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DEFICIENCY OF MACROPHAGE MIGRATION INHIBITORY FACTOR (MIF) INHIBITS CYTOKINE-INDUCED IL-1B GENERATION IN MURINE PANCREATIC ISLET CELLS

IVANA STOJANOVIĆ, TAMARA SAKSIDA and STANISLAVA STOŠIĆ-GRUJIČIĆ

Department of Immunology, Institute for Biological Research "Siniša Stanković", University of Belgrade, 11108 Belgrade, Serbia

Abstract - In diabetes, pancreatic islets are subjected to high levels of inflammatory mediators that can lead to beta cell destruction. We recently showed that pancreatic islet cells derived from MIF-deficient (MIF-KO) mice are resistant to apoptosis induction by the cytotoxic stimuli of cytokines. Here we show that MIF-KO islets under cytokine (IFN-γ+TNF-α+IL-1β) stimulation express and secrete significantly lower amounts of IL-1β, while the expression of caspase-1 mRNA is not influenced by MIF deficiency. These data suggest that MIF-KO islets possess an innate defect in the process of IL-1 β synthesis and secretion.

Key words: Pancreatic islets, Macrophage Migration Inhibitory Factor (MIF), IL-1β, caspase-1

INTRODUCTION

Diabetes mellitus refers to a group of chronic diseases, where Type 1 diabetes (T1D) and Type 2 diabetes (T2D) constitute its two main forms (Brooks-Worrell and Palmer, 2011). In T1D, damage to the beta cells occurs via T lymphocyte and macrophage infiltration of the islets of Langerhans and exposure to the proinflammatory mediators that these cells produce, such as cytokines (TNF-α, IL-1β, IL-17, IL-18, IFN-γ, MIF) and reactive oxygen and nitrogen species (Cnop et al., 2005; Eizirik et al., 2009). Moreover, the beta cells themselves contribute to the production of some of the cytokines, chemokines and nitric oxide under a proinflammatory stimulus (Kaminitz et al., 2007). In T2D, slow progressive low-grade inflammatory processes take place, and the beta cells succumb to a predominantly mitochondria-dependent apoptosis associated with the increase in circulating proinflammatory cytokines and free fatty acids or glucose (Stumvoll et al., 2005; Masters et al., 2010). Thus, although the instigating factors that lead to beta cell destruction in T1D and T2D are distinct, both forms of diabetes present an inflammatory pathogenesis in which the beta cells perish (reviewed in Donath et al., 2008). Moreover, IL-1β seems to be a common mediator involved in beta cell destruction in both types of diabetes (Dinarello et al., 2010).

Macrophage migration inhibitory factor (MIF) is a proinflammatory cytokine of the innate immune system that plays a major role in the onset and development of several inflammation-based diseases, including diabetes (reviewed in: Cvetković and Stošić-Grujičić, 2006; Stošić-Grujičić et al., 2009; Stojanović et al., 2012). One of the main characteristics of MIF is its ability to recruit cells of both innate and acquired immunity to the site of inflammation and to amplify the production of various proinflammatory mediators such as TNF-α, IL-1β, IFN-γ, IL-17, nitric oxide (Calandra and Bucala, 1997), thus positioning MIF at the top of the inflammatory cascade. The final stage of this cascade is the activation of pro-apoptot-

ic signals that may lead to beta cell death. Indeed, we have demonstrated that neutralization, or deletion of MIF prevents beta cell death in diabetes in mice by the modulation of cytokines shown to be relevant to disease pathogenesis (Cvetković et al., 2005; Stošić-Grujičić et al., 2008). Our *in vitro* studies further supported the role of MIF in the functional suppression and propagation of insulin-producing pancreatic islet cell death (Stojanović et al., 2008; Saksida et al., 2011). Interestingly, this protein with extraordinarily versatile functions was found to be present within the insulin secretory granules in the beta cells, and to act as an autocrine factor to stimulate insulin release (Waeber et al., 1997). However, beside this function, one can postulate that MIF could influence the final stage of the cytokine-mediated apoptotic cascade. On the other hand, diabetes, as an IL-1β-mediated disease, is often termed "auto-inflammatory" and the dominant finding is the release of the active form of IL-1β. This led us to examine the possible involvement of MIF in the local control of this important proinflammatory and cytotoxic mediator. In order to study the causal relationship between MIF and IL-1β within the target tissue, we compared the expression of IL-1β between the pancreatic islets obtained from wild type C57BL/6 mice and that of genetically deficient MIF-KO mice. We demonstrated that the absence of MIF affected the production of IL-1β, which could be one more explanation for the previously observed resistance of MIF-deficient islets to apoptosis induction.

MATERIALS AND METHODS

Materials

Unless otherwise stated, all chemicals were purchased from Sigma-Aldrich, St Louis, MO, USA.

Isolation of pancreatic islets and treatments

The experiments were approved by the Ethics Committee for Animal Experimentation at the Belgrade University and conducted in accordance with local and international legislation regarding the wellbeing of laboratory animals. Pancreatic islets were prepared from homozygous *mif* gene-deficient (MIF-KO) mice (Fingerle-Rowson et al., 2003), as well as from their age-matched wild-type (WT) C57BL/6 counterparts. The islets were isolated using a collagenase V digestion technique, followed by handpicking (Rydgren and Sandler, 2002). Before performing the experiments, the islets were cultured overnight in RPMI-1640 medium containing 10% FCS (PAA Chemicals, Pasching, Austria), 10 mM HEPES, 5 μM 2-mercaptoethanol, 2 mM L-glutamine, 1 mM sodium pyruvate, 100 IU/ml penicillin and 100 μg/ml streptomycin in a humidified (5% CO_2, 95% air) atmosphere at 37°C in 6-well non-adhesive culture plates, or in 96-well tissue culture plates, as will be indicated in the Results. The pancreatic islets were treated with a mixture of murine cytokines IFN-γ+TNF-α+IL-1β (R&D, Minneapolis, MN, USA), 10 ng/ml each.

RNA isolation, reverse transcription and PCR

Total RNA was isolated from the pancreatic islets (groups of 80) with TRIzol reagent (Genosys, Woodlands, TX) according to the manufacturer's instructions. RNA was reverse-transcribed using Moloney leukemia virus reverse transcriptase and random hexamers (Pharmacia, Uppsala, Sweden). PCR amplification of cDNA with primers specific for the gene in question and β-actin as a housekeeping gene was carried out in a Mastercycler Gradient thermal cycler (Eppendorf, Hamburg, Germany) as follows: 30 s of denaturation at 95°C, 30 s of annealing at 58°C, and 30 s of extension at 72°C. For each gene, preliminary experiments were conducted to ascertain that amplification of the cDNA was in the linear range under the respective cycling conditions. Primer pair sequences are given in Table 1. The PCR products were visualized by electrophoresis in 2.5% agarose gels containing ethidium bromide; the gels were photographed by GelDoc (Biorad, Hercules, CA, USA) and the results were analyzed by densitometry using Kodak 1D 3.6 software.

Determination of IL-1β levels in culture media

IL-1β concentrations in the supernatants of cultured pancreatic islets were measured by sandwich ELISA

according to the manufacturer's instructions. The paired IL-1β antibodies specific for mouse IL-1β were from R&D. The results were calculated using a standard curve, constructed using known concentrations of recombinant mouse IL-1β (R&D).

Statistical analysis

The results from at least 3 experiments were presented as means ± SD. Differences were assessed using the unpaired Student's *t*-test. A *P* value less than 0.05 was taken to be statistically significant. The statistical package used was Statistica 7.0 (StatSoft Inc., Tulsa, OK, USA).

RESULTS

IL-1β is produced by the beta cells in an inflammatory *milieu* and operates in a paracrine and autocrine fashion to induce apoptosis in beta cells (Kaminitz et al., 2007; Pirot et al., 2008). To determine the causal relationship between MIF and IL-1β generation within a pancreatic islet, we used the pancreatic islets isolated from MIF-KO and C57BL/6 mice (WT). To mimic an inflammatory insult, the islets were treated with a cytokine cocktail (TNF-α+IL-1β+IFN-γ). After 6 h of incubation, we investigated the expression of IL-1β mRNA in untreated islets and their cytokine-stimulated counterparts. While basal expression of IL-1β mRNA was similar in WT and MIF-KO islet cells, upon exposure to the cytotoxic cytokine insult the MIF-KO islets expressed lower levels of IL-1β mRNA compared to the WT islets (Fig. 1a).

We then examined the secretion of IL-1β upon cytokine stimulation, as well as basal secretion of the protein. In order to prevent interference with IL-1β detection, we used rat recombinant IL-1β for stimulation, while for detection by ELISA, antibody pairs specific for mouse IL-1β were used. After 24 h of cultivation, a significant amount of IL-1β was detected in the culture supernatants of both MIF-KO and WT islets. However, upon exposure to the cytokine cocktail, the secretion of the formed protein was almost completely suppressed in the MIF-KO islets (Fig. 1b), while it was upregulated in the WT islets.

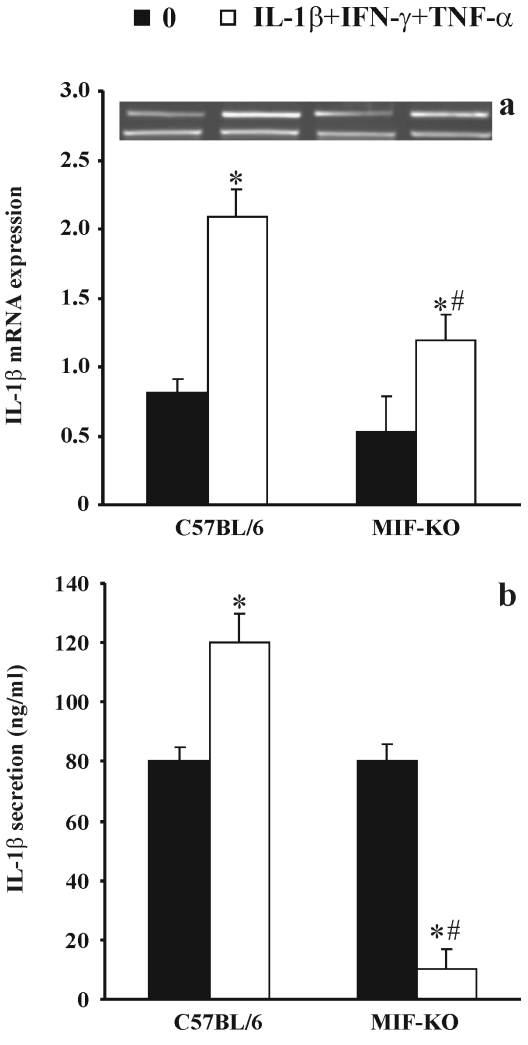

Fig. 1. MIF absence affects IL-1β generation. Pancreatic islets from C57BL/6 or MIF-KO mice were incubated in the presence or absence of a cytokine mixture (IL-1β+IFN-γ+TNF-α- 10 ng/ml each) for determination of IL-1β mRNA expression (in groups of 100) by PCR after 6 h (a), and IL-1β secretion (in groups of 20) by ELISA after 24 h (b). Data are presented as the mean ± SD; * indicates P<0.05 cytokine-treated *vs*. untreated (0), while # indicates P<0.05 MIF-KO *vs*. C57BL/6 islets. Photos of PCR amplicons were displayed above the corresponding graphs; lower lane – β-actin, upper line – gene in question.

As caspase-1 is necessary for the activation of IL-1β, we explored its expression and found that caspase-1 mRNA is not constitutively expressed in the islets of Langerhans of both mouse strains (Fig. 2). In addition, after cytokine treatment the stimulation

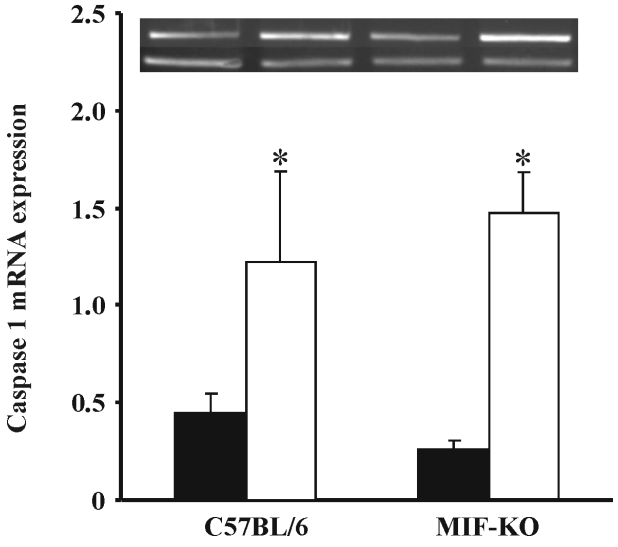

Fig. 2. MIF absence does not affect caspase-1 mRNA expression. Pancreatic islets from C57BL/6 or MIF-KO mice were incubated in the presence or absence of cytokine mixture (IL-1β+IFN-γ+TNF-α- 10 ng/ml each) for determination of caspase-1 mRNA expression (in groups of 100) by PCR after 6 h of incubation. Data are presented as the mean ± SD; * indicates $P<0.05$ cytokine-treated *vs.* untreated (0). Photos of PCR amplicons were displayed above the adequate graphs; lower lane – β-actin, upper line – gene in question.

of caspase-1 mRNA expression was similar in both MIF-deficient and MIF-producing islets (Fig. 2).

DISCUSSION

In diabetes, uncontrolled inflammation may cause cellular dysfunction and aggravate tissue damage. There is evidence that cytokines exist in "cascades" and that interrupting just one cytokine can interrupt the whole cascade (Dinarello, 2010). We previously reported that the absence of MIF could rescue beta cells from the damaging effects of a cytokine-rich *milieu* (Stojanović et al., 2008; Stojanović et al., 2012 and Stojanović et al., in press). In the current study, we demonstrated that the absence of MIF downregulates cytokine-induced IL-1β expression and secretion within pancreatic islets. Since IL-1β is the most potent beta cell cytotoxic cytokine, our results suggest a dominant role for MIF in the final step of the inflammatory cascade within the target tissue, which may lead to beta cell dysfunction and destruction in a setting of localized inflammation.

IL-1β under physiologic circumstances plays an important role in the daily maintenance of beta cell mass and function (Maedler et al., 2006). However, the long-term and pathologically elevated levels of islet IL-1β associated with inflammation of the islet lead to decreased beta cell function and mass (Donath et al., 2010). Therefore, IL-1β is one of the principal cytokines implicated in both T1D and T2D (Mandrup-Poulsen et al., 1986; Dinarello et al., 2010). A key feature of these two diseases is the failure to control the processing and secretion of IL-1β. On the other hand, MIF is known to activate downstream proinflammatory cytokines such as IL-1β, TNF-α, and IL-17, IFN-γ (Flaster et al., 2007; Stojanović et al., 2009). Interestingly, both MIF and IL-1β are produced by the beta cells themselves (Waeber et al., 1997; Heitmeier et al., 2001). In the present study, we found that MIF is the cytokine that directly regulates IL-1β generation within the islet cells. Although IL-1β mRNA expression was upregulated in the cytokine-stimulated MIF-KO islets, it was much lower in comparison to MIF sufficient counterparts. Moreover, the secretion of IL-1β was suppressed in the presence of cytokines, suggesting a block in protein synthesis, activation of IL-1β, or a defect in the secretory mechanism. From this, one may conclude that MIF is the cytokine that mediates a significant loss in beta-cell function and/or mass.

IL-1β activity is tightly controlled and requires the conversion of the primary transcript, the inactive IL-1β precursor, to the active cytokine by limited proteolysis. This can take place extracellularly by serine proteases, or intracellularly by the IL-1-converting enzyme, also known as caspase-1 (Dinarello, 2011). When caspase-1 is activated, the IL-1β precursor is cleaved and secretion takes place. Therefore, the rate-limiting step in the processing and release of active IL-1β may be at the level of this enzyme. However, in contrast to IL-1β mRNA expression, we found that caspase-1 expression was fully preserved in stimulated MIF-KO islets, suggesting that the actions of TNF-α and IFN-γ were suf-

Table 1. Primer pairs sequences

Gene	Primer pairs	PCR product (bp)	GenBank acc. no.
β-actin	5'-TCCTTCTTGGGTATGG-3' 5'-ACGCAGCTCAGTAACAG-3'	358	NM_007393.3
Caspase 1	5'CACAAGACTTCTGACAGTAC-3' 5'-AAGTCACAAGACCAGGCATA-3'	426	NM_009807.2
IL-1β	5'-GAGCATCCAGCTTCAAATCTC-3' 5'-GAAGACAAACCGCTTTTCCATC-3'	520	NM_008361.3

ficient for enzyme induction. IL-1β itself increases the synthesis of caspase-1 as well as its own IL-1β precursor (Karlsen et al., 2000). A similar case may exist for the beta cell (Maedler et al., 2002), although IL-1-induced caspase-1 expression has not yet been demonstrable in the pancreatic islet. Since caspase-1 was fully expressed in the MIF-KO islets, it could be assumed that activation of IL-1β was operative. However, elevated levels of caspase-1 mRNA does not necessarily imply the presence of the active enzyme product necessary for IL-1β maturation, since activation of caspase-1 is tightly controlled by a molecular complex, the inflammasome (Agostini et al., 2004). Indeed, in the pancreas, the intracellular accumulation of ROS due to hyperglycemia activates the NLRP3 inflammasome and promotes the release of IL-1β from beta cells (Masters et al., 2010). Nevertheless, the rate-limiting steps in the secretion of IL-1β of MIF-KO islet cells might be at the transcriptional and translational levels, as well as at the level of secretion itself.

CONCLUSION

The inability of the IL-1β, TNF-α and IFN-γ cocktail to sufficiently activate IL-1β expression and secretion in MIF-KO islet cells proves a dominant and indispensable role of MIF as a key factor of IL-1 generation in the pancreas. These data suggest that MIF-KO islets possess an innate defect in the process of IL-1β synthesis or secretion. Reducing IL-1β activity may hold promise for correcting dysfunctional beta-cell production of insulin in diabetes, including a possibility that the suppression of IL-1β-mediated inflammation in the microenvironment of the islet allows their regeneration.

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