International Immunopharmacology (2008) **8**, 859–865

www.elsevier.com/locate/intimp

Development of a novel method to measure macrophage migration inhibitory factor (MIF) in sera of patients with rheumatoid arthritis by combined electrochemical immunosensor

Shuhui Li [a,1], Ronghua Zhang [b,1], Peng Li [a,1], Weijing Yi [a], Zhujun Zhang [a], Sha Chen [a], Shaobo Su [c], Lijun Zhao [d], Chuanmin Hu [a,*]

[a] *Department of Clinical Biochemistry, Laboratory Sciences, the Third Military Medical University, Chongqing, Gaotanyan Street 30, Shapingba District, Chongqing, 400038, PR China*
[b] *Department of Rheumatology, Southwest Hospital, The Third Military Medical University, Chongqing, 400038, PR China*
[c] *Institute of Inflammation & Immune Disease, Shantou University Medical College, Shantou, 515031, PR China*
[d] *Department of Chemistry and Biochemistry, University of North Carolina, Greensboro, NC. 27402-6170, USA*

Received 17 January 2008; received in revised form 31 January 2008; accepted 31 January 2008

KEYWORDS
Macrophage migration inhibitory factor (MIF);
Monoclonal antibody;
IgM;
Electrochemical immunosensor;
Rheumatoid arthritis (RA)

Abstract

Macrophage migration inhibitory factor (MIF) has a multitude of biological activity and is associated with a number of inflammatory and immune diseases, including rheumatoid arthritis (RA). The increased serum levels of MIF in patients not only suggest this protein as a marker for disease progression, but also as a potential therapeutic target. The aim of this study is to develop a novel electrochemical method to more precisely and conveniently measure MIF in patient sera. An IgM murine monoclonal antibody (mAb) against human MIF was prepared and used in the electrochemical immunosensor, with modified gold electrode coated with compounds of gold nanoparticles, titanium dioxide nanoparticles and thionine (NGP-NTiP-Thi) followed by adsorption of anti-MIF antibodies with IgM or IgG1 isotype. The IgM immunosensor recognized MIF in a linear relationship in the range of 0.03 and 230 ng/mL with the lower limit ($S/N=3$) of 0.02 ng/mL. The measurement showed considerable levels of sensitivity, selectivity, stability and long-term maintenance of bioactivity, as shown by testing with serum MIF in RA patients as compared to healthy donors. The performance of the IgM immunosensor was also superior to IgG1 sensor. Thus, we have developed a novel measurement approach for serum MIF, which may have great potential in the clinic for monitoring the course of diseases associated with increased MIF.

* Corresponding author. Tel.: +86 23 68753761.
E-mail address: chuminhu@163.com (C. Hu).
[1] These authors contributed equally to this work.

1567-5769/$ - see front matter
doi:10.1016/j.intimp.2008.01.032

1. Introduction

Macrophage migration inhibitory factor (MIF) was discovered in 1966 by two independent laboratories [1,2] and characterized as a T cell-derived mediator that inhibits the random movement of macrophages in vitro. MIF is produced by many effector cells of the immune system, most notably the macrophages [3]. After the cloning of complementary DNA from human T lymphocytes [4] and determination of protein sequence and chemical characteristics [5], MIF has attracted much attention and many of its actions were discovered. In recent years, MIF has emerged as a key factor regulating inflammatory responses both by directly activating immune cells, and by participating in biological responses triggered by other stimuli. An increasing amount of literature implicated MIF in the pathogenesis of sepsis, inflammation, and autoimmune diseases, suggesting that MIF might be considered as a therapeutic target in human diseases [6]. In patients with RA, high concentrations of MIF were found both in sera and in synovial fluid and the levels of detected MIF significantly correlated with disease severity [7–9]. In addition, a functional polymorphism in the promoter of MIF gene has been shown to be associated with disease severity in RA patients [10,11]. These observations indicate a crucial role for MIF in the pathogenesis of RA [12], as a mediator and a marker in RA disease progression. In this context, it will be important to develop sensitive and convenient methods for the measurement of MIF in body fluid.

However, current available method for determination of MIF uses enzyme-linked immunosorbent assay (ELISA), which has limitations such as relying on the label of antigen or antibody, radiation hazards, long analysis time, expensive instruments and the need for skillful operators [13]. In recent years, electrochemical immunosensors are of great interest due to their simple pretreatment procedures, fast analytical time, precise measurement and small sized instruments [14–16]. In this study, we report a novel MIF measurement methods using IgM mAb electrochemical immunosensor based on gold nanoparticles, titanium dioxide nanoparticles and thionine (NGP-NTiP-Thi).

2. Materials and methods

2.1. Serum samples

Human serum samples were obtained from 70 RA patients and 19 healthy volunteers (aged 17–76). The study protocols were approved by local Institutional Review Board (IRB), and informed consent was obtained from patients. The sera were isolated by 20 min centrifugation at 3000 rpm and kept frozen at −80 °C until MIF analysis. The severity of disease in RA patients was represented by score 28 (DAS28) system based to the clinical criteria [17,18]. The RA patients were divided into low, moderate and high disease severity groups scored as 2.6–3.2, 3.2–5.1, and >5.1, respectively.

2.2. RNA extraction and purification

Peripheral blood mononuclear cells (PBMC) were isolated within 1 h after collection by gradient centrifugation of the blood on Ficoll Hypaque-1077 (Sigma). Total RNA from 1×10^6 PBMC was extracted and purified by the TRIzol Reagent kit (GIBCOBRL) according to the manufacturer's recommendation.

2.3. cDNA synthesis

Purified total RNA (1.0 μg) was denatured for 5 min at 65 °C and immediately cooled on ice. Reverse transcription was performed using the ReverTra-Plus-™ (TOYOBO) according to the manufacturer's protocol. After random primers were annealed for 10 min at 30 °C, cDNA synthesis was performed for 60 min at 42 °C followed by enzyme deactivation for 5 min at 85 °C.

2.4. Gene cloning and prokaryotic expression of rhMIF

PCR was performed in a 50 μl reaction mix containing 2 μl synthesized cDNA, 5 μl 10×KOD-Plus PCR buffer (TOYOBO), 0.2 mM each of primers (sense: 5′-CGCGGATCCTGCGGCTCTTAGGCGAAGG-3′; anti-sense: 5′-CGCCATATGCCGATGTTCATCGTAAACAC-3′), 2.5 mM $MgCl_2$, 0.2 mM each dNTP and 1 U KOD-Plus polymerase (TOYOBO). The PCR conditions were as follows: initial denaturation at 94 °C for 3 min, 35 PCR cycles with denaturation at 94 °C for 0.5 min, annealing at 55 °C for 0.5 min and extension at 68 °C for 1 min, followed by a final extension at 68 °C for 10 min. The PCR products were analyzed in 1.5% agarose ethidium bromide-stained gel. In order to confirm the nucleotide sequence, the amplified products were gel purified using a kit (Wizard's PCR Preps DNA Purification System, Promega) and cloned using a TA cloning system (Promega) generating pGEM-T easy /rhMIF after adding 3'-A overhangs to PCR product by A-attachment Mix (TOYOBO). The inserts were sequenced from both ends by automated Applied Biosystem Sequencer (ABI377, Invitrogen). For all sequences derived from PCR-amplified DNA, at least five clones from separate reactions were sequenced in order to minimize errors.

2.5. Prokaryotic expression and purification of rhMIF

The confirmed human MIF cDNA segment was inserted into the prokaryotic expression vector pET11b. *E. coli*. BL21 were transformed with the MIF-containing vectors, cultured, and induced to express human (h) MIF protein with isopropyl-beta-D-thiogalactopyranoside (IPTG). The lysate of *E. coli* containing hMIF protein was subjected to cation exchange chromatography, and hydrophobic chromatography. The identity of rhMIF protein was determined by an IgG1 type anti-MIF monoclonal antibody (a generous gift from Dr. Richard Bucula, Yale University, New Haven, CT).

2.6. Preparation of anti-rhMIF monoclonal antibody

The purified rhMIF was used for immunization of BALB/c mice. Two injections were given s.c. to each mouse on the day 1 with 50 μg of rhMIF in CFA (Complete Freund's Adjuvant) and on the day 28 with 50 μg of rhMIF in IFA (Incomplete Freund's Adjuvant). Seven days after the second injection, the blood of the immunized mice was taken from tail veins and the presence of anti-MIF antibodies was determined by indirect ELISA. The mouse with the highest anti-MIF titer was boosted i.v. on day 38 with 50 μg rhMIF in 0.9% NaCl). Fusion was performed 3 days later using fresh mouse splenocytes (5×10^7) and SP2/0 myeloma cells. ELISAs, fusions and cloning of anti-MIF antibody producing hybridoma cell lines were then performed. The immunoglobulin subclass was determined using mouse hybridoma isotyping reagents (Sigma, St. Louis, USA).

2.7. Purification of anti-rhMIF IgM monoclonal antibody

For ascites production, BALB/c mice were intraperitoneally injected with 2×10^7 MIF secreting hybridoma cells. After 18 days, ascitic fluid was collected. Anti-rhMIF IgM mAb was purified by affinity chromatography according to IgM purification procedures (Amersham Biosciences). Briefly, ascitic fluid was diluted with 9 times of binding buffer (20 mM sodium phosphate, 0.8 M $(NH_4)_2SO_4$, pH 7.5) and then filtered through a 0.45 μm filter. HiTrap IgM Purification HP 1 mL