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"Biochemistry of Control in Life and Technology"

Proceedings

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“***Biochemistry of Control in Life and Technology***”

PROGRAMME

10:00-10:10	Welcome message Marija Gavrović-Jankulović (President of the Serbian Biochemical Society)
10:10-10:40	Ario de Marco University of Nova Gorica, Vipava, Slovenia **Nanobodies: Towards rational design of immune-reagents** (FEBS3+ Lecture)
10:40-11:00	Vladimir P. Beškoski Faculty of Chemistry, University of Belgrade **Classical biochemistry / biotechnology and molecular biochemistry / biotechnology of environmental microorganisms in degradation of petroleum products and persistent organic pollutants**
11:00-11:20	Jakub Nowak NanoTemper Technologies, Krakow, Poland **Microscale thermophoresis and NanoDSF as advanced methods in life science research**
11:20-11:50	Coffee break
11:50-12:10	Marija Vidović Institute for Multidisciplinary Research, University of Belgrade **Sun as a stressor and/or regulator of plant metabolism: responses to UV radiation and high light**

12:10-12:30	Vladimir B. Mihailović Faculty of Science, University of Kragujevac **Phytochemical characterization and biological activities of some *Gentiana* plants from Serbia**
12:30-12:50	Marija Genčić Faculty of Science and Mathematics, University of Niš **Phytochemical re-examination of well-studied medicinal plants as an useful approach in the discovery of (novel) potentially bioactive natural products – The case of *Inula helenium* L.**
12:50-13:10	Dalibor Stanković The Vinča Institute of Nuclear Sciences, University of Belgrade **Nano-structured materials and their application in the detection of biological compounds**
13.10-13.40	Poster session
13.40-14.00	Cocktail
14:00-14:20	Ivana Beara Faculty of Sciences, University of Novi Sad **Battle against inflammation: polyphenols targeting selective inhibition of cyclooxygenase-2**
14:20-14:40	Tamara Saksida Institute for Biological Research “Siniša Stanković”, University of Belgrade **The role of macrophage migration inhibitory factor in the development of obesity and altered intestinal permeability**

14:40-15:00	Žanka Bojić Trbojević Institute for the Application of Nuclear Energy - INEP, University of Belgrade **Galectin-1 ligands in human trophoblasts**
15.00-15:20	Discussion and concluding remarks

The role of macrophage migration inhibitory factor in the development of obesity and altered intestinal permeability

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Macrophage migration inhibitory factor (MIF) is a molecule expressed both by the immune cells, like T, B lymphocytes and macrophages, and non-immune cells, like adipocytes, hepatocytes and beta cells of pancreatic islets. It has actions in the innate and adaptive immunity, such as a part in regulating the interleukin-17 expression and production, but also in the development of chemically induced type 1 diabetes in mice. This paper summarizes our results on the role of MIF in the development of obesity and type 2 diabetes, done *in vitro* on beta cell models and murine pancreatic islets, as well as *in vivo*, when mice with MIF deletion (MIF-KO) and their wild type (wt) counterparts were on a high fat diet. It is considered that obesity can develop as a consequence of altered intestinal permeability, so potential leakage of the intestinal barrier is investigated in the MIF-KO and wt mice. Also, the interplay between MIF and regulatory T cells, as an important regulator of inflammation in the adipose tissue, is explored at the level of visceral adipose tissue.

Introduction

Macrophage migration inhibitory factor (MIF) is a cytokine that is ubiquitously expressed, both by the immune and non-immune cells. Importantly, MIF is expressed by pancreatic beta cells and by adipose tissue cells. It is well known for its pro-inflammatory effects on other immune cells: MIF promotes the inflammatory response by inducing the expression of macrophage surface receptors (Toll-like receptor 4, receptor for TNF-α and for interleukin (IL)-1) and by increasing the release of other pro-inflammatory mediators (TNF-α, prostaglandin-E2, cytochrome oxidase-2). Besides from its actions on innate immunity, MIF is implicated in adaptive immunity as it is released by T lymphocytes, it promotes B- and T-cell proliferation and induces expression of CD25 (IL-2R). Also, MIF is recognized as a negative regulator of the immunosuppressive actions of glucocorticoids. In line with this, MIF has been implicated in the development of many acute inflammatory and auto-immune diseases, as well as chronic inflammatory metabolic disorders. Work in our laboratory showed that MIF has a critical role in the immune-mediated beta-cell destruction in the animal model of human type 1 diabetes mellitus [1]. Lymphocytes from mice treated with the MIF inhibitors exhibited reduction of both islet antigen-specific

proliferative responses and adhesive cell-cell interactions. Neutralization of MIF down-regulated the *ex vivo* secretion of the proinflammatory mediators, TNF-α, IFN-γ, and nitric oxide, while augmenting production of the antiinflammatory cytokine, IL-10. MIF can act via its receptor CD74, and controls the recruitment of inflammatory cells via CXCR2 and CXCR4 signaling. Furthermore, MIF can exert pro-inflammatory effects through its enzymatic tautomerase and oxidoreductase activity. MIF is a stimulator of another potent pro-inflammatory cytokine, IL-17 [2], so it could be positioned on the top of a pro-inflammatory cascade. T helper type 17 (Th17) cells produce IL-17 and have a critical role in immunity to extracellular bacteria and the pathogenesis of several autoimmune disorders. The effect of MIF on IL-17 production was dependent on p38, extracellular signal-regulated kinase, Jun N-terminal kinase and Janus kinase 2/signal transducer and activator of transcription 3, and not on nuclear factor-kappaB and nuclear factor of activated T cells signaling [2]. Several lines of evidence provide indications that MIF, besides its role in inflammation, may have a role in energy metabolism. As mentioned, MIF is expressed in metabolically active tissues such as the adipose tissue and the liver. Its expression by adipocytes is regulated by glucose and insulin and it has been shown to have catabolic effects in muscle. Importantly, MIF co-localizes with insulin within the secretory granules of pancreatic beta cells. Our investigations established that MIF binds insulin within beta cell and that pre-incubation of recombinant MIF with insulin promotes the formation of insulin hexamers [3]. Based on these results, it is presumed that MIF enables proper insulin folding which results in insulin full activity.

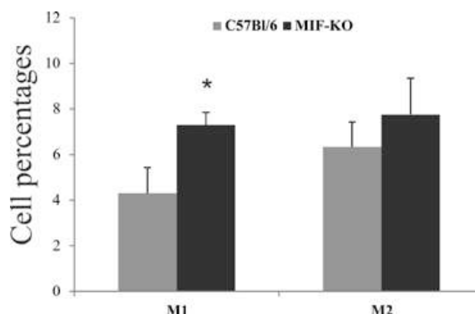

Figure 1. Percentages of M1 ($F4/80^+CD40^+$) and M2 ($F4/80^+CD206^+$) macrophages in visceral adipose tissue of MIF-KO and C57Bl/6 mice measured by flow cytometry. Results obtained from 6 animals per group.*$p<0.05$ represents statistically significant difference versus C57Bl/6 mice.

MIF in obesity and type 2 diabetes

Human studies have shown a positive association between obesity and circulating MIF levels, generally reporting increased circulating MIF in obese individuals compared with healthy lean controls. Also, MIF mRNA expression in the mononuclear cells is elevated in the obese subjects [4]. So, having all this in mind it was of interest to investigate its role in the development of obesity and type 2 diabetes. Starting point were the *in vitro* investigations done on pancreatic islets and insulinoma cell lines, as models for beta cells

of pancreatic islets. When these cells are exposed to high concentrations of saturated fatty acid, palmitic acid, as they are exposed during the progression of these diseases, they succumb to apoptotic death [5]. The cells can be protected by the inhibition of MIF: whether chemical inhibition (ISO-1) is employed, or whether neutralizing anti-MIF antibody is used, or whether we use small interfering RNAs, or it is deleted at gene level (usage of cells from MIF-KO mice), the end result is the same: taking MIF out the equation rescues beta cells from toxic fatty acid insult. Protection from the induced apoptosis is mediated by altered activation of the caspase pathway and had correlated with changes in the level of Bcl-2 family members[5]. Interestingly, ablation of MIF is protective even in the circumstances of the exposure to high glucose concentrations [6]. Diabetes patients or pre-diabetic individuals cannot regulate glucose metabolism and have variations in blood glucose concentration, which is considered as another harmful stimulus for beta cells. *In vitro* investigation that mimics this situation is the exposure of beta cells to high glucose concentrations. Again, MIF neutralization or deletion is a rescue for beta cells[6]. So, one could hope for a similar situation to happen *in vivo*. In order to test this, MIF-KO and wild type control mice were feed a high fat diet. Such an approach is a model for diet induced obesity and type 2 diabetes. High fat feeding of C57Bl/6 mice was accompanied with an up-regulation of MIF in pancreatic islets[5]. On the other hand, MIF-KO mice developed obesity even without high fat food. Starting from the 6th months of their lives they have higher body mass compared to wild type controls [7]. Also, even without high fat feeding they develop glucose intolerance and hyperglycemia. What could underlie such a phenomenon? MIF and glucocorticoids are on a balance: MIF is (so far) the only cytokine that can antagonize anti-inflammatory actions of GCs. On the other hand, when GCs are high, as they are in Cushing's disease, the patients exhibit obesity and metabolic syndrome. So, we measured corticosterone in sera of our MIF-KO colony[8]. In the situation of innate absence of MIF, GCs were higher than in wild type controls. Furthermore, when MIF-KO mice were treated with GC antagonist, RU486, they handled more efficiently glucose burden, measured by the *i.p.* glucose tolerance test. Also, treating mice with the antagonist restored euglycemia [8]. This could be one possible explanation for the observed discrepancy of *in vitro* and *in vivo* results. In the simplified *in vitro* situation MIF ablation may be sufficient to protect beta cells from toxic insults such as fatty acids or glucose, but in the more complicated setting of *in vivo* investigation, one must account for other factors, such as hormones, redundancy of cytokines, etc.

What happens at the level of target tissue, adipose tissue?

During the progression of obesity adipose cells experience hypertrophy and hyperplasia, to cope with the high energy demands forced upon them with the intake of high caloric diet. In a lean individual adipose tissue is held in a quiescent state by the action of both the innate and adaptive immune systems, notably the anti-inflammatory macrophages (type 2 macrophages) and T regulatory (Treg) cells. But, as obesity progresses this fat depot takes on a pro-inflammatory tenor, hosting a variety of innate and adaptive effector-cell types, such as neutrophils, pro-inflammatory macrophages, CD8^{+} T lymphocytes and Th1 cells.

Obesity increases adipose tissue macrophage numbers and these macrophages, not adipocytes, produce majority of cytokines in response to obesity. Namely, these are the pro-inflammatory, type 1 macrophages. Indeed, visceral adipose tissue of MIF-KO mice had significantly higher proportion and number of type 1 macrophages, compared to wild type controls (Figure 1). At the same time with the increase in macrophage numbers, obesity is accompanied by a significant drop in the population of Treg cells in visceral adipose tissue but not elsewhere; and systemic reduction or augmentation of Treg cells increases or decreases adipose-tissue inflammation and insulin resistance, respectively [9,10], thus showing a significant role for Treg cells in metabolic processes. Interestingly, visceral adipose tissue of MIF-KO mice harbored significantly more Treg cells than the visceral adipose tissue of C57Bl/6 mice (Figure 2).

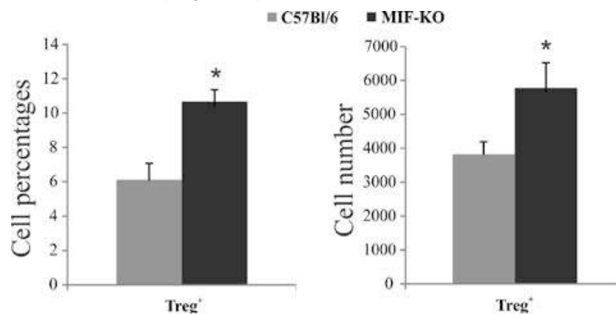

Figure 2. Cell percentages and number of Treg cells in the visceral adipose tissue MIF-KO and C57Bl/6 mice. Treg subpopulation analyzed by flow cytometry as a proportion of $FoxP3^+$ cells within $CD4^+CD25^{high}$ cells. Results obtained from 3 animals per group.*$p<0.05$ represents statistically significant difference versus C57Bl/6 mice.

In order to answer why these cells were so abundantly present in the adipose tissue, we measured their proliferation with BrdU test. The animals received an *i.p.* injection of BrdU, a deoxy analog of thymidine that incorporates to DNA during replication. After 24h proliferation of Treg cells residing in visceral adipose tissue, denoted with FoxP3 transcription factor, was measured on a flow cytometer. As Treg cells of MIF-KO mice had comparable proliferation as the cells of C57Bl/6 mice (Figure 3) we can conclude that the reason for the Treg abundance in the visceral adipose tissue of MIF-KO mice is not their increased *in situ* proliferation. Instead, it might be that the migration of Treg cells to the adipose tissue in MIF-KO mice is increased or that Treg cells are developing by conversion from conventional $CD4^+$ cells. These hypotheses are to be investigated in the future. The functionality of these Treg cells is further questioned, as in spite of their abundance the MIF-KO mice are obese. We tested the IL-10 production of Treg cells in adipose tissue, because IL-10 is the main cytokine of Treg's suppressive action. Indeed, Treg cells isolated from visceral adipose tissue of MIF-KO mice had significantly lower percentages and numbers of $IL\text{-}10^+$ cells (Figure 4). These findings imply a connection between the Treg cells and MIF in the adipose tissue that should be further delineated.

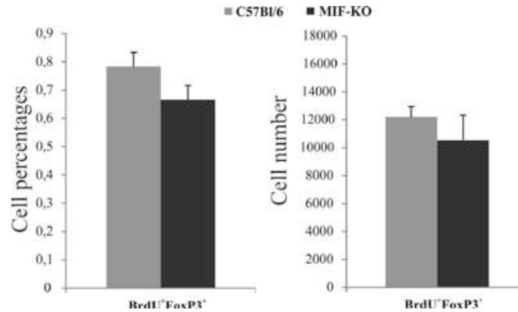

Figure 3. Cell percentages and number of cells positive for BrdU and FoxP3 in the visceral adipose tissue MIF-KO and C57Bl/6 mice. Mice received an *i.p.* injection of BrdU (400mg/kg boy weight) and after 24 hours cells were analyzed on a flow cytometer. Results obtained from 3 animals per group.

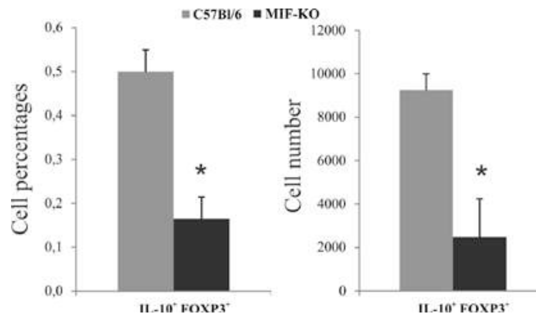

Figure 4. Cell percentages and number of cells positive for IL10 and FoxP3 in the visceral adipose tissue MIF-KO and C57Bl/6 mice measured by flow cytometry. Results obtained from 3 animals per group.*p<0.05 represents statistically significant difference versus C57Bl/6 mice.

How can one connect obesity and intestinal permeability?

Obesity is a health problem of the modern age, it is connected with the way people live (sedentary) and eat (high fat food). High caloric food is a burden for the organism itself, as the organism must process and store the ingested energy. Apart from the obvious hypertrophy and hyperplasia of adipocytes that fat food imposes, such type of food can change the composition of microbiota. There are animal studies that investigated effects of high-fat diets on the intestinal permeability and on the composition of gut microbiota. Consistently, energy-rich high-fat diets enhanced intestinal permeability. This increased intestinal permeability reflected disturbances of the gastrointestinal barrier. When gut barrier is dysfunctional it enables the entry of toxins from the intestinal lumen, such as LPS— a structural part of gram-negative bacteria cell walls. As a consequence, these high blood endotoxins levels can trigger local inflammation or gain access to circulation and induce systemic inflammation through cytokine release. Continuous infusion of endotoxins

or a high endotoxin level induced by a high-fat diet was shown to trigger the development of obesity and insulin resistance [11]. The authors coined the term metabolic endotoxemia, to differentiate it from the higher endotoxinemic levels found in sepsis. To test intestinal permeability of MIF-KO mice, we applied FITC-dextran *per os* to the obese MIF-KO mice. Indeed, MIF-KO mice had a higher incidence of increased intestinal permeability (7 out of 10 tested mice) than C57Bl/6 mice. These results suggest that MIF absence provoked an increase in intestinal permeability that could be linked to increased obesity of MIF-KO mice.

Conclusion

MIF is a pleiotropic molecule with versatile functions. Its role in the development of diabetes and obesity is doubtless. On one hand, MIF deletion/inhibition may be protective for beta cells in the early stages of obesity/type 2 diabetes progression, but its ablation does not seem favorable at the level of adipose tissue. Intestinal permeability is impaired without MIF and this could contribute to the observed adiposity in MIF-KO mice. If Treg cells are indeed less functional without MIF, then the potentiation of MIF may be a novel approach to enhance Treg cells.

Acknowledgements

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