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SHORT COMMUNICATION

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Increased levels of mannan-binding lectin in type 1 diabetic patients with incipient and overt nephropathy

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Abstract *Aims/hypothesis:* Diabetic nephropathy is associated with insulin resistance, and low-grade inflammation and activation of the complement system may contribute to this cascade. Mannan-binding lectin (MBL) activates the complement system, and elevated MBL concentrations have been observed in normoalbuminuric type 1 diabetic patients. The aim of this study was to assess whether MBL is associated with diabetic nephropathy in type 1 diabetes, and whether there is an association between MBL and low-grade inflammatory markers or insulin resistance. *Methods:* A total of 191 type 1 diabetic patients from the Finnish Diabetic Nephropathy Study were divided into three groups based upon their AER. Patients with normal AER (n=67) did not take antihypertensive medication, while patients with microalbuminuria (n=62) or macroalbuminuria (n=62) were all treated with an ACE inhibitor. As a measure of insulin sensitivity we used estimated glucose disposal rate. MBL was measured by an immunofluorometric assay, C-reactive protein by a radioimmunoassay and IL-6 by high-sensitivity enzyme immunoassay. *Results:* Patients with normal AER (median [interquartile range]: 1,154 μg/l [180–2,202 μg/l]) had lower levels of MBL than patients with microalbuminuria (1,713 μg/l [724–2,760 μg/l]; p=0.029) or macroalbuminuria (1,648 μg/l [568–3,394 μg/l]; p=0.019). There was a significant correlation between MBL and estimated glucose disposal rate, but not between MBL and C-reactive protein or IL-6 levels in univariate analysis. However, in a multiple regression analysis, HbA1c was the single variable independently associated with MBL (β±SEM: 0.26±0.08; p=0.003). *Conclusions/interpretation:* MBL concentrations are increased in type 1 diabetic patients with diabetic nephropathy. MBL was not associated with low-grade inflammatory markers.

Keywords Low-grade inflammation · Mannan-binding lectin · Nephropathy · Type 1 diabetic patient

Abbreviations CRP: C-reactive protein · eGDR: estimated glucose disposal rate · FinnDiane: Finnish Diabetic Nephropathy Study · MBL: mannan-binding lectin

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Introduction

The true pathogenetic mechanisms of diabetic nephropathy are not known. However, low-grade inflammation and endothelial dysfunction may be a common denominator of microvascular and macrovascular complications [1–3], and it has been suggested that the activation of the complement system contributes to this cascade of inflammation [4].

The complement system can be activated by three different pathways, of which the lectin pathway probably antedates the classical and alternative pathways [5]. Mannan-binding lectin (MBL) is synthesised by the liver and secreted into the bloodstream [6]. MBL has binding sites for carbohydrates such as mannose and *N*-acetylglucosamine [6, 7]. If the carbohydrates are present in the correct pattern, e.g., as on the surface of micro-organisms, the binding of MBL will result in activation of the complement by MBL-associated proteases [6]. The serum concentration of MBL is highly variable in humans and the variability is largely due to structural polymorphisms leading to amino acid replacements in the collagen-like region, which decreases MBL assembly and stability but is also under the influence of polymorphisms in the promoter region [8].

Patients with type 1 diabetes and normal AER show increased levels of MBL when compared with healthy subjects; further, the MBL concentration was associated with AER even within the normoalbuminuric range while no correlation between MBL and C-reactive protein (CRP) was observed [4].

There are no studies focusing on MBL in incipient and overt nephropathy, where the levels of the low-grade inflammatory markers CRP and IL-6 are elevated when compared with healthy control subjects [3]. It is worth noting that diabetic nephropathy is associated with insulin resistance [9] as is chronic inflammation [10]. However, whether MBL is linked to insulin resistance in diabetic nephropathy is not known.

Therefore, the aim of the present study was to evaluate whether MBL is associated with diabetic nephropathy in type 1 diabetes, and whether there is an association between MBL, low-grade inflammatory markers and insulin resistance.

Subjects and methods

Subjects A total of 191 type 1 diabetic patients were selected from the ongoing nationwide, multicentre Finnish Diabetic Nephropathy Study (FinnDiane) and divided into three groups according to their AER from three consecutive overnight or 24-h urine collections as described earlier [3].

Type 1 diabetes was defined as an onset of diabetes before the age of 35 years and permanent insulin treatment initiated within 1 year of diagnosis. Patients with normal AER were not allowed to be taking antihypertensive medication and were not allowed to have signs of cardiovascular disease. Patients with microalbuminuria and macroalbuminuria were required to be having treatment with ACE inhibitors, which may attenuate low-grade inflammation. Of the initial study population of 1,616 type 1 diabetic patients, 401 met these criteria, and from this cohort we randomly selected 191 patients to be representative of a wide range of AER. The duration of diabetes had to be at least 10 years. The ethical committees of all participating centres approved the study protocol. Written informed consent was obtained from each patient and the study was performed in accordance with the guidelines of the Declaration of Helsinki as revised in 2000.

Methods Data on medication, cardiovascular status, diabetic complications, hypertension and cardiovascular disease were obtained using a standardised questionnaire, which was completed by the patient's attending physician based upon medical files. Blood pressure was measured twice in the sitting position using a mercury sphygmomanometer after a rest of at least 10 min. Height and weight were recorded, and blood was drawn for the measurement of HbA_1c, lipids, creatinine, inflammatory markers and MBL.

HbA_1c and lipids were measured by enzymatic methods at the local hospitals. Serum creatinine was determined using a modified Jaffé reaction. As a measure of insulin sensitivity we calculated the estimated glucose disposal rate (eGDR) using an equation developed by Williams et al. [11], modified for HbA_1c. To define the severity of renal disease in addition to AER we estimated GFR using the Cockroft–Gault formula [12]. CRP was measured by radioimmunoassay and IL-6 by high-sensitivity enzyme immunoassay as previously described [3]. Serum MBL concentrations were measured by an in-house time-resolved immunofluorometric assay [13]. Intra-assay and interassay coefficients of variation were below 5% and 10% respectively.

Statistical analysis Data are expressed as means±SD for normally distributed values, and median (range) or median (interquartile range) for non-normally distributed values. Frequencies are given as percentages. Differences between groups for normally distributed variables were tested using ANOVA, and nonparametric data with the Kruskal–Wallis test. Correlations were calculated using the Pearson correlation coefficient. Multiple linear regression analysis with MBL as the dependent variable was used to assess independent relationships. The distribution of triglycerides, creatinine, AER, CRP, IL-6 and MBL were all skewed and therefore logarithmically transformed before being entered into regression analyses. All calculations were performed using a BMDP statistical package (BMDP Statistical Software, Los Angeles, CA, USA). A p value below 0.05 was considered statistically significant.

Results

The clinical characteristics of the patients are shown in Table 1. Since there were no differences in MBL or low-grade inflammatory markers between males and females, the data are pooled.

MBL concentrations are depicted in Fig. 1. MBL increased in a strikingly similar fashion to CRP. Although there was a significant difference in MBL between normoalbuminuric and microalbuminuric patients (p=0.029) as well as between normoalbuminuric and macroalbuminuric patients (p=0.019), there was no difference between

Table 1 Clinical characteristics of 191 type 1 diabetic patients

	Normoalbuminuria	Microalbuminuria	Macroalbuminuria
n (M/F)	67 (36/31)	62 (36/26)	62 (33/29)
Age (years)	37.1±8.0	36.6±7.9	34.0±7.4[d,g]
Age at onset (years)	14.9±8.2	13.8±8.0	11.6±6.4[e]
BMI (kg/m^2)	24.0±3.0	25.3±2.9[b]	25.3±3.8
WHR	0.85±0.08	0.88±0.08	0.89±0.08[d]
Systolic BP (mm Hg)	129±11	136±19[a]	138±18[e]
Diastolic BP (mm Hg)	78±8	83±11[b]	84±9[f]
eGDR (mg kg^{-1} min^{-2})	7.6±2.1	4.5±1.6[c]	3.9±1.6[f,g]
HbA_1c (%)	8.1±1.2	8.7±1.5[a]	9.4±1.9[f,g]
Total cholesterol (mmol/l)	4.92±0.79	5.07±1.03	5.66±1.26[f,h]
HDL cholesterol (mmol/l)	1.63±0.41	1.60±0.48	1.41±0.46[e,g]
Triglycerides (mmol/l)	0.9 (0.4–2.0)	1.0 (0.5–3.4)	1.5 (0.6–9.1)[f,i]
Creatinine (μmol/l)	82 (47–114)	89 (65–120)[b]	109 (70–675)[f,i]
GFR, Cockcroft–Gault (ml min^{-1}1.73 m^{-2})	101±20	100±23	72±33[f,i]
AER (mg/24 h)*	9 (2–85)	97 (3–418)	719 (10–6,069)
CRP (mg/l)	1.7 (0.1–8.0)	2.5 (0.1–7.8)[a]	2.4 (0.1–18.5)[e]
IL-6 (ng/l)	1.6 (0.3–10.3)	2.0 (0.8–19.5)[a]	2.6 (0.6–16.4)[f, h]

Data are means±SD or median (range)
*Classification of patients was based on three consecutive measurements. The values of AER are taken from the last collection of urine, and the patients with microalbuminuria or macroalbuminuria were all on ACE inhibitors. Normal AER was defined as an AER persistently below 20 μg/min or 30 mg/24 h, microalbuminuria as an AER between 20 and 200 μg/min or 30 and 300 mg/24 h, and macroalbuminuria as an AER above 200 μg/min or 300 mg/24 h in at least two out of three urine collections
[a]$p<0.05$, [b]$p<0.01$, [c]$p<0.001$ normoalbuminuria vs. microalbuminuria
[d]$p<0.05$, [e]$p<0.01$, [f]$p<0.001$ normoalbuminuria vs. macroalbuminuria
[g]$p<0.05$, [h]$p<0.01$, [i]$p<0.001$ microalbuminuria vs. macroalbuminuria

patients with microalbuminuria and those with macroalbuminuria.

In univariate analysis, MBL correlated with eGDR (r=−0.17; $p<0.05$), HbA_1c (r=0.21; $p<0.01$) and HDL cholesterol (r=−0.19; $p<0.05$). However, no significant correlations were observed between MBL and the inflammatory markers CRP (r=−0.01; p=0.92) or IL-6 (r=0.10; p=0.17), duration of diabetes, WHR, systolic or diastolic blood pressure, creatinine, GFR, total cholesterol, triglycerides or AER (data not shown). MBL did not correlate with AER in the combined group of patients with microalbuminuria or macroalbuminuria (data not shown). When

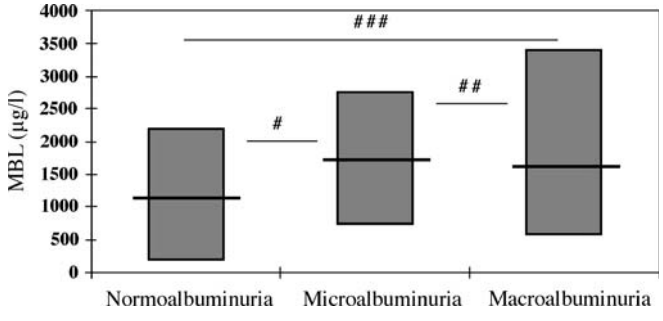

Fig. 1 MBL levels (median with interquartile range) in 191 type 1 diabetic patients without (1,154 μg/l [180–2,202]) and with diabetic nephropathy further divided into incipient (microalbuminuria: 1,713 μg/l [724–2,760]) and overt (macroalbuminuria: 1,648 μg/l [568–3,394]) nephropathy. [#]p=0.029 normoalbuminuria vs microalbuminuria; [##] NS microalbuminuria vs macroalbuminuria; [###]p=0.019 normoalbuminuria vs macroalbuminuria

HbA_1c, eGDR and HDL cholesterol were entered into the multiple regression analysis model, HbA_1c was the only variable independently associated with MBL (β±SEM: 0.26±0.08; p=0.003).

Discussion

We report increased circulating levels of MBL in type 1 diabetic patients with diabetic nephropathy. There was no relationship between MBL and inflammatory markers or between MBL and insulin resistance. In contrast, HbA_1c, a known risk factor for diabetic nephropathy, was independently related to the MBL levels.

The finding that MBL correlated with glycaemic control but not with AER in patients with microalbuminuria and macroalbuminuria contrasts with that shown in patients with normal AER [4]. One possible explanation for these conflicting results may be the difference in HbA_1c concentrations between our patient groups. The fact that MBL was not associated with AER is also in line with the finding that MBL was not associated with either IL-6 or CRP in this study. In this context it is worth noting that we have previously shown an association of AER with the inflammatory markers CRP and IL-6 in diabetic patients with incipient or overt nephropathy [3]. One explanation for the lack of correlation between MBL and inflammatory markers could also come from the simple fact that CRP may inhibit the production of MBL [14, 15]. On the other hand, it has been suggested that MBL and CRP are

coordinated in the acute phase response, since CRP modulates the cascade in which the complement regulatory protein H regulates MBL-initiated cytolysis [15].

Even if we could not confirm a relationship between MBL and eGDR in the multivariate analysis, there was a negative correlation between MBL and eGDR as well as between MBL and HDL cholesterol in the simple regression analysis, suggesting that MBL may be a marker of insulin resistance. Such a notion is supported by the known positive association between insulin resistance and diabetic nephropathy in type 1 diabetes [9].

In diabetic nephropathy we do not yet know whether MBL plays an active role in the pathogenesis or the progression of already prevalent disease. On the other hand, it has been suggested that MBL plays an unfavourable role in other kidney diseases such as IgA nephropathy and Henoch–Schönlein purpura. MBL binding to IgA results in complement activation, and the deposition of MBL in association with IgA nephropathy is found in the mesangial area of the kidneys [16, 17]. IgA is a heavily glycosylated molecule with more mannose type N-linked glycan chains on the cell surface to be recognised and bound by MBL [18]. Whether MBL is also found in the diabetic kidney is still not known, but it can be speculated that MBL ligands are present in diabetic kidneys, leading to a deposition of MBL in the kidney or even other target organs with deleterious effects. The mechanism could be that hyperglycaemia stimulates the production of *N*-acetylglucosamine through the hexosamine pathway, and as a consequence an abundance of various secretory and cell membrane glycoproteins are modified by N-linked glycosylation, enabling these proteins to be targets for MBL.

In conclusion, MBL concentrations are increased in type 1 diabetic patients with diabetic nephropathy. Whether MBL also plays a pathogenetic role is still not known, but its independent association with glycaemic control raises the possibility that it may have deleterious effects. No independent direct associations between MBL and inflammatory markers or insulin resistance were observed.

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References

1. Festa A, D'Agostino R Jr, Howard G, Mykkänen L, Tracy RP, Haffner SM (2000) Inflammation and microalbuminuria in nondiabetic and type 2 diabetic subjects: the Insulin Resistance Atherosclerosis Study. Kidney Int 58:1703–1710
2. Stehouwer CDA, Gall M-A, Twisk JWR, Knudsen E, Emeis JJ, Parving H-H (2002) Increased urinary albumin excretion, endothelial dysfunction, and chronic low-grade inflammation in type 2 diabetes. Diabetes 51:1157–1165
3. Saraheimo M, Teppo AM, Forsblom C, Fagerudd J, Groop P-H (2003) Diabetic nephropathy is associated with low-grade inflammation in type 1 diabetic patients. Diabetologia 46:1402–1407
4. Hansen TR, Thiel S, Knudsen ST et al (2003) Elevated levels of mannan-binding lectin in patients with type 1 diabetes. J Clin Endocrinol Metab 88:4861–4867
5. Ji X, Azumi K, Sasaki M, Nonaka M (1997) Ancient origin of the complement lectin pathway revealed by molecular cloning of mannan binding protein-associated serine protease from urochordate, the Japanese ascidian Halocynthia roretzi. Proc Natl Acad Sci USA 94:6340–6345
6. Kilpatrick DC (2002) Mannan-binding lectin: clinical significance and applications. Biochim Biophys Acta 1572:401–413
7. Kozutsumi Y, Kawasaki T, Yamashina I (1980) Isolation and characterization of a mannan-binding protein from rabbit serum. Biochem Biophys Res Commun 95:658–664
8. Presanis JS, Kojima M, Sim RB (2003) Biochemistry and genetics of mannan-binding lectin (MBL). Biochem Soc Trans 31:748–752
9. Orchard TJ, Chang Y, Ferrell RE, Petro N, Ellis DE (2002) Nephropathy in type 1 diabetes: a manifestation of insulin resistance and multiple genetic susceptibilities? Kidney Int 62:963–970

10. Pickup JC, Mattock MB, Chusney GD, Burt D (1997) NIDDM as a disease of the innate immune system: association of the acute-phase reactants and interleukin-6 with metabolic syndrome X. Diabetologia 40:1286–1292
11. Williams KV, Erbey JR, Becker D, Arslanian S, Orchard TJ (2000) Can clinical factors estimate insulin resistance in type 1 diabetes? Diabetes 49:626–632
12. Cockroft DW, Gault MH (1976) Prediction of creatinine clearance from serum creatinine. Nephron 16:31–41
13. Thiel S, Moller-Kristensen M, Jensen L, Jensenius JC (2002) Assays for the functional activity of the mannan-binding lectin pathway of complement activation. Immunobiology 205:446–454
14. Steel DM, Whitehead AS (1994) The major acute phase reactants: C-reactive protein, serum amyloid P component and serum amyloid A protein. Immunol Today 15:81–88
15. Suankratay C, Mold C, Zhang Y, Potempa LA, Lint TF, Gewurz H (1998) Complement regulation in innate immunity and the acute phase response: inhibition of mannan-binding lectin-initiated complement cytolysis by C-reactive protein. Clin Exp Immunol 113:353–359
16. Matsuda M, Shikata K, Wada J et al (1998) Deposition of mannan binding protein and mannan binding protein-mediated complement activation in the glomeruli of patients with IgA-nephropathy. Nephron 80:408–413
17. Endo M, Ohi H, Ohsawa I, Fujita T, Matsushita M, Fujita T (2000) Complement activation through the lectin pathway in patients with Henoch–Shönlein purpura nephritis. Am J Kidney Dis 35:401–407
18. Van den Steen P, Opdenakker G, Wormald MR, Dwek RA, Rudd PM (2001) Matrix remodeling enzymes, the protease cascade and glycosylation. Biochim Biophys Acta 1528:61–67