Increased Circulating Intercellular Adhesion Molecule-1 Levels in Type II Diabetic Patients: The Possible Role of Metabolic Control and Oxidative Stress

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Blood levels of the circulating form of the integrin intercellular adhesion molecule-1 (ICAM-1), malondialdehyde (MDA), and hemoglobin A_{1c} (HbA_{1c}) were studied at baseline and 3 months after improved metabolic control in 25 type II diabetic patients without signs of macroangiopathy, and were compared with those in 15 matched healthy normal controls. Circulating ICAM-1 and MDA levels were increased in diabetic patients, both at baseline and 3 months later. However, with improving metabolic control HbA_{1c}, circulating ICAM-1, and MDA significantly decreased. A significant correlation between circulating ICAM-1, HbA_{1c}, and MDA was found in diabetic patients at each time. Multiple regression analysis considering circulating ICAM-1 as the dependent variable and HbA_{1c} and MDA as independent variables, showed a significant correlation between the three variables at each time. Similar correlations were found in control subjects. These data show increased levels of circulating ICAM-1 in type II diabetic patients, independent of the presence of macroangiopathy. Moreover, these results suggest that oxidative stress and metabolic control might participate in determining increased circulating ICAM-1 levels in both type II diabetic patients and normal subjects.

INTERCELLULAR ADHESION molecule-1 (ICAM-1) is one of the most important intercellular adhesion molecules.[1] ICAM-1 expression is normally present at low levels on several cell types, but can be upregulated by a variety of inflammatory stimuli.[2] It is well known that inflammation is accompanied by oxidative stress,[3] and oxidative stress has been recently hypothesized to play an important role in the pathogenesis of diabetic complications.[4-6] A circulating form of ICAM-1 (c-ICAM-1) has been described,[7] and elevated levels of this molecule have been reported in recent-onset insulin-dependent diabetes.[8]

In this study, we evaluated cICAM-1 levels in type II (insulin-independent) diabetic patients and the influence of glucose control and oxidative stress on this parameter.

SUBJECTS AND METHODS

Twenty-five type II diabetic patients on diet and hypoglycemic-agent therapy (11 men and 14 women aged 58.9 ± 1.4 years [mean ± SE]; duration of diabetes, 11.0 ± 1.3 years; body mass index [BMI], 26.2 ± 1.2) and selected according to National Diabetes Data Group criteria[9] gave informed consent to this study, which was approved by the Ethics Committee of our Institution. Fifteen healthy normal subjects matched for sex (seven men and eight women), age (59.4 ± 1.6 years), and BMI (25.9 ± 1.4) served as a control group.

None of the selected subjects of this study were on antioxidant supplementation or had clinically symptomatic macroangiopathy, as judged by pathological changes in the resting electrocardiogram, or a previous history of cardiac angina, intermittent claudication, or myocardial or cerebral infarction. None had microalbuminuria or macroalbuminuria.

In all subjects, fasting plasma glucose, hemoglobin A_{1c} (HbA_{1c}), malondialdehyde (MDA), cholesterol, triglycerides, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, and cICAM-1 levels were evaluated at the start of the study. Better blood glucose control was attempted after the initial studies in each patient, by the use of two or three injections of unmodified (soluble, regular) human insulin and mixtures of unmodified and NPH human insulin. All patients performed blood glucose monitoring on themselves using a dexstrometer (Boehringer Mannheim, Milan, Italy), and came to our unit weekly for assessment of the therapy. All parameters were then evaluated after 3 months of improved metabolic control.

Plasma glucose was assayed by the glucose oxidase method. HbA_{1c} level was measured by aminophenylboronic acid affinity chromatography.[10] Cholesterol and triglyceride concentrations were measured enzymatically on a clinical chemistry analyzer (MONARCH; Instrumentation Laboratory, Lexington, MA). HDL cholesterol level was measured after precipitation of apolipoprotein B–containing lipoproteins with heparin sulfate and manganese chloride.[11] LDL cholesterol level was calculated from the Friedewald formula.[12] MDA was evaluated by the high-sensitivity fluorometric method as reported by Conti et al.[13] Intraassay and interassay coefficients of variation for this method were 6.5% and 8.8%, respectively.

cICAM-1 plasma levels were evaluated by an enzyme-linked immunosorbent assay method (British Bio-technology, Abington Oxon, UK). Statistical analysis was performed by paired and unpaired Student's t test and simple and multiple regression analysis.

RESULTS

cICAM-1, MDA, and triglyceride levels were significantly increased in diabetic patients in comparison to controls, both at baseline and after 3 months of improved metabolic control (Table 1). However, at that time, cICAM-1, HbA_{1c}, triglycerides, and MDA were significantly decreased in diabetic patients with respect to baseline values (Table 1). A significant direct correlation between cICAM-1 levels and HbA_{1c} was found in these patients at both periods of examination ($r = .61$, $P < .001$ and $r = .52$, $P < .008$, respectively; Fig 1). Moreover, a

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Table 1. cICAM-1 and MDA Plasma Levels in Diabetic Patients and Control Subjects

	MDA (μmol/L)	cICAM-1 (ng/mL)	HbA_{1c} (%)	Triglycerides (mmol/L)	Cholesterol (mmol/L)	HDL (mmol/L)	LDL (mmol/L)
Control subjects (n = 15)	0.40 ± 0.02	187.1 ± 6.5	5.2 ± 0.1	1.4 ± 0.1	5.7 ± 0.8	1.06 ± 0.06	3.9 ± 0.8
Diabetic patients (n = 25)	0.65 ± 0.03†	249.5 ± 8.4†	8.1 ± 0.3	2.1 ± 0.2	5.8 ± 1.2	1.06 ± 0.08	4.1 ± 0.9
Baseline	$P < .001$	$P < .002$	$P < .01$				
3 months later	0.50 ± 0.03*	220.2 ± 8.5*	7.6 ± 0.3	1.8 ± 0.1	5.7 ± 1.1	1.08 ± 0.07	4.2 ± 1.0

NOTE. Data are expressed as the mean ± SE.

*$P < .05$ *v* controls.

†$P < .001$ *v* controls.

significant correlation was also found between cICAM-1 levels and MDA ($r = .47$, $P < .01$ and $r = .49$, $P < .01$, respectively) and between MDA and HbA_{1c} ($r = .52$, $P < .008$ and $r = .40$, $P < .05$, respectively). A linear correlation between cICAM-1 and triglycerides was shown at baseline ($r = .54$, $P < .006$), but not after 3 months of treatment ($r = .38$, P = NS). No correlation was found between cICAM-1 levels and disease duration ($r = .03$ and $r = .07$, respectively), age ($r = .14$ and $r = .24$, respectively), and BMI ($r = .17$ and $r = .12$, respectively).

In normal subjects, a direct correlation was found between cICAM-1 plasma levels and both HbA_{1c} ($r = .63$, $P < .01$) and MDA ($r = .66$, $P < .001$; Fig 2). No correlation was found between cICAM-1 and age ($r = .18$) and BMI ($r = .11$).

Multiple regression analysis with cICAM-1 as the dependent variable and HbA_{1c} and MDA as independent variables was significant in diabetic patients in both periods of examination (F ratio = 7.67, $P < .003$ and F ratio = 6.41, $P < .006$, respectively) and in normal healthy subjects (F ratio = 4.89, $P < .03$).

DISCUSSION

Multiple lines of evidence indicate that inflammatory reactions are modulated by the interaction of circulating leukocytes with adhesion molecules on the luminal surface of blood vessels. These vascular cell adhesion molecules arrest circulating leukocytes and thus perform the first step in their recruitment to infected or otherwise inflamed tissue sites.[1] In recent studies, elevated cICAM-1 levels have been described during ongoing inflammation or tissue damage.[7] Increased cICAM-1 levels have been described in patients with recent-onset insulin-dependent diabetes and in first-degree relatives at risk for it.[8] These data suggested that elevated cICAM-1 in these subjects may reflect ongoing immune processes.[8]

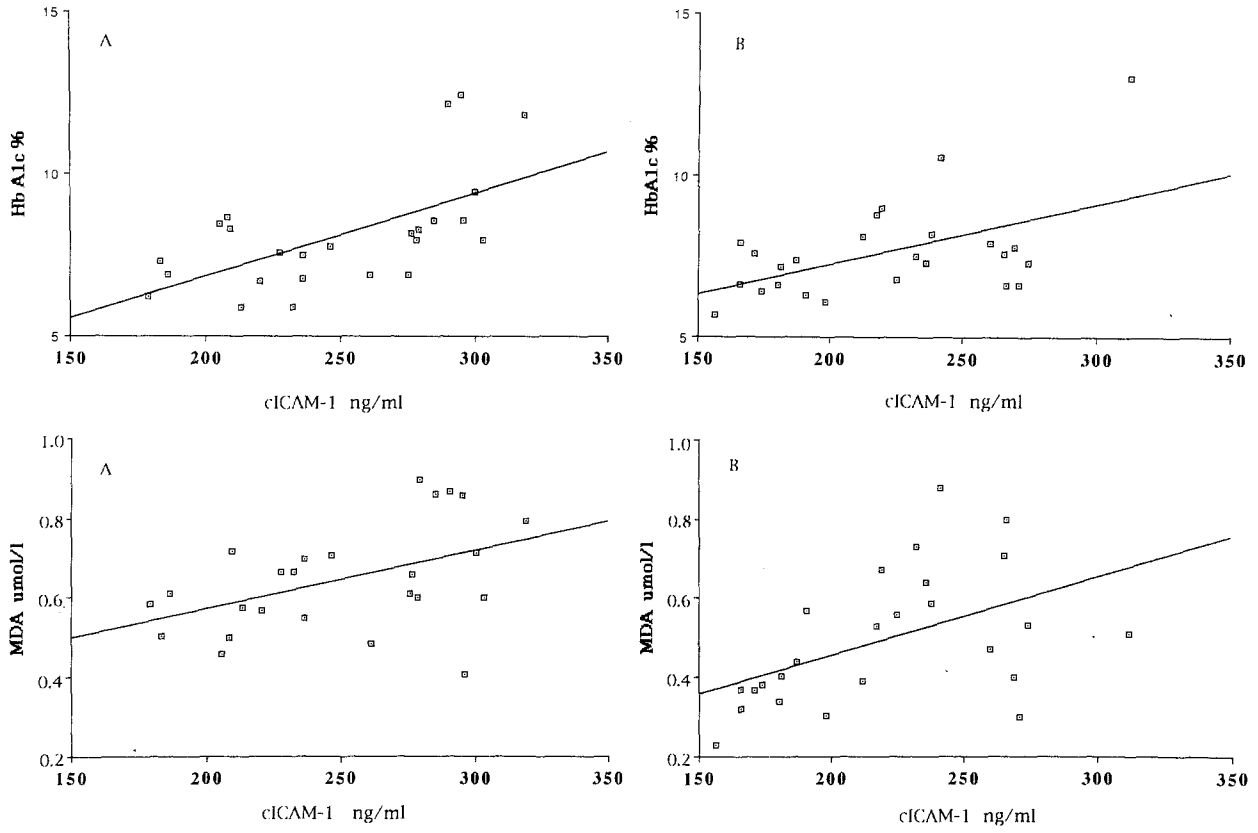

Fig 1. Correlation between circulating ICAM-1 levels and HbA_{1c} and MDA in type II diabetic patients at baseline (A) and 3 months later (B).

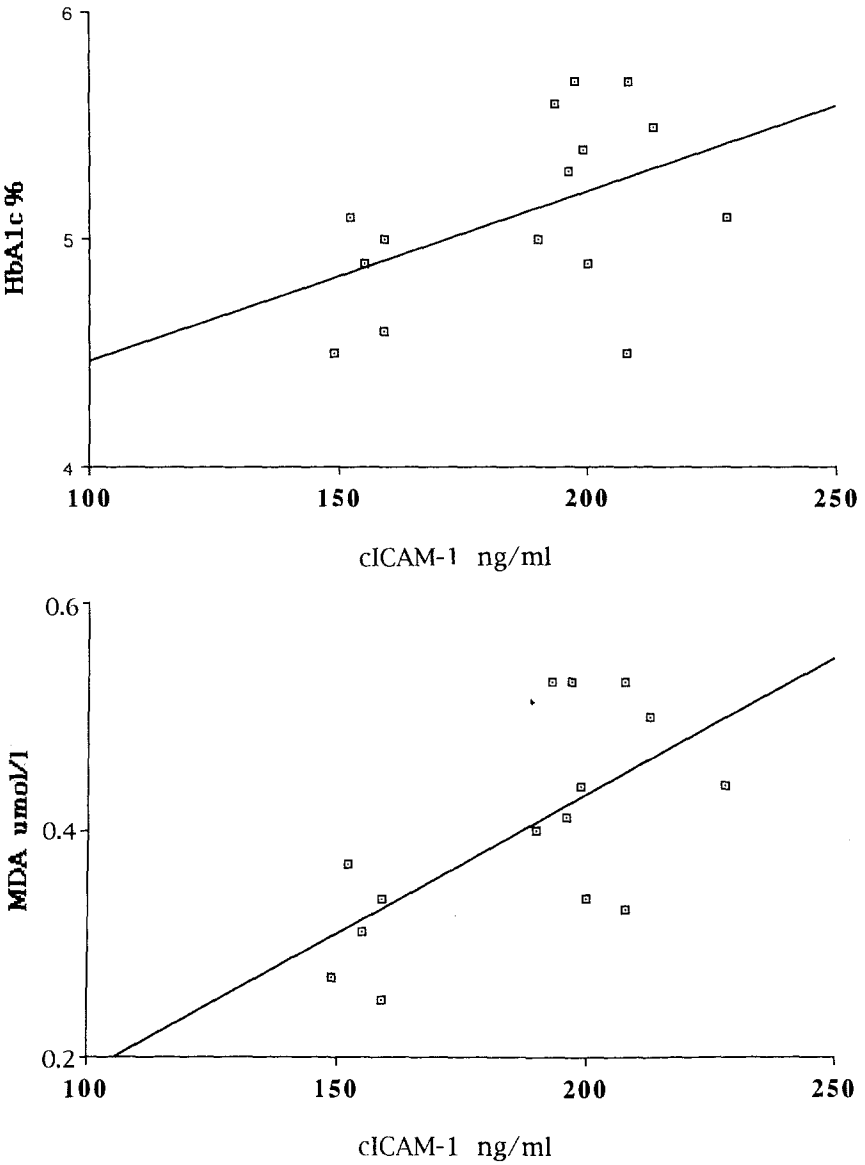

Fig 2. Correlation between circulating ICAM-1 levels and HbA_{1c} and MDA in healthy normal subjects.

In our study, we demonstrated elevated cICAM-1 levels in type II diabetic patients, independent of the presence of macroangiopathy. This result seems of particular interest, since ICAM-1 overexpression has been implicated in atherogenesis, even in diabetes mellitus.[14] The increase in cICAM-1 levels thus might reflect an early phase of activation of the atherogenetic process, which is a common phenomenon in diabetic patients.

cICAM-1 levels correlated with HbA_{1c} in normal subjects and in diabetic patients at both periods of observation. cICAM-1 levels decreased, improving metabolic control in diabetic patients. These results suggest that glycemic control may influence cICAM-1 plasma levels. These data might be considered consistent with the evidence that blood glucose has been proved to be associated with increased incidence of cardiovascular disease in both diabetic[15-16] and nondiabetic subjects.[17-18]

It has been recently hypothesized that oxidative stress has an important pathogenetic role in the development of both microangiopathic and macroangiopathic complications in diabetes mellitus.[4-6] It is well known that oxidative stress is present in the inflammatory processes[3] and participates in the development of atherosclerosis.[19] MDA is the most common tool to assess oxidative stress in humans.[13] In our study, and according to many previous studies,[6] MDA levels are increased in diabetic patients, suggesting that in these subjects an oxidative stress is present. Moreover, our data confirm that with improving metabolic control in diabetic patients, MDA decreases.[20] MDA levels correlated with cICAM-1 concentrations in both normal and diabetic subjects. These data led us to hypothesize that oxidative stress also might condition the increase in cICAM-1 levels.

Since it is well known that diabetic subjects have endothelial cell dysfunction,[21] the increase of cICAM-1 levels may be a reflection of endothelial cell dysfunction in diabetes. This point of view is consistent with the evidence that free

radicals may be the mediators of the (proatherogenic) endothelial cell dysfunction caused by elevated glucose.[22-25] Multiple regression analysis supports the hypothesis that both glucose and oxidative stress may cooperate in generating increased cICAM-1 plasma levels in diabetes.

Our data show that increased cICAM-1 plasma levels are present in type II diabetic patients. They correlate with HbA_{1c} and MDA in both normal and diabetic subjects. With improving metabolic control in diabetic patients, cICAM-1 levels decrease. Since ICAM-1 overexpression has been involved in the atherogenesis, our finding suggests that oxidative stress and metabolic control may play an important role as pathogenetic factors in the vascular complications of diabetes mellitus.[6]

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