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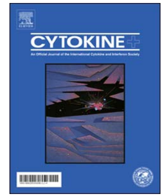

Proinflammatory cytokine MIF plays a role in the pathogenesis of type-2 diabetes mellitus, but does not affect hepatic mitochondrial function

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ABSTRACT

Background: Macrophage migration inhibitory factor (MIF) is a proinflammatory cytokine that plays an important role in the pathogenesis of type 2 diabetes mellitus (T2DM). Although the effect of high glucose on liver function has been described, the role of MIF in hepatic mitochondrial function during T2DM has not been studied.
Objective: We examine the influence of MIF to hepatic mitochondrial function in T2DM mouse model.
Methods: WT and *Mif*$^{-/-}$ BALB/c mice were treated with a single dose of streptozotocin (STZ). After an 8-week follow-up, serum glucose, proinflammatory cytokines, C-reactive protein (CRP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) enzyme quantification, and liver histological analyses were performed. Liver mitochondria were extracted, and mitochondrial function was evaluated by oximetry, swelling and peroxide production.
Results: Following treatment with STZ, WT mice (WT/STZ) developed significant hyperglycemia and high serum levels of MIF, tumor necrosis factor (TNF)-α, interleukin-β (IL-β), and CRP. Liver damage enzymes ALT and AST were found at high levels. In contrast, *Mif*$^{-/-}$STZ lacked serum MIF levels and showed smaller increases in blood glucose, less TNF-α, IL-1β, CPR, ALT and AST, and failure to develop clinical signs of disease compared to the WT/STZ group. Mitochondria extracted from the *Mif*$^{-/-}$STZ liver showed similar respiratory control (RC) to WT/STZ or healthy mice with glutamate/malate or succinate as substrates. The four respiratory chain complexes also had comparable activities. WT/STZ-isolated mitochondria showed low swelling with calcium compared to mitochondria from *Mif*$^{-/-}$STZ or healthy mice. Peroxide production was comparable in all groups.
Conclusion: These results show although high systemic levels of MIF contribute to the development of T2DM pathology, the liver mitochondria remain unaltered. Importantly, the absence of MIF reduced the pathology of T2DM, also without altering liver mitochondrial function. These support MIF as a therapeutic target for the treatment of this disease in humans.

1. Introduction

T2DM, is a major comorbidity of obesity that predisposes patients to significant end-organ damage [1–3]. Several studies have shown that the development of T2DM is associated with impaired responsiveness to insulin and the subsequent failure of pancreatic β cells to secrete adequate amounts of insulin to maintain blood glucose levels [4]. In addition, T2DM is associated with high serum levels of several inflammatory cytokines, such as IL-12, TNF-α, interferon (IFN)-γ and MIF [5–7].

Abbreviations: AGEs, advanced glycation end products; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GLUT2, glucose transporter 2; CI, complex I; CIII, complex III; DBH_2, decylubiquinol; G/M, glutamate/malate; NAFLD, non-alcoholic fatty liver disease; RC, respiratory control

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MIF is a ubiquitously expressed protein that in recent years has gained substantial attention as a pivotal mediator of innate and adaptive immune response. MIF acts as a proinflammatory cytokine to promote the expression of other inflammatory cytokines, including IL-1β TNF-α, IL-2, IL-6, IL-8, IFN-γ and inducible nitric oxide synthase (iNOS), and inhibits the anti-inflammatory effects of glucocorticoids [8,9].

Accumulating evidence of its role in the regulation of inflammatory cytokines, leads one to postulate that MIF represents an important link between chronic inflammation and pathology in multiple inflammatory and autoimmune diseases [10] including, rheumatoid arthritis [10,11], inflammatory bowel diseases [12], chronic and ulcerative colitis [13,14], immunologically induced kidney diseases [15], glomerulonephritis [16,17], Guillain-Barré syndrome [18] and autoimmune encephalomyelitis [19], among others. In this line, anti-MIF monoclonal antibodies (mAb) have been effective in the treatment of some of these pathologies in experimental murine models. For example, in acute experimental autoimmune encephalomyelitis in rats, the treatment with anti-MIF mAb resulted in impaired homing of T cells to the central nervous system due to the down-regulation of VCAM-1, and impaired the effector functions of T cells [20]. In addition, the administration anti-MIF mAb or MIF chemical inhibitor ISO1 both reduced the cumulative severity score, and the course of murine experimental allergic neuritis [18]. Both observations support the idea that neutralization of MIF has therapeutic effects associated to down-regulation of the inflammatory-autoimmune Th1 response.

Moreover, it has been demonstrated that MIF is involved in the development and progression of type 1 diabetes mellitus (T1DM) [21,22], as well as T2DM [7]. MIF has been detected increased in the sera of patients with T2DM [7,23–25], and has been discovered as an important molecule in obesity-associated adipose tissue inflammation that leads to the development of insulin resistance associated to T2DM [26].

Chronic T2DM has significant adverse effects on various organs, but the molecular mechanisms involved in each case are unclear [25,27–29]. The liver has been implicated in metabolic syndrome because it receives sugars as absorbed nutrients that can alter liver function through caloric excess, or by the specific natures of the nutrient components [30]. Thus, alterations in liver function, and liver disease could contribute to the emergence of T2DM. However, there is no evidence of the influence of MIF to hepatic mitochondrial function in T2DM.

Mitochondria are especially abundant in the liver and can be damaged by high fructose, fatty acids, and amino acids that are not eliminated by the liver [30]. Therefore, alterations to mitochondrial function are associated with liver pathogenesis [31].

Considering the importance of T2DM-associated systemic inflammation and glucose abnormalities toward affecting hepatic function, we asked whether MIF could play a role in mitochondrial function in this organ. To address this, we developed a T2DM mouse model with a single injection of a standard dose of STZ in WT and *Mif*$^{-/-}$ mice and analyzed the mitochondrial function in the livers of these groups. Here, we present our analysis of a diabetic model that was not subjected to a high-calorie diet. Instead, animals were fed a normal balanced diet, while they maintained high blood sugar levels throughout the experiment. The results show that, although MIF fuels severe inflammatory and functional hepatic damage in a mouse model of T2DM, the mitochondrial function remains unaltered, providing evidence of the tight control of this organelle.

2. Materials and methods

2.1. Animals

BALB/c mice were purchased from Envigo Laboratories (Mexico City, Mexico) and maintained in a pathogen-free environment at our animal facilities in accordance with institutional guidelines. *Mif*$^{-/-}$ mice were developed as previously described [32] and backcrossed for more than 10 generations to a BALB/c genetic background. All mice were genetically validated before being used. Animals were fed with the standard diet base formula, the Teklad Custom Diet, from Envigo Teklad Diets, (Madison, WI, USA). This study was approved and carried out in strict accordance with the guidelines for the Care and Use of Laboratory Animals adopted by the U.S. National Institutes of Health and the Mexican Regulation of Animal Care and maintenance (NOM-062-ZOO-1999, 2001) and was approved by the Ethics Committee at FES-Iztacala, UNAM (1027/27/06/2014). All euthanasia was performed under a CO_2/O_2 excess atmosphere, and all efforts were made to minimize suffering.

2.2. Induction of type-2 diabetes mellitus

Seven-week-old male *Mif*$^{-/-}$ and WT mice were fasted for 12 h before the induction of diabetes with a single i.p. injection of STZ at 130 mg/kg (Sigma-Aldrich, St. Louis, MO, USA), which was freshly dissolved in 0.05 M citrate buffer at pH 4.5, by a previously described protocol [33]. In preparation of this work, we performed dose response curves (Fig. S1) to establish a single dose of STZ to induce T2DM. Healthy mice from each strain were injected with equivalent volumes of citrate buffer as negative controls. It is important to note that a small increment in the dose (160 mg/kg) caused an increase in blood glucose levels up to 600 mg/dl as early as one week post-treatment and WT and *Mif*$^{-/-}$ mice died around day 7 post STZ-treatment. At this high dose, the STZ induced a toxic damage to pancreatic islets and death of experimental mice. *Mif*$^{-/-}$ mice were not protected against the “toxic” form of diabetes, consistent with a previous report [21].

2.3. Pancreas histopathology

Healthy *Mif*$^{-/-}$ and WT mice were euthanized, and each pancreas was isolated, fixed overnight in formaldehyde and embedded in paraffin, after which 5-µm-thick transverse sections were mounted onto slides and subsequently stained with hematoxylin-eosin. Slides were analyzed by light microscopy. Other slides were incubated in a humidified chamber overnight at 4 °C with mouse anti-Insulin primary antibodies at 1:500 dilution (all from Sigma-Aldrich). The slides were then washed and incubated for 2 h at room temperature with the secondary antibody (biotin-conjugated goat anti-mouse IgG). To detect the peroxidase-labeled antibody, we used a DAB solution (10 ml PBS + 0.006 g DAB + 10 µL 30% H_2O_2 per liter of dH_2O). Sections were analyzed under an Axiostar plus light microscope (Carl Zeiss, Oberkochen, Germany) equipped with a digital video camera. We assessed the number and perimeter lengths of the islets of Langerhans on 10 slides per mice from the experimental and heathy mice. The total number of islets counted on the 10 slides from heathy mice was considered as 100% and was used as a reference for all experimental group comparisons. For immunofluorescent labeling, tissue sections were incubated in a humidified chamber overnight at 4 °C with primary antibodies raised against mouse anti-GLUT2 (Sigma-Aldrich) at a 1:2000 dilution. The next day, samples were washed and incubated for 1 h with the secondary antibody, FITC-conjugated anti-guinea pig (1:100 dilution). Tissues were mounted with medium containing 15 mM NaN_3 (Fluorescence Mounting Medium, DAKO Agilent Pathology Solutions, Santa Clara, CA, USA). For negative controls, we omitted primary or secondary antibodies. Slides were analyzed under a fluorescence microscope (DM IRB, Leica, Wetzlar, Germany).

2.4. Pancreatic islet isolation for mRNA analysis

Pancreatic islets were isolated by collagenase digestion and a discontinuous Ficoll-density gradient (Sigma-Aldrich) as previously described [34]. Total RNA was extracted from isolated islets with TRIzol

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