Relationships of Plasma Interleukin-18 Concentrations to Hyperhomocysteinemia and Carotid Intimal-Media Wall Thickness in Patients With Type 2 Diabetes

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OBJECTIVE — We compared plasma interleukin (IL)-18 concentrations in patients with type 2 diabetes with those in age-matched control subjects and investigated whether plasma IL-18 was associated with plasma total homocysteine (tHcy) concentration or carotid intimal-media wall thickness (IMT), an early marker of atherosclerosis, in these patients.

RESEARCH DESIGN AND METHODS — We measured plasma IL-18 in 103 type 2 diabetic patients and 45 age-matched control subjects. We also measured patients' plasma tHcy and serum high-sensitivity C-reactive protein (hs-CRP). IMT was evaluated for both common carotid arteries.

RESULTS — Plasma IL-18 was significantly higher in diabetic patients than in control subjects (203 ± 153 vs. 118 ± 37 pg/ml, $P < 0.001$). High IL-18 was defined as equaling or exceeding the mean + 2 SD of plasma IL-18 in control subjects (192 pg/ml). Patients with high IL-18 showed a greater carotid IMT than those with normal IL-18. Carotid plaques were more numerous in diabetic patients with high IL-18 than in those with normal IL-18. Plasma tHcy concentrations were significantly higher in patients with high IL-18 than in those with normal IL-18. Univariate and multivariate analyses showed a strong independent association between tHcy and IL-18. Plasma IL-18 also correlated positively with serum hs-CRP.

CONCLUSIONS — In patients with type 2 diabetes, plasma IL-18 concentrations are greater than in nondiabetic subjects. Plasma IL-18 is an independent determinant of plasma tHcy, which is linked independently with atherosclerotic carotid wall thickening.

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Patients with type 2 diabetes have a high incidence of atherosclerosis, which leads to increased morbidity and mortality from coronary artery disease (CAD), cerebrovascular disease, and peripheral vascular disease (PVD) (1–3). Atherosclerosis is a chronic low-grade inflammatory disease (4–6). Plasma concentrations of several inflammatory markers such as C-reactive protein (CRP) and interleukin (IL)-6 have been linked with future cardiovascular disease (CVD) in a variety of clinical settings (7,8). IL-18 stimulates release of interferon-γ, shows potent activities on inflammatory and vascular cells, and is considered a proinflammatory cytokine (9). Recently, increased expression of IL-18 has been reported in carotid ulcerated atherosclerotic plaques (10), suggesting that IL-18 also plays a role in plaque destabilization. A recent study (11) identified high serum IL-18 concentrations as a strong predictor of death from cardiovascular causes in patients with CAD.

Hyperhomocysteinemia has been associated with atherothrombotic vascular diseases such as CAD, stroke, and PVD (12–14). A previous study (15) demonstrated that moderate hyperhomocysteinemia is a stronger risk factor for CVD in patients with type 2 diabetes than in nondiabetic subjects, suggesting a synergistic effect of diabetes with hyperhomocysteinemia that accelerates the development of atherosclerosis. Although homocysteine can exert vascular toxicity via several mechanisms (16), hyperhomocysteinemia was not associated with serum high-sensitivity CRP (hs-CRP), a marker of low-grade inflammation in previous studies (17,18). Moreover, no reports have examined the associations of plasma IL-18 with CVD or total homocysteine (tHcy) concentrations in patients with type 2 diabetes.

The present study was therefore undertaken to compare plasma concentrations of IL-18 in patients with type 2 diabetes with those in age-matched control subjects and to investigate whether plasma IL-18 is associated with tHcy concentration or carotid intimal-media wall thickness (IMT), an early marker of atherosclerosis (19), in patients with type 2 diabetes.

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Abbreviations: CAD, coronary artery disease; CRP, C-reactive protein; CVD, cardiovascular disease; FPG, fasting plasma glucose; HOMA-IR, homeostasis model assessment of insulin resistance; hs-CRP, high-sensitivity CRP; IL, interleukin; IMT, intimal-media wall thickness; PVD, peripheral vascular disease; tHcy, total homocysteine; UAE, urinary albumin excretion.

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

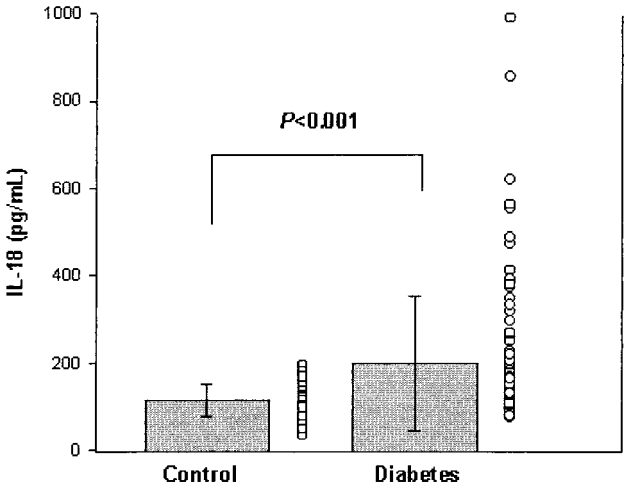

Figure 1—*Plasma IL−18 concentrations in patients with type 2 diabetes and in age-matched nondiabetic control subjects.*

RESEARCH DESIGN AND METHODS

— We studied 103 type 2 diabetic patients (47 women and 56 men). The diabetic patients were referred to the diabetes outpatient clinic at the Dokkyo University Hospital for glycemic control. The diagnosis of type 2 diabetes was made according to the criteria of the World Health Organization. All patients who fulfilled the following inclusion criteria were considered for the study: no episodes of ketoacidosis, initial diagnosis of diabetes at >30 years of age, insulin therapy, if any, initiated after at least 5 years of known disease, and no demonstrable antibodies to glutamic acid decarboxylase. Age and diabetes duration (mean ± SD) were 59.2 ± 12.2 and 11.6 ± 8.2 years, respectively. BMI was 23.7 ± 3.8 kg/m^2. CVD was defined as CAD, stroke, and PVD. CAD was defined as a history of myocardial infarction, coronary artery bypass grafting, or an abnormal coronary angiogram. Stroke was defined as a history of ischemic stroke confirmed by cerebral computed tomography or magnetic resonance imaging. PVD was defined as a history of peripheral artery reconstruction or amputation of a foot. Twenty-six of the diabetic patients had CVD.

Forty-six of the diabetic patients had hypertension, defined as systolic blood pressure >140 mmHg and/or diastolic blood pressure >90 mmHg or, alternatively, as treatment with one or more antihypertensive agents. The latter included ACE inhibitors (n = 14), calcium blockers (n = 24), and angiotensin receptor blockers (n = 7).

As a control group, 45 nondiabetic subjects were selected to match the overall age and sex distribution of the diabetic group. Their age was 55.1 ± 11.3 years, and their BMI was 23.8 ± 3.9 kg/m^2. All patients gave informed consent. The study was approved by the Dokkyo University Institutional Review Board.

Venous blood was obtained between 6:00 and 7:00 A.M. after an overnight fast. Plasma IL-18 concentrations were measured using a commercially available enzyme-linked immunosorbent assay (MBL, Nagoya, Japan) (intra- and interassay coefficients of variation [CV] 5.03 and 6.25%, respectively). Plasma concentrations of IL-6 were measured by a chemiluminescent enzyme assay (CLEIA kit; Fujirebio, Tokyo, Japan) (intra- and interassay CV 5.24 and 6.83%). Serum hs-CRP was determined by an immunonephelometricassay kit (Dade Behring, Marburg, Germany) (intra- and interassay CV 1.72 and 2.80%). Plasma concentrations of tHcy were measured in EDTA-anticoagulanted plasma using high-pressure liquid chromatography (intra- and interassay CV 1.44 and 5.09%). Plasma insulin levels were determined by a radioimmunoassay. The insulin resistance was evaluated by the homeostasis model assessment (HOMA-IR), which was calculated as follows: HOMA-IR = fasting plasma insulin level (μU/ml) × fasting plasma glucose (FPG) (mmol/l)/22.5.

Urinary albumin excretion (UAE) concentrations after a 24-h collection were measured with an immunoturbidimetric assay. According to the rate of UAE in a 24-h collection, normoalbuminuria was defined as UAE <30 mg/24 h (n = 45), microalbuminuria as UAE 30–299 mg/24 h (n = 40), and macroalbuminuria as UAE >300 mg/24 h (n = 18).

Carotid IMT of the common carotid artery was determined using duplex ultrasonography with a high-resolution 7.5-MHz transducer (SSA-380A; Toshiba, Tokyo, Japan). Carotid IMT was defined as the distance from the leading edge of the first echogenic line to the leading edge of the second echogenic line in the sonographic image (20). Measurements of IMT were made at each of the three sites of the greatest thickness on both sides. Carotid IMT was defined as the mean of these maximal IMT measurements. Plaques were defined as the presence of focal, severe wall thickening (IMT >2.0 mm), wall irregularity, and calcification. All scans were performed by a trained physician (U. Iigo).

Statistical analysis

Data are presented as mean ± SD or median and interquartile range. Differences were analyzed by Student's unpaired t test or the Mann-Whitney U test. Correlation was determined by linear regression analysis or multivariate analysis. Significance of differences in prevalence between groups was analyzed by a χ^2 test. Logarithmic transformation of plasma IL-18, hs-CRP, and UAE was used to render the distribution normal for the parametric tests. A P value <0.05 was accepted as indicating statistical significance.

RESULTS

— Plasma concentrations of IL-18 were significantly higher in diabetic patients than in age-matched control subjects (203 ± 153 vs. 118 ± 37 pg/ml, P < 0.001) (Fig. 1). Plasma concentrations of tHcy were also significantly higher in diabetic patients than in age-matched control subjects (8.96 ± 3.04 vs. 6.92 ± 1.36 μmol/l, P < 0.0001). Carotid IMT was significantly greater in diabetic patients than in control subjects (0.89 ± 0.42 vs. 0.59 ± 0.11 mm, P < 0.0001).

Using the mean + 2 SD for IL-18 concentrations in the control group as a cutoff value, patients with IL-18 concentrations ≥192 pg/ml were defined as the high–IL-18 group (n = 37) and those with values <192 pg/ml as the normal–IL-18 group (n = 66). We then compared clinical variables between diabetic patients in the two groups. As shown in Table 1, no differences in age, diabetes duration, or BMI were

Table 1—*Characteristics of type 2 diabetic patients with normal and high plasma IL-18 concentrations*

Variables	Normal IL-18	High IL-18
n (M/F)	66 (32/34)	37 (24/13)
Age (years)	58.2 ± 12.5	60.9 ± 11.5
BMI (kg/m^2)	23.5 ± 3.9	24.0 ± 3.7
Diabetes duration (years)	10.0 (6.0–15.0)	10.5 (3.5–16.0)
FPG (mmol/l)	9.8 ± 3.2	11.5 ± 5.2*
HbA_{1c} (%)	9.5 ± 1.9	9.8 ± 2.2
Total cholesterol (mmol/l)	5.2 ± 1.2	5.4 ± 2.0
Triglyceride (mmol/l)	1.9 (1.3–2.6)	2.1 (1.5–2.8)
HDL cholesterol (mmol/l)	1.3 ± 0.4	1.2 ± 0.3
C-peptide (nmol/l)	0.66 ± 0.40	0.80 ± 0.42
Creatinine clearance (ml/min)	82.3 ± 32.0	71.8 ± 30.4
UAE (mg/24 h)	45 (11–191)	40 (14–130)
tHcy (μmol/l)	8.3 ± 2.6	10.1 ± 3.5†
Mean carotid IMT (mm)	0.75 ± 0.24	1.02 ± 0.55†
Plaque	8 (12)	12 (32)*
Hypertension	26 (29)	20 (54)
CVD	14 (21)	11 (30)
Treatment (D/OHA/insulin)	18/34/14	11/20/6

Data are mean ± SD, median (interquartile ranges), or *n* (%). *P <0.05, †P < 0.01 vs. normal IL-18. D, diet alone; OHA, oral hypoglycemic agents.

present between the two groups. FPG was significantly higher in patients with high IL-18 than in those with normal IL-18 (P < 0.05). We found no differences in blood lipid profile between the two groups. Creatinine clearance was also similar between the two groups. Plasma concentrations of tHcy were significantly higher in patients with high IL-18 than in those with normal IL-18 (P < 0.01). Carotid IMT was significantly greater in patients with high IL-18 than in those with normal IL-18 (P < 0.01). The number of plaques was higher in patients with high IL-18 than in those with normal IL-18 (P < 0.05). However, we found no significant difference in prevalence of CVD between the two groups.

By linear regression analysis, we found a significant correlation between plasma IL-18 and FPG (P < 0.05) or total cholesterol (P < 0.05) in the diabetic patients (Table 2). Furthermore, plasma concentrations of IL-18 correlated positively with serum hs-CRP (r = 0.27, P = 0.0096) (Fig. 2*A*) and with plasma tHcy (r = 0.41, P < 0.0001) (Fig. 2*B*). Plasma concentrations of IL-18 tended to correlate positively with mean carotid IMT (r = 0.22, P = 0.0564) (Table 2).

To determine independent factors for plasma concentrations of IL-18, we performed multivariate analysis controlling for age, diabetes duration, blood pressure, blood lipids, glycemic control, and renal function. In a model that explained 51.1% of variation of plasma IL-18, age (partial coefficient = 0.277, P = 0.045), FPG (partial coefficient = 0.305, P = 0.025), and plasma tHcy (partial coefficient = 0.545, P = 0.000) were independent determinants of plasma IL-18 in patients with type 2 diabetes (Table 3).

Next, we divided the diabetic patients into two groups according to presence of CVD. As shown in Table 4, we found no difference in age, duration of diabetes, glycemic control, or blood lipid profile between diabetic patients with and without CVD. However, creatinine clearance was significantly lower in patients with CVD than in those without CVD (P < 0.05). Both hs-CRP and IL-6 were significantly higher in patients with CVD than in those without CVD (P < 0.01, respectively). However, we found no significant difference in plasma IL-18 concentration between these two groups. Plasma concentrations of tHcy tended to be higher in the patients with CVD than in those without CVD. Hypertension was more prevalent in patients with CVD (P < 0.01).

CONCLUSIONS — The present study demonstrated for the first time that plasma IL-18 concentrations were significantly higher in type 2 diabetic patients than in age-matched control subjects. Mechanisms responsible for elevation of plasma IL-18 in diabetic patients remain to be determined. However, the present study showed that FPG was higher in the patients with high IL-18 than in those with normal IL-18. Furthermore, multivariate and univariate analyses showed a significant positive correlation between plasma IL-18 and FPG. A hyperglycemic glucose clamp study has shown that plasma IL-18 increases within 2 h of clamping in both control subjects and subjects with impaired glucose tolerance (21). However, we found no significant correlation between plasma IL-18 and HbA_{1c}, a measure of average blood glucose concentration during the preceding 6–8 weeks, in the diabetic patients. One possible explanation is that current hyperglycemia, as opposed to chronic hyperglycemia, may affect plasma concentrations of IL-18 in the diabetic patients, since the hyperglycemic clamp study also showed plasma IL-18 concentrations rising to a peak at 2 h and returning to basal levels at 3 h (21). Although hyperglycemia appears likely to contribute to elevation of plasma IL-18 concen-

Table 2—*Univariate analysis of relationships between plasma IL-18 and characteristics of patients with type 2 diabetes*

	IL-18	
Variables	*r*	*P*
Age (years)	0.1113	0.2801
BMI (kg/m^2)	0.1735	0.0990
Diabetes duration (years)	−0.0848	0.4405
SBP (mmHg)	0.1027	0.3381
DBP (mmHg)	0.0893	0.4502
FPG (mmol/l)	0.2405	0.0182
HbA_{1c} (%)	0.0504	0.6274
Total cholesterol (mmol/l)	0.2315	0.0234
Triglyceride (mmol/l)	0.1440	0.1637
HDL cholesterol (mmol/l)	−0.1369	0.1858
HOMA-IR	0.1788	0.0889
Fasting C-peptide (nmol/l)	0.1652	0.1155
Creatinine clearance (ml/min)	−0.126	0.1855
UAE (log_{10} mg/24 h)	0.1027	0.3302
Mean carotid IMT (mm)	0.217	0.0564

DBP, diastolic blood pressure; SBP, systolic blood pressure.

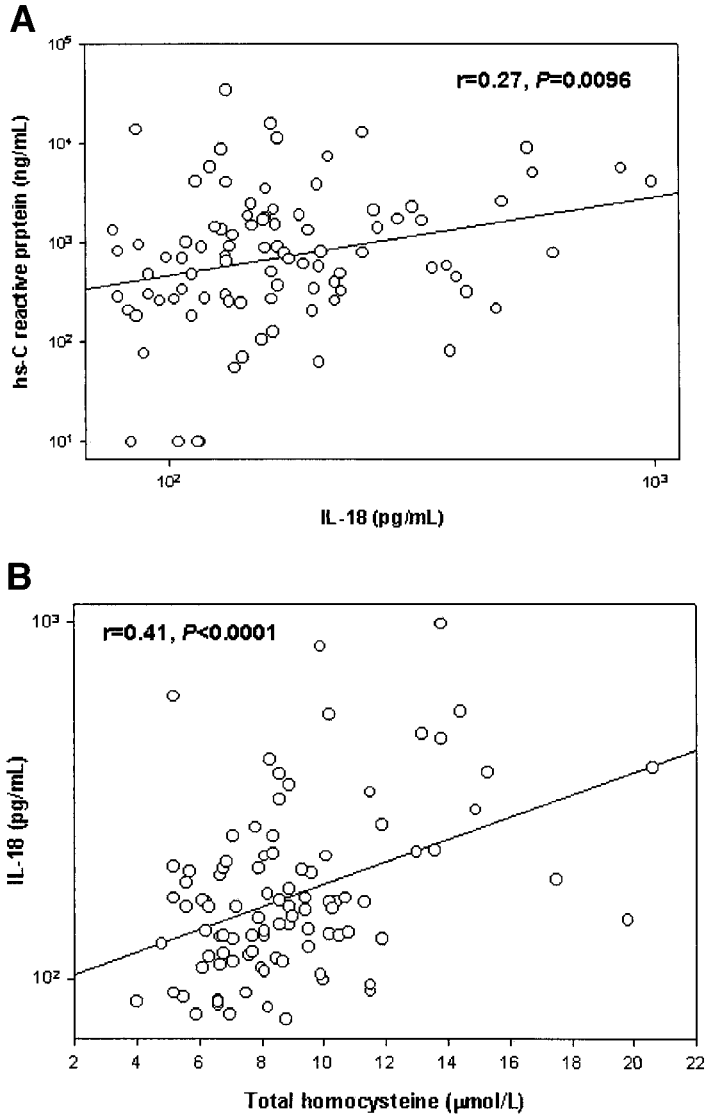

Figure 2—*Correlation between plasma IL-18 concentrations and hs-CRP* (A) *or tHcy* (B) *in patients with type 2 diabetes.*

trations in patients with type 2 diabetes, it would be helpful to evaluate changes in plasma IL-18 concentrations after lowering fasting plasma glucose. We then examined the relationship between plasma IL-18 and insulin resistance, a measure of HOMA-IR, in diabetic patients. We found no significant correlation between plasma IL-18 and HOMA-IR or fasting C-peptide.

We found that plasma concentrations of IL-18 correlated positively with plasma concentrations of tHcy in patients with type 2 diabetes. Furthermore, multivariate analysis showed that plasma tHcy is an independent determinant of plasma IL-18. This is the first study to demonstrate a possible relationship between plasma IL-18 and tHcy in type 2 diabetic patients. Hyperhomocysteinemia is associated with high incidence of CAD, stroke, and PVD (12–14). Several studies have reported that in patients with type 2 diabetes, elevated plasma tHcy is also associated with increased prevalence of CVD (22,23). Furthermore, previous studies have reported that plasma concentrations of tHcy are elevated in type 2 diabetic patients, especially those with diabetic nephropathy (24,25). We also found that plasma concentrations of tHcy were significantly higher in diabetic patients than in control subjects. Taking these findings together, elevated plasma tHcy concentrations may be associated with an increase in plasma IL-18 in patients with type 2 diabetes. Mechanisms underlying the relationship between plasma IL-18 and tHcy concentrations in patients with type 2 diabetes remain unclear. However, since IL-18 is produced mainly by monocytes and macrophages (26), increased plasma homocysteine would be likely to stimulate IL–18 secretion by these cell types. However, effects of homocysteine on IL-18 secretion by cultured monocytes or macrophages have not been reported, so such an in vitro study should be carried out. It would also be helpful to determine whether the lowering of homocysteine concentrations with folic acid would result in lower concentrations of IL-18. In patients with type 2 diabetes, the synergistic effects of hyperglycemia and hyperhomocysteinemia most likely contribute to the elevation of IL-18 in plasma.

Evidence is accumulating that IL-18 is a proatherogenic cytokine associated with the development of CVD. A recent study demonstrated increased IL-18 expression in human atherosclerotic plaques in association with plaque destabilization (10). In a mouse model, exogenous IL-18 administration increased atherosclerotic lesion size in apolipoprotein $E^{-/-}$ mice through release of interferon-γ (27). An in vitro study has shown that IL-18 signaling mediated by IL-18 receptors on endothelial cells or smooth

Table 3.—*Multivariate analysis of relationships between plasma IL-18 concentrations and selected variables in type 2 diabetic patients*

Variables	Partial coefficient	*P*
Age (years)	0.277	0.045
Diabetes duration (years)	−0.054	0.704
BMI (kg/m^2)	0.093	0.510
SBP (mmHg)	−0.155	0.267
DBP (mmHg)	−0.030	0.833
FPG (mmol/l)	0.308	0.025
HbA_{1c} (%)	−0.063	0.655
Total cholesterol (mmol/l)	0.217	0.119
Triglyceride (mmol/l)	−0.100	0.475
HDL cholesterol (mmol/l)	−0.081	0.562
UAE ($\log_{10}$ mg/24 h)	−0.184	0.188
Creatinine clearance (ml/min)	−0.083	0.555
Serum C-peptide (nmol/l)	0.064	0.461
tHcy (μmol/l)	0.545	0.000

R^2 = 0.511 (adjusted multiple R^2 = 0.376). DBP, diastolic blood pressure; SBP, systolic blood pressure.

Table 4—*Demographic clinical and laboratory data for type 2 diabetic patients with or without cardiovascular disease*

	No CVD	CVD
n (M/F)	77 (40/37)	26 (16/10)
Age (years)	57.5 ± 12.7	64.2 ± 9.0
BMI (kg/m^2)	23.5 ± 3.75	24.4 ± 4.08
Diabetes duration (years)	10.0 (6.0–15.0)	10.0 (4.5–14.5)
FPG (mmol/l)	10.2 ± 3.53	11.0 ± 5.45
HbA_{1c} (%)	9.76 ± 2.03	9.20 ± 1.91
Creatinine clearance (ml/min)	82.3 ± 30.7	67.3 ± 31.6*
Total cholesterol (mmol/l)	5.24 ± 1.11	5.48 ± 2.33
Triglyceride (mmol/l)	1.84 (1.30–2.60)	2.01 (1.59–3.05)
HDL cholesterol (mmol/l)	1.26 ± 0.32	1.18 ± 0.36
hs-CRP (log_{10} ng/ml)	2.73 ± 0.66	3.27 ± 0.65†
IL-6 (pg/ml)	2.30 (1.50–3.60)	3.80 (2.65–5.65)†
IL-18 (pg/ml)	152.5 (114.5–205.0)	161.0 (133.0–219.5)
tHcy (μmol/l)	8.7 ± 3.0	10.0 ± 2.8
Hypertension	30 (39)	19 (73)†
Treatment (D/OHA/insulin)	23/42/12	6/12/8

Data are mean ± SD, median (interquartile ranges), or *n* (%). *$P < 0.05$, †$P < 0.01$. D, diet alone; OHA, oral hypoglycemic agents.

muscle cells induced atherogenesis via IL-6 and IL-8 release, as well as expression of adhesion molecules and matrix metalloproteinase (28). Furthermore, a prospective clinical study demonstrated that plasma IL-18 concentration was a strong predictor of cardiovascular death in patients with stable or unstable angina (11). Thus, elevated plasma concentrations of IL-18 might be associated with acceleration of atherosclerosis in type 2 diabetic patients. The present study also showed that diabetic patients with high IL-18 had a greater carotid IMT than those with normal IL-18. Furthermore, numbers of carotid plaques were higher in diabetic patients with high IL-18 than in those with normal IL-18. Carotid IMT, a marker of early atherosclerosis, not only shows a strong relationship with CVD risk factors but also predicts cardiovascular events such as myocardial infarction (29–31). However, we found no significant difference in the prevalence of CVD between the patients with high IL-18 and those with normal IL-18. Conversely, no significant difference in plasma IL-18 was demonstrated between patients with and without CVD. We therefore could not confirm an association of plasma IL-18 with the presence of clinically manifested atherosclerotic disease. These results suggest that elevated plasma IL-18 concentrations may be associated at least with early carotid atherosclerosis, but not advanced atherosclerosis, in patients with type 2 diabetes.

However, plasma tHcy itself has been demonstrated to be an independent risk factor for increased carotid artery wall thickness (32–34). We found that plasma tHcy was significantly higher in patients with high IL-18 than in those with normal IL-18. Thus, we could not determine whether IL-18 or homocysteine was the predominant contributor to increased carotid IMT in type 2 diabetic patients. Although several mechanisms for homocysteine-induced vascular disease have been proposed, including atherogenesis resulting from endothelial dysfunction (35), smooth muscle cell proliferation (36), and hypercoagulation (37), we speculate that inflammation or plaque instability mediated by elevated plasma IL-18 concentration represents a novel mechanism by which hyperhomocysteinemia could accelerate development of atherosclerosis in diabetic patients.

The cross-sectional nature of the relationship between plasma IL-18 and hyperhomocysteinemia or carotid IMT is a major limitation in the present study. The causal relationship cannot be proven by cross-sectional data. A prospective study is required to confirm the causality among plasma IL-18 and hyperhomocysteinemia or carotid IMT.

Increased circulating IL-18 in type 2 diabetic patients, possibly a consequence of elevated tHcy concentrations in plasma, may contribute at a relatively early stage to the development of tHcy-associated atherosclerotic disease. Unlike other cytokines, elevated plasma IL-18 may be associated with atherosclerotic plaque instability, which may cause acute coronary syndrome. Our study implies that practitioners treating type 2 diabetes should be aware of the risk of hyperhomocysteinemia in diabetic patients with high plasma IL-18. Suppression or antagonism of IL-18 might prove clinically beneficial.

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