Association of Systemic Concentrations of Macrophage Migration Inhibitory Factor With Impaired Glucose Tolerance and Type 2 Diabetes

Results from the Cooperative Health Research in the Region of Augsburg, Survey 4 (KORA S4)

Christian Herder, phd[1]
Hubert Kolb, phd[1]
Wolfgang Koenig, md[2]
Burkhard Haastert, phd[3]
Sylvia Müller-Scholze, phd[1]
Wolfgang Rathmann, md, msph[3]
Rolf Holle, phd[4]
Barbara Thorand, phd, mph[5]
H.-Erich Wichmann, md, phd[5]

OBJECTIVE — Macrophage migration inhibitory factor (MIF) is a central cytokine in innate immunity. MIF expression can be regulated by glucose and insulin, but data on the association with type 2 diabetes are sparse. The aim of this study was to test whether MIF is associated with impaired glucose tolerance (IGT) and type 2 diabetes and whether these associations are independent of metabolic and immunological risk factors and to compare the associations of MIF and IGT/type 2 diabetes with those of C-reactive protein (CRP) and interleukin-6 (IL-6) with IGT/type 2 diabetes.

RESEARCH DESIGN AND METHODS — The Cooperative Health Research in the Region of Augsburg/Kooperative Gesundheitsforschung im Raum Augsburg, Survey 4 (KORA S4) is a population-based survey performed in Southern Germany (1999–2001). Of 1,653 participants aged 55–74 years, 236 patients with type 2 diabetes, 242 subjects with IGT, and 244 normoglycemic control subjects matched for age and sex were included in this cross-sectional study. Serum concentrations of MIF were measured by enzyme-linked immunosorbent assay.

RESULTS — Serum MIF concentrations are highly increased in individuals with IGT and type 2 diabetes. The associations of MIF with IGT and type 2 diabetes were independent of classical risk factors and of CRP and IL-6 and were much stronger before and after multivariate adjustment than the associations of CRP and IL-6 with IGT and type 2 diabetes.

CONCLUSIONS — Our data suggest that elevations of systemic MIF concentrations precede the onset of type 2 diabetes. This finding may be relevant because MIF has been reported to contribute to the development of type 2 diabetes–related diseases such as atherosclerosis and cancer.

Diabetes Care 29:368–371, 2006

Given the fact that macrophage migration inhibitory factor (MIF) was initially discovered almost four decades ago, surprisingly little is known about the physiological function of this protein. MIF represents an essential proinflammatory mediator of innate immunity in antimicrobial defense and in the stress response (ref. 1 and references therein), but in addition has tautomerase/isomerase and thiol oxidoreductase activities (2) and might participate in antigen presentation (3).

Several studies demonstrated an association of MIF expression with obesity. It has been reported that murine adipocytes express MIF (4), and we recently also identified mature human adipocytes as a cellular source of MIF (5). Importantly, constitutive expression levels were positively correlated with donor BMI, which suggests that MIF may be an obesity-dependent mediator of macrophage infiltration of obese adipose tissue. Ghanim et al. (6) found elevated MIF mRNA in peripheral blood mononuclear cells of obese patients and increased MIF plasma concentrations in obesity, which could be lowered by the antidiabetic drug metformin (7). Although it is indeed known that MIF expression can be regulated by insulin and may be associated with insulin resistance (8–10), data on the potential association between MIF and type 2 diabetes are sparse (11).

Therefore, the two main aims of this case-control study based on the Cooperative Health Research in the Region of Augsburg, Survey 4 (KORA S4) were *1*) to test whether MIF is associated with IGT

From the [1]German Diabetes Clinic, German Diabetes Center, Leibniz Center at Heinrich Heine University, Düsseldorf, Germany; the [2]Department of Internal Medicine II–Cardiology, University of Ulm Medical Center, Ulm, Germany; the [3]Institute of Biometrics and Epidemiology, German Diabetes Center, Leibniz Center at Heinrich Heine University, Düsseldorf, Germany; the [4]Institute of Health Economics and Health Care Management, GSF–National Research Center for Environment and Health, Neuherberg, Germany; and the [5]Institute of Epidemiology, GSF–National Research Center for Environment and Health, Neuherberg, Germany.

Address correspondence and reprint requests to Dr. Christian Herder, German Diabetes Clinic, German Diabetes Center, Leibniz Center at Heinrich Heine University, Auf'm Hennekamp 65, 40225 Düsseldorf, Germany. E-mail: christian.herder@ddz.uni-duesseldorf.de.

Received for publication 8 August 2005 and accepted in revised form 31 October 2005.

Abbreviations: CRP, C-reactive protein; IGT, impaired glucose tolerance; IL-6, interleukin-6; KORA, Cooperative Health Research in the Region of Augsburg; MIF, macrophage migration inhibitory factor; OGTT, oral glucose tolerance test.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Table 1—*Concentrations of circulating MIF, CRP, and IL-6 in the KORA S4 study population*

Immune mediator	Control subjects	IGT	Type 2 diabetes
MIF (ng/ml)	4.97 (2.36–8.46) (n = 241)	7.95 (5.10–12.72) (n = 239)*	10.96 (7.28–15.86) (n = 230)*†
CRP (mg/l)	1.27 (0.68–2.98) (n = 243)	2.39 (1.25–4.44) (n = 241)*	2.52 (1.13–5.63) (n = 228)*
IL-6 (pg/ml)	1.69 (0.68–2.90) (n = 241)	2.32 (1.32–3.54) (n = 240)*	2.48 (1.28–4.80) (n = 230)*

Concentrations are given as median (25th–75th percentile). *$P < 0.001$ vs. control subjects; †$P < 0.001$ vs. IGT subjects (Wilcoxon test).

and/or type 2 diabetes independent of metabolic syndrome–related clinical and immunological risk factors and 2) to compare odds ratios (ORs) of association with those for C-reactive protein (CRP) and interleukin-6 (IL-6) as previously described risk factors of type 2 diabetes.

RESEARCH DESIGN AND METHODS

— The KORA S4 (previously designated KORA Survey 2000 or KORA S2000) study population, laboratory, and statistical methods have been described extensively (12,13). Briefly, the KORA S4 studied a sample of the adult general population of German nationality in the region of Augsburg recruited from October 1999 to April 2001. The study was conducted in accordance with the Declaration of Helsinki as revised in 1996, including written informed consent of all participants and approval by the local ethics committee. In the age range of 55–74 years, 1,653 persons participated in a standardized interview followed by biochemical and clinical analyses. An oral glucose tolerance test (OGTT) and biochemical and immunological analyses were performed as described (12). The standardized protocol for the OGTT required fasting from 10.00 P.M. in the evening before the visit until 8.00–11.00 A.M. the next day. This standardization is important because circulating MIF levels vary by circadian rhythm (14). Diabetes was diagnosed according to the 1999 World Health Organization criteria. A total of 236 individuals with type 2 diabetes (137 men and 99 women) and 242 with IGT (130 men and 112 women) were available for analysis; 244 normoglycemic control subjects (137 men and 107 women) were randomly selected after frequency matching for age and sex. Blood samples were fasting in nondiabetic subjects and in 120 subjects with type 2 diabetes diagnosed during the survey and mostly nonfasting in 116 subjects with type 2 diabetes previously diagnosed by their treating physicians (mean ± SD duration from diagnosis 9.1 ± 6.7 years). Subjects who had positive results for GAD autoantibodies were excluded.

Sociodemographic, clinical, and laboratory measurements

Standardized interviews and medical examinations were conducted by trained medical staff (mainly nurses). All assessment procedures and standard laboratory methods used to determine LDL cholesterol, HDL cholesterol, uric acid, plasma CRP, and serum IL-6 have been described elsewhere in detail (12,13). Hypertension was defined as use of antihypertensive treatment or systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg. Data on CRP and IL-6 have been published before (12). Serum concentrations of MIF were measured by sandwich enzyme-linked immunosorbent assay using an antibody pair and recombinant MIF from R&D Systems (Wiesbaden, Germany). Intra- and interassay variations were <10 and 17.4%, respectively. Samples with MIF or IL-6 concentrations below the detection limit of 34.7 and 0.24 pg/ml, respectively, were assigned a value of 0.5 of the detection limit (n = 10 for MIF; n = 46 for IL-6).

Statistical analyses

Data with Gaussian distribution were described by means ± SD and all other continuous variables by median and 25th–75th percentiles. Differences among subjects with IGT or type 2 diabetes versus control subjects were analyzed by unpaired t test or Wilcoxon test, respectively. For dichotomous variables, absolute numbers were given and the corresponding probabilities were compared with Fisher's exact test (all tests were two-sided). The analysis of the associations of increasing MIF serum concentrations and diabetes risk was assessed after subdivision of the sample into quartiles based on the distribution of normoglycemic control subjects. Multiple logistic regression models were fitted using diabetes or IGT as dependent and the chemokines as independent variables (trend: quartiles as ordinal variables; quartile analysis: quartiles as indicator variables). These models were estimated on the subpopulations of diabetic subjects and control subjects or subjects with IGT and control subjects, respectively. Adjustment for clinical and biochemical confounders was performed by including them as independent variables in the logistic regression models. ORs and 95% CIs were estimated from the logistic regression models. The level of significance was set at 0.05. Calculations were carried out using the SAS statistical package version 8.2 TS2M0.

RESULTS

— The study population (n = 722) is slightly larger than the previously described group used for immunogenetic analyses (n = 704) (15), but both populations do not differ statistically. The lower number is mainly due to missing DNA samples for some individuals, who were, however, included in the serological analysis. Individuals with type 2 diabetes or IGT showed significantly elevated mean or median levels of BMI, waist-to-hip ratio, body fat content, insulin resistance measured by homeostasis model assessment, HbA_{1c}, fasting triglyceride levels, systolic and diastolic blood pressure, uric acid, leukocyte count, CRP, and IL-6, whereas HDL cholesterol concentrations were significantly lower than in normoglycemic control subjects. In addition, patients with type 2 diabetes exhibited significantly elevated fat-free mass and decreased total and LDL cholesterol compared with control subjects (data not shown; see ref. 15). The IGT and type 2 diabetes groups were not significantly different from the control subjects in their smoking behavior, alcohol consumption, or frequency of respiratory infections or other inflammatory conditions during the week before the examination.

Concentrations of circulating MIF, CRP, and IL-6 in the study population

Levels of circulating MIF, CRP, and IL-6 were significantly increased in subjects with IGT or type 2 diabetes compared

Table 2—*Association of MIF, CRP, and IL-6 with type 2 diabetes and IGT (multiple logistic regression models)*

Model	Quartile 1	Quartile 2	Quartile 3	Quartile 4	*P* (trend)
MIF: type 2 diabetic vs. control subjects					
M1	1.0	2.23 (0.71–7.03)	10.54 (3.90–28.43)*	25.57 (9.65–67.76)*	<0.0001
M2	1.0	2.32 (0.70–7.68)	11.83 (4.11–34.04)*	27.30 (9.57–77.87)*	<0.0001
MIF: IGT vs. control subjects					
M1	1.0	3.40 (1.60–7.22)†	4.99 (2.42–10.31)*	7.60 (3.73–15.48)*	<0.0001
M2	1.0	3.11 (1.45–6.69)†	4.93 (2.36–10.30)*	6.87 (3.33–14.20)*	<0.0001
CRP: type 2 diabetic vs. control subjects					
M1	1.0	3.09 (1.48–6.43)†	3.26 (1.58–6.74)†	4.30 (2.12–8.71)*	0.0003
M2	1.0	3.32 (1.49–7.40)†	2.68 (1.22–5.89)‡	3.61 (1.64–7.91)†	0.0089
CRP: IGT vs. control subjects					
M1	1.0	1.29 (0.67–2.49)	2.55 (1.39–4.68)†	2.48 (1.35–4.57)†	0.0006
M2	1.0	1.26 (0.63–2.50)	2.27 (1.21–4.28)‡	2.18 (1.14–4.16)‡	0.0060
IL-6: type 2 diabetic vs. control subjects					
M1	1.0	0.95 (0.49–1.84)	0.99 (0.52–1.86)	1.98 (1.08–3.62)‡	0.0160
M2	1.0	0.73 (0.35–1.51)	0.56 (0.27–1.15)	0.87 (0.42–1.79)	0.6870
IL-6: IGT vs. control subjects					
M1	1.0	1.51 (0.82–2.79)	1.74 (0.96–3.16)	2.25 (1.25–4.05)†	0.0070
M2	1.0	1.37 (0.72–2.62)	1.35 (0.72–2.55)	1.53 (0.81–2.91)	0.2422

Data are ORs (95% CIs) for association of type 2 diabetes or IGT with MIF, CRP, or IL-6. Quartiles are based on data from normoglycemic control subjects (see Table 1 for quartile boundaries). Model 1 was adjusted for BMI, sex, age, hypertension, LDL cholesterol, HDL cholesterol, and uric acid; model 2 is model 1 with additional adjustment for log values of the other two immune mediators. *$P < 0.001$, †$P < 0.01$, ‡$P < 0.05$ vs. quartile 1.

with normoglycemic control subjects ($P < 0.001$ in all cases) (Table 1). MIF concentrations shown as median (25th–75th percentiles) in control subjects, IGT, and type 2 diabetes were 4.97 (2.36–8.46), 7.95 (5.10–12.72), and 10.96 (7.28–15.86) ng/ml, respectively. CRP concentrations in control subjects, IGT, and type 2 diabetes were 1.27 (0.68–2.98), 2.39 (1.25–4.44), and 2.52 (1.13–5.63) mg/l, respectively. IL-6 concentrations in these three groups were 1.69 (0.68–2.90), 2.32 (1.32–3.54), and 2.48 (1.28–4.80) pg/ml, respectively. In contrast to CRP and IL-6, there was also a highly significant increase in MIF concentrations in the type 2 diabetes group compared with the IGT group ($P < 0.001$) (Table 1).

Association of MIF, CRP, and IL-6 with IGT and type 2 diabetes

Table 2 demonstrates the risk for IGT or type 2 diabetes by ORs and 95% CI for increasing quartiles of MIF, CRP, or IL-6 concentrations. After adjustment for sex, age, BMI, hypertension, LDL cholesterol, HDL cholesterol, and uric acid (model 1), MIF, CRP and IL-6 were significantly associated with both type 2 diabetes and IGT. With respect to type 2 diabetes risk, the comparison of extreme quartiles yielded ORs (95% CI) for MIF, CRP, and IL-6 of 25.57 (9.65–67.76), 4.30 (2.12–8.71), and 1.98 (1.08–3.62), respectively. Analogous values for the association with IGT were 7.60 (3.73–15.48), 2.48 (1.35–4.57), and 2.25 (1.25–4.05), respectively.

To test whether these associations were independent of the other immune mediators investigated in this study, the fully adjusted model 2 included the adjustment for the log-transformed concentrations of the other two immune markers. This adjustment had hardly any impact on the extremely strong association of MIF with IGT and type 2 diabetes, attenuated the association of CRP with IGT and type 2 diabetes, and led to nonsignificant results for the association of IL-6 with IGT and type 2 diabetes (Table 2).

CONCLUSIONS — Our data show clearly that individuals with IGT and type 2 diabetes exhibit significantly elevated serum levels of MIF and suggest a stepwise increase of systemic MIF concentrations from normoglycemia to IGT and then to type 2 diabetes. The significance of this finding is underlined by the comparison with the known type 2 diabetes risk factors CRP and IL-6, because *1*) the association of MIF with IGT and type 2 diabetes appears considerably stronger than that for CRP and IL-6 and therefore discriminates better between healthy individuals and patients with IGT or type 2 diabetes before and after multivariate adjustment for metabolic syndrome–related parameters and *2*) this association is independent of these two inflammatory variables. Thus, results of our study considerably extend other evidence from smaller studies published as letters linking elevated concentrations of circulating MIF with insulin resistance (10) and type 2 diabetes (11).

The principal limitation of our case-control study is the cross-sectional design. Hence, we cannot identify true risk factors. However, the assessment of diabetes status by OGTT and the inclusion of subjects with IGT revealed highly significantly elevated MIF levels in IGT. In addition, the use of a well-characterized study population (12,13) allowed the comparison of MIF with CRP and IL-6 and demonstrated the independence of the association of MIF with IGT and type 2 diabetes from these established inflammatory markers.

Several lines of evidence indicate that elevated MIF expression may have a significant impact on the immune status of an individual and the development of various inflammation-related diseases. Essential physiological functions of MIF in natural host defense mechanisms against invading pathogens include regulation of leukocyte infiltration, induction of cell activation, and secretion of proinflammatory cytokines and promotion of survival of inflammatory cells (1). However, elevated MIF concentrations may contribute to hyperinsulinemia in proinflammatory states of insulin resistance because glucose-stimulated release of MIF from islet

β-cells has been reported to enhance insulin secretion in pancreatic β-cell lines and isolated rat islets (16). In addition, MIF participates in the development of atherosclerosis and restenosis in different mouse models (17–19) and regulates cell proliferation and differentiation, promotes tumor growth and neovascularization, and may link type 2 diabetes and increased incidence of cancer (20).

Taken together, our data strongly suggest that elevations of systemic MIF precede the onset of type 2 diabetes, but prospective studies are required to test the hypothesis that measurement of MIF may be useful for disease prediction. The finding of elevated MIF levels in individuals with IGT and type 2 diabetes may be important because MIF is involved in the development of diseases such as atherosclerosis and cancer, which are more prevalent in diabetic patients than in nondiabetic subjects.

Acknowledgments— The KORA research platform was initiated and financed by the GSF–National Research Center for Environment and Health, which is funded by the German Federal Ministry of Education and Research and by the State of Bavaria. The work was supported by the Deutsche Forschungsgemeinschaft, the European Foundation for the Study of Diabetes, the German Federal Ministry of Health and Social Security, the German Federal Ministry of Education, Science, Research and Technology, the Ministry of Science and Research of the State North Rhine-Westphalia and the Department of Internal Medicine II-Cardiology at the University of Ulm. The KORA S4 and, in particular, the diabetes substudy (OGTT) was coinitiated and cofinanced by the German Diabetes Center, Leibniz Center for Diabetes Research at Heinrich-Heine-University Düsseldorf, which is funded by the German Federal Ministry of Health and Social Security and the Ministry of Science and Research of the State North Rhine-Westphalia.

We thank Petra Weskamp and Gerlinde Trischler for expert technical assistance. We appreciate the voluntary contribution of all study participants.

References

1. Calandra T, Roger T: Macrophage migration inhibitory factor: a regulator of innate immunity. *Nat Rev Immunol* 3:791–800, 2003
2. Rosengren E, Bucala R, Aman P, Jacobsson L, Odh G, Metz CN, Rorsman H: The immunoregulatory mediator macrophage migration inhibitory factor (MIF) catalyzes a tautomerization reaction. *Mol Med* 2:143–149, 1996
3. Potolicchio I, Santambrogio L, Strominger JL: Molecular interaction and enzymatic activity of macrophage migration inhibitory factor with immunorelevant peptides. *J Biol Chem* 278:30889–30895, 2003
4. Hirokawa J, Sakaue S, Tagami S, Kawakami Y, Sakai M, Nishi S, Nishihira J: Identification of macrophage migration inhibitory factor in adipose tissue and its induction by tumor necrosis factor-alpha. *Biochem Biophys Res Commun* 235:94–98, 1997
5. Skurk T, Herder C, Kraft I, Müller-Scholze S, Hauner H, Kolb H: Production and release of macrophage migration inhibitory factor from human adipocytes. *Endocrinology* 146:1006–1011, 2005
6. Ghanim H, Aljada A, Hofmeyer D, Syed T, Mohanty P, Dandona P: Circulating mononuclear cells in the obese are in a proinflammatory state. *Circulation* 110: 1564–1571, 2004
7. Dandona P, Aljada A, Ghanim H, Mohanty P, Tripathy C, Hofmeyer D, Chaudhuri A: Increased plasma concentration of macrophage migration inhibitory factor (MIF) and MIF mRNA in mononuclear cells in the obese and the suppressive action of metformin. *J Clin Endocrinol Metab* 89:5043–5047, 2004
8. Sakaue S, Nishihira J, Hirokawa J, Yoshimura H, Honda T, Aoki K, Tagami S, Kawakami Y: Regulation of macrophage migration inhibitory factor (MIF) expression by glucose and insulin in adipocytes in vitro. *Mol Med* 5:361–371, 1999
9. Benigni F, Atsumi T, Calandra T, Metz C, Echtenacher B, Peng T, Bucala R: The proinflammatory mediator macrophage migration inhibitory factor induces glucose catabolism in muscle. *J Clin Invest* 106:1291–1300, 2000
10. Vozarova B, Stefan N, Hanson R, Lindsay RS, Bogardus C, Tataranni PA, Metz C, Bucala R: Plasma concentrations of macrophage migration inhibitory factor are elevated in Pima Indians compared to Caucasians and are associated with insulin resistance. *Diabetologia* 45:1739–1741, 2002
11. Yabunaka N, Nishihira J, Mizue Y, Tsuji M, Kumagai M, Ohtsuka Y, Imamura M, Asaka M: Elevated serum content of macrophage migration inhibitory factor in patients with type 2 diabetes. *Diabetes Care* 23:256–258, 2002
12. Herder C, Haastert B, Muller-Scholze S, Koenig W, Thorand B, Holle R, Wichmann HE, Scherbaum WA, Martin S, Kolb H: Association of systemic chemokine concentrations with impaired glucose tolerance and type 2 diabetes: results from the Cooperative Health Research in the Region of Augsburg Survey S4 (KORA S4). *Diabetes* 54 (Suppl. 2):S11–S17, 2005
13. Rathmann W, Haastert B, Icks A, Löwel H, Meisinger C, Holle R, Giani G: High prevalence of undiagnosed diabetes mellitus in Southern Germany: target populations for efficient screening: the KORA survey 2000. *Diabetologia* 46:182–189, 2003
14. Petrovsky N, Socha L, Silva D, Grossman AB, Metz C, Bucala R: Macrophage migration inhibitory factor exhibits a pronounced circadian rhythm relevant to its role as a glucocorticoid counter-regulator. *Immunol Cell Biol* 81:137–143, 2003
15. Illig T, Bongardt F, Schöpfer A, Müller-Scholze S, Rathmann W, Koenig W, Thorand B, Vollmert C, Holle R, Kolb H, Herder C, members of the Kooperative Gesundheitsforschung im Raum Augsburg/Cooperative Research in the Region of Augsburg (KORA): Significant association of the interleukin-6 gene polymorphisms C-174G and A-598G with type 2 diabetes. *J Clin Endocrinol Metab* 89: 5053–5058, 2004
16. Waeber G, Calandra T, Roduit R, Haefliger JA, Bonny C, Thompson N, Thorens B, Temler E, Meinhardt A, Bacher M, Metz CN, Nicod P, Bucala R: Insulin secretion is regulated by the glucose-dependent production of islet β cell macrophage migration inhibitory factor. *Proc Natl Acad Sci U S A* 94:4782–4787, 1997
17. Schober A, Bernhagen J, Thiele M, Zeiffer U, Knarren S, Roller M, Bucala R, Weber C: Stabilization of atherosclerotic plaques by blockade of macrophage migration inhibitory factor after vascular injury in apolipoprotein E-deficient mice. *Circulation* 109:380–385, 2004
18. Chen Z, Sakuma M, Zago AC, Zhang X, Shi C, Leng L, Mizue Y, Bucala R, Simon D: Evidence for a role of macrophage migration inhibitory factor in vascular disease. *Arterioscler Thromb Vasc Biol* 24:709–714, 2004
19. Pan JH, Sukhova GK, Yang JT, Wang B, Xie T, Fu H, Zhang Y, Satoskar AR, David JR, Metz CN, Bucala R, Fang K, Simon DI, Chapman HA, Libby P, Shi GP: Macrophage migration inhibitory factor deficiency impairs atherosclerosis in low-density lipoprotein receptor-deficient mice. *Circulation* 109:3149–3153, 2004
20. Mitchell RA: Mechanisms and effectors of MIF-dependent promotion of tumourigenesis. *Cell Signal* 16:13–19, 2004