrada-Chávez · José Francisco Muñoz-Valle

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Abstract Rheumatoid arthritis (RA) is an inflammatory autoimmune disease of unknown etiology. Many cytokines have been found to be associated with RA pathogenesis and among them is macrophage migration inhibitory factor (MIF). The aim of this study was to determine whether MIF serum levels are associated with RA course, clinical activity, and clinical biomarkers of the disease. MIF levels were

determined in serum samples of 54 RA patients and 78 healthy subjects (HS) by enzyme-linked immunosorbent assay (ELISA). Disease activity was evaluated using the DAS28 score. Patients were subgrouped according to disease activity and years of evolution of disease. Statistical analysis was carried out by SPSS 10.0 and GraphPad Prism 5 software. RA patients presented increased levels of MIF as compared to HS. MIF levels were raised on early stages of RA and tend to decrease according to years of evolution. Moreover, MIF levels positively correlated with rheumatoid factor in RA patients and with C reactive protein in all individuals studied. Our findings suggest that MIF plays a role in early stages of RA.

M. A. Llamas-Covarrubias · I. P. Guzmán-Guzmán
Doctorado en Ciencias Biomédicas, Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, Sierra Mojada #950, Col. Independencia, 44348 Guadalajara, Jalisco, Mexico

Y. Valle · R. E. Navarro-Hernández · J. F. Muñoz-Valle
IIRSME, Departamento de Biología Molecular y Genómica, Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, Sierra Mojada #950, Col. Independencia, 44348 Guadalajara, Jalisco, Mexico

M. G. Ramírez-Dueñas
Laboratorio de Inmunología, Departamento de Fisiología, Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, Sierra Mojada #950, Col. Independencia, 44348 Guadalajara, Jalisco, Mexico

H. Rangel-Villalobos
Instituto de Investigación en Genética Molecular, Centro Universitario de la Ciénega, Universidad de Guadalajara, Av. Universidad #1115, Col. Lindavista, Ocotlán, Jalisco, Mexico

C. Estrada-Chávez
Centro de Investigación y Asistencia en Tecnológica y Diseño del Estado de Jalisco, Av. Normalistas #800 Colonia Colinas de la Normal, 44270 Guadalajara, Jalisco, Mexico

J. F. Muñoz-Valle (✉)
IIRSME, Departamento de Biología Molecular y Genómica, Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, Insurgentes 244-1, Colonia Lomas de Atemajac, 45178 Zapopan, Jalisco, Mexico
e-mail: biologiamolecular@hotmail.com

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Abbreviations

RA	Rheumatoid arthritis
MIF	Macrophage migration inhibitory factor
HS	Healthy subjects
RF	Rheumatoid factor
CRP	C reactive protein
MMPs	Matrix metalloproteinases
FLS	Fibroblast like synoviocytes
DMARDs	Disease-modifying antirheumatic drugs
NSAIDs	Non-steroidal anti-inflammatory drugs
PLA ₂	Phospholipase A ₂
COX-2	Cyclooxygenase 2

Introduction

Rheumatoid arthritis (RA) is a systemic autoimmune disease affecting about 1% of the population worldwide.

It is characterized by chronic synovial inflammation of multiple joints, which leads to joint destruction and severe disability [1]. Its etiology has not been completely understood yet, but there has been accumulative evidence suggesting a critical role for cytokines on its pathogenesis [1].

Macrophage migration inhibitory factor (MIF) was one of the first cytokines to be discovered [2], but its functions and biologic importance remained unknown for a long period. At present, it is recognized as a potent pro-inflammatory cytokine secreted by T-cells, macrophages, and other inflammatory cells. MIF functions include: the promotion of tumor necrosis factor α (TNF α), interleukin 1 β (IL-1 β), IL-2, IL-6, IL-8, and interferon gamma (IFN γ) secretion, as well as phospholipase A₂ (PLA₂), cyclooxygenase 2 (COX-2), and some matrix metalloproteinases (MMPs). In addition, MIF has been shown to counter regulate the immunosuppressive action of glucocorticoids [3, 4].

Due to its proinflammatory actions, MIF is implicated in a number of autoimmune diseases including Sjögren syndrome, systemic lupus erythematosus and RA [5, 6].

Data revealed that MIF is overexpressed in serum, synovial fluid, and fibroblast-like synoviocytes (FLS) of RA patients. Furthermore, the observation that FLS MIF induces TNF α secretion by mononuclear cells suggests that this cytokine is an upstream member of the RA cytokine cascade [7].

Herein, we investigated the association of MIF serum levels with RA evolution, as well as with disease activity assessed through DAS28 score. The influence of MIF levels on clinical biomarkers of the disease was also evaluated.

Materials and methods

Subjects

Peripheral blood was obtained from 54 RA patients classified according to the American College of Rheumatology criteria, and 78 healthy subjects (HS). All participants signed an informed consent prior to their inclusion in the study, and none of them were being treated with glucocorticoids. The study protocol was designed according to the ethical principles established in Helsinki Declaration and it was approved by the investigation, ethics, and biosecurity committee of the Health Sciences University Center of the University of Guadalajara. Disease activity was evaluated in the RA group at the time of inclusion through the DAS28 score, which ranges from 2 to 10, were a score of 3.2 and above is considered clinically important [8]. C reactive protein (CRP) and rheumatoid factor (RF) were quantified by nephelometry using the IMMAGE[®] Immunochemistry system (Beckman Coulter, Inc. Fullerton, CA, USA).

Determination of MIF serum levels

MIF serum levels were quantified by ELISA assay according to the manufacturer's instructions (R&D Systems, Minneapolis, MN, USA). The range of detection was of 0.156–10 ng/mL and the sensitivity of 0.017 ng/mL. Samples were diluted 10 times before the assay.

Evaluation of MIF association with RA course

To assess if there is an association between disease evolution and MIF levels in RA, patients were classified according to years of disease evolution in: <1 versus >1 year, <2 versus >2 years, <3 versus >3 years, <4 versus >4 years and <5 versus >5 years; then MIF levels were compared among groups.

Statistical analysis

Differences in MIF levels between groups and according to disease evolution were evaluated by unpaired student's t test. Association of MIF levels with disease activity was assessed by ANOVA. Spearman's correlation was applied

Table 1 Clinical features of the patients with rheumatoid arthritis (RA)

Clinical parameters	
Total number	54
Age (years)	45 ± 13 (22–77)
Sex	
Men	5
Women	49
Duration of disease (years)	10.4 ± 8.7 (0.11–32)
Painful joints (0–28)	8 ± 9 (0–28)
Tender joints (0–28)	7 ± 6 (0–24)
DAS-28 score	5.08 ± 1.73 (1.13–8.42)
Treatment	
DMARDs ^a	45/54 (83.3%)
Methotrexate	37/45 (82.2%)
Chloroquine	32/45 (71.1%)
Hydroxychloroquine	1/45 (2.2%)
Sulfasalazine	16/45 (35.5%)
Leflunomide	2/45 (4.4%)
NSAIDs ^b	34/54 (63%)

Data are expressed as average ± standard deviation and range

^a Disease-modifying antirheumatic drugs

^b Non-steroidal anti-inflammatory drugs

to test the relationship between MIF and clinical markers of the disease. Probability values less than 0.05 were considered statistically significant. The analysis was performed using SPSS version 10.0 and GraphPad Prism 5 software.

Results

Clinical and demographic characteristics

A total of 54 RA patients (49 women, 5 men) with an average age of 45 years (range, 22–77) were included. The clinical features of these patients are depicted in Table 1. Patients were diagnosed with RA for an average of 10.4 years and the mean DAS-28 score was of 5.08, which indicates that they were coursing with a moderate disease activity. The control group was composed of 48 women and 30 men with an average age of 34 years (range, 18–61).

Elevated MIF serum levels in RA patients

Significantly increased levels of the cytokine were found in the RA group compared to the HS group [median (range):

9.5 ng/ml (7.7–12.0) vs. 8.2 ng/ml (5.8–10.7), $P = 0.031$; data not shown].

To assess MIF relationship with disease activity, we stratified patients according to the DAS28 score. The groups obtained were: remission ($\text{DAS28} < 2.6$; $n = 5$), low activity ($2.6 < \text{DAS28} \leq 3.2$; $n = 4$), moderate activity ($3.2 < \text{DAS28} < 5.1$; $n = 16$), and high activity ($\text{DAS28} \geq 5.1$; $n = 27$). We found no statistical differences in MIF levels between groups ($P = 0.339$; data not shown).

Relationship between MIF serum levels and disease evolution

In order to investigate the relationship of MIF levels with RA evolution, patients were grouped according to years of evolution of the disease (Fig. 1a–e). A significant increase of MIF levels in early stages of RA was detected ($P < 0.05$). Moreover, throughout the course of the disease, MIF levels tend to decrease. This finding was further confirmed when a negative correlation was found between years of disease evolution and MIF levels ($r = -0.336$; $P = 0.014$) (Fig. 2a).

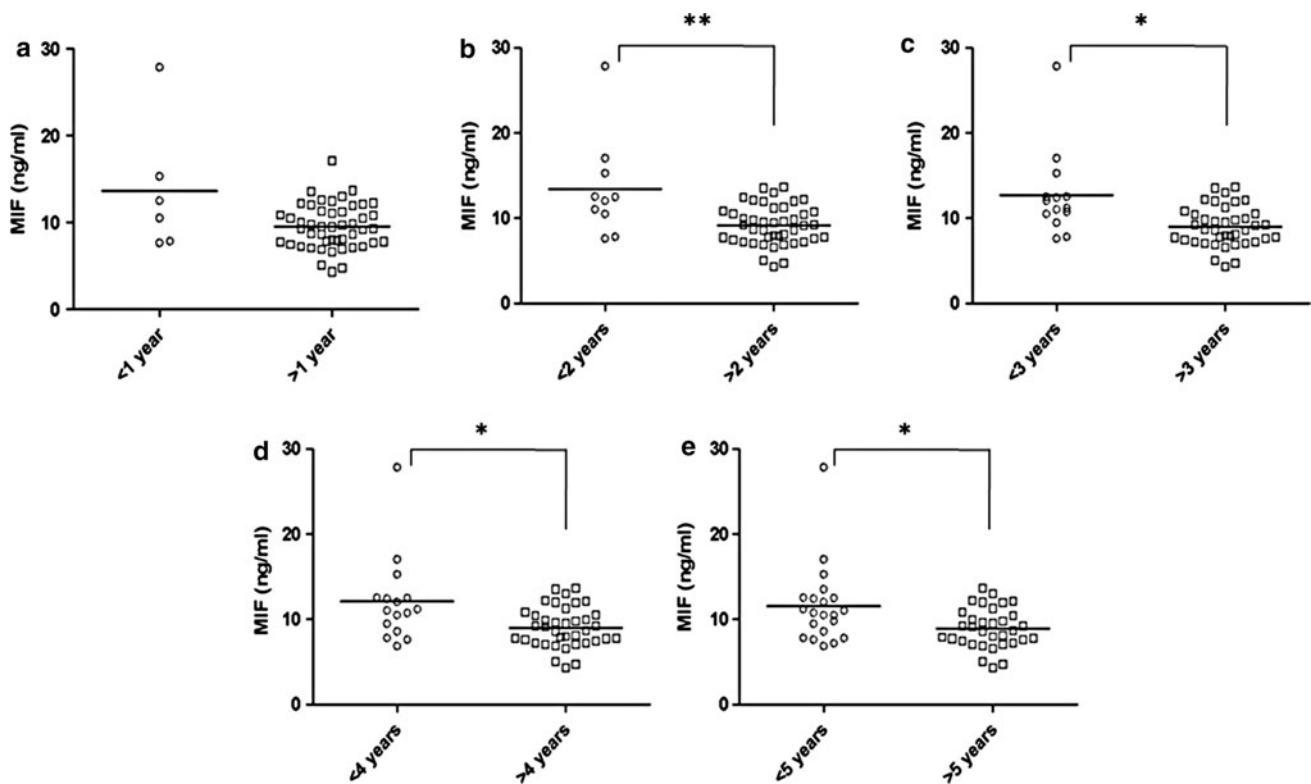


Fig. 1 Association of MIF levels with years of RA evolution. Significantly increased levels of MIF were detected in early stages of RA as compared to later stages. **a** <1 year versus >1 year of RA evolution,

b <2 years versus >2 years of RA evolution, **c** <3 years versus >3 years of RA evolution, **d** <4 years versus >4 years of RA evolution, **e** <5 years versus >5 years of RA evolution. * $P < 0.05$; ** $P < 0.01$

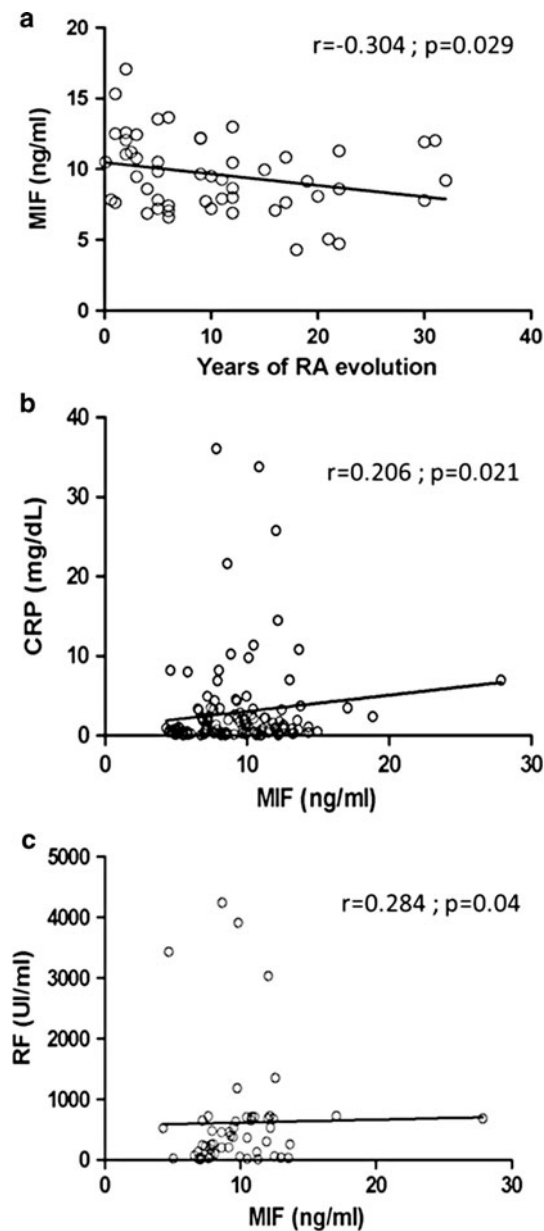


Fig. 2 Correlation of MIF levels with disease evolution, CRP, and RF. A negative correlation between MIF levels and disease evolution was identified in the RA group (a). On the other hand, a weak positive correlation between MIF and CRP was detected in all individuals (b), and finally MIF levels also correlated with RF in the RA group (c). *r* and *P* values were obtained by Spearman's correlation test

An analysis of correlations was also performed in each group of disease evolution. Significant correlations were found between MIF and RF; and MIF and DAS28 in patients with <3 and <4 years of evolution of disease (data not shown). Furthermore, we found that MIF serum levels correlated positively with CRP when evaluating all individuals included in the study ($r = 0.206$; $P = 0.02$) (Fig. 2b); and with RF titers in the RA group ($r = 0.284$; $P = 0.04$) (Fig. 2c).

Discussion

Cytokines play a key role in RA pathogenesis by their ability to regulate fundamental events both at the onset and progression of the disease, such as: macrophage, B cell and T cell activation, cell migration to the inflammation site, angiogenesis, and secretion of effector molecules [8]. The introduction of therapeutic interventions focused on cytokine blockade, has shown the importance of this molecules on RA [9–11].

MIF has recently arisen as a candidate in the treatment of several inflammatory and autoimmune diseases [2, 3, 5]. In animal models of arthritis, MIF blockade has shown an important inhibitory effect on disease incidence, severity, and mortality [12–14]. In the present study, increased MIF serum levels were detected in RA, especially in early stages of disease. This finding suggests that MIF may play a key role in RA onset when the inflammatory process is beginning to occur, and then, once the inflammation is well established other mediators such as $\text{TNF } \alpha$ may act to maintain the state. Our hypothesis is supported by the discovery that MIF is an upstream regulator of $\text{TNF } \alpha$ secretion in RA [7]. Moreover, Martinez et al. showed that the C allele of the MIF -173G>C polymorphism is associated with early onset RA [15], and it was previously detected that carriers of this allele show higher levels of circulating MIF [16]. Hence, MIF is emerging as an important player on RA onset.

Although this was a cross-sectional study, our findings together with the evidence previously discussed, suggests that MIF blockade would be of special importance on the treatment of early RA. Nevertheless, further studies are required to confirm the role of MIF in early RA.

We also analyzed the association of MIF levels with clinical markers of disease. A positive correlation was found between MIF and CRP in all the individuals. This finding is consistent with the previous report that MIF can directly stimulate hepatocytes to secrete acute phase reactants, including CRP [17], and Morand et al. [18] also detected a correlation between synovial MIF levels and circulating CRP. Nevertheless, CRP levels are widely dependent on the treatment employed, and our study group was heterogeneous in that respect, so this finding must be interpreted with caution.

Besides the role MIF plays in innate and cellular immunity, it is also an important component in humoral immunity. For instance, MIF suppression has shown an inhibitory effect on antibodies production [19], moreover, in Sjögren patients, MIF serum levels correlated with γ -globulin percentage [5]. In the present study, a positive correlation was detected among MIF levels and RF titers in RA patients, thus underlying the importance of this cytokine on humoral immunity.

In conclusion, this study supports the role of MIF in RA pathogenesis and it is the first one that shows an association of this cytokine especially on early stages of disease, suggesting that therapeutic interventions directed against MIF would be critical on the treatment of early RA. A weak association with CRP and RF was also determined, suggesting that MIF might be useful as a biologic marker in RA.

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Conflict of interest Authors do not have conflict of interest.

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