

Platelet-Monocyte Aggregates are Independently Associated with Occurrence of Carotid Plaques in Type 2 Diabetic Patients

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Recent evidence suggests important roles for platelet activation in the progression of atherosclerosis. We have recently shown that P-selectin expression or the presence of platelet-monocyte aggregates, a well-characterized marker of platelet activation, is associated with carotid atherosclerosis in the general population. It is not clear, however, whether platelet activation is also associated with carotid atherosclerosis in patients with type 2 diabetes. In the present study, we measured circulating levels of platelet-monocyte aggregates in 120 patients with type 2 diabetes and 120 age- and gender-matched non-diabetic subjects, and examined their association with carotid atherosclerosis determined by arterial ultrasound. The percentage of platelet-monocyte aggregates was analyzed by CD41-positivity determined by whole-blood flow cytometry. Diabetic subjects ($7.73 \pm 4.04\%$, mean \pm SD) showed significantly higher percentages of platelet-monocyte aggregates than non-diabetic subjects ($6.03 \pm 4.38\%$). The percentage of these aggregates was significantly and positively correlated with HbA_{1c} in both diabetic and non-diabetic subjects, with the association independent of other clinical factors. Logistic multiple regression analyses revealed that platelet-monocyte aggregates were significantly associated with the presence of carotid plaques independent of the status of glycemic control in diabetic subjects. Thus, an increase in platelet-monocyte aggregation in type 2 diabetic patients appears to be involved in the pathophysiology of carotid atherosclerosis. *J Atheroscler Thromb*, 2005; 12: 344–352.

Key words: Platelet activation, Atherosclerosis, Flow cytometry, Pathophysiology

Introduction

Cardiovascular events are known to be more frequent in diabetic patients (1, 2). Platelet activation resulting from plaque disruption is important in the pathogenesis and clinical outcome of arterial thrombotic diseases including acute coronary syndrome (3). Altered platelet func-

tion reported in diabetic patients appears to be involved in the pathogenesis and clinical outcome of arterial thrombotic diseases (reviewed in) (4, 5). There have been three studies revealing the beneficial effects of aspirin, an anti-platelet agent, on cardiovascular events (6–8). Based on these trials, the American Diabetes Association has recommended low-dose aspirin therapy as a primary prevention strategy in diabetics at high risk for cardiovascular events (9). There is also a question of “aspirin resistance” in diabetic patients (10, 11), making further study fundamental to understand more about the pathophysiology of platelet function in diabetic patients.

Activated platelets can bind and form aggregates with leukocytes, and numbers of these aggregates are shown to be increased in patients with coronary artery disease

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(12, 13). Platelet P-selectin, a 140-kDa selectin family member (14), and the leukocyte counter-receptor P-selectin glycoprotein ligand-1 (PSGL-1) (15) appear to be involved in the formation of aggregates, and may alter leukocyte recruitment and activation patterns (16–18). The expression of P-selectin has been considered a good marker of platelet activation, since P-selectin translocates to the surface of the plasmatic membrane from the α -granule membrane upon platelet activation. Increased percentages of P-selectin-positive platelets in diabetic patients have been reported by several groups including ours (19–21). It has been proposed that platelet-monocyte aggregates may be a more sensitive marker of platelet activation than platelet P-selectin expression, since degranulated platelets rapidly lose surface P-selectin *in vivo* (22, 23).

Recently, evidence has emerged that activated platelets contribute to the progression of atherosclerosis in apoE-deficient mice (24–26). We have also found in a human study that the expression of P-selectin on platelets is positively associated with the thickness of the atherosclerotic wall in the carotid artery (21). These findings suggest that activated platelets play crucial roles in the progression of spontaneous atherosclerosis as well as arterial thrombosis. However, the relation between platelet activation and atherosclerotic parameters in diabetic patients has not been reported.

The purpose of the present study was to examine the association of platelet-monocyte aggregates in 120 patients with type 2 diabetes, and compare the results with those for 120 age- and gender-matched non-diabetic subjects. We found that the percentage of platelet-leukocyte aggregates was significantly increased in diabetic patients. Moreover, we showed for the first time that the level of platelet-monocyte aggregates is an independent factor associated with the occurrence of carotid plaques even in diabetic subjects.

Subjects

This study was approved by the Ethics Committee at Osaka City University Graduate School of Medicine (approval No. 307), and informed consent was obtained from all subjects enrolled in the study. Type 2 diabetic subjects ($n = 120$; 60 males and 60 females) and age-, gender-matched non-diabetic control ($n = 120$; 60 males and 60 females) were the participants of a medical check performed at the Diabetes Center in Osaka City University Hospital (Osaka, Japan) and the Osaka Health Promotion Center (Osaka, Japan), respectively. Clinical characteristics are summarized in Table 1. Type 2 diabetes was defined by the criteria proposed by the American Diabetes Association (27). The diabetic group had a significantly higher body mass index, higher systolic blood pressure, and lower plasma level of HDL-cholesterol. The

diabetic group also contained more hypertensive subjects (blood pressure higher than 140/90 mmHg or the use of agents for treatment) and less subjects with hypercholesterolemia (total cholesterol higher than 5.2 mmol/l or the use of any drugs for treatment).

Methods

Measurement of platelet-monocyte aggregates and platelet P-selectin expression

Platelet-monocyte aggregates were analyzed by whole-blood flow cytometry (Becton-Dickinson FACS Calibur) essentially as described previously (23). In brief, peripheral blood samples were collected in 0.313% sodium citrate and fixed in 1.0% formaldehyde/PBS for 30 minutes at 4°C. The samples were then diluted 4.6-fold with distilled water to lyse the erythrocytes. After being washed twice with FACS buffer (0.2% bovine serum albumin, 0.1% sodium azide/PBS), the cells were stained with anti-CD14, a monocyte marker, and anti-CD41 antibody as a platelet identifier. Typical flow cytometric plots for CD14 and CD41 double-staining are shown in Fig. 1. In preliminary experiments, a majority of the cells in the monocyte gate (Fig. 1A) were CD14-positive ($94.2 \pm 2.7\%$, $n = 18$, mean \pm SD). Thus, CD14 staining was omitted for analyzing the samples in this study. The percentage of monocyte-platelet aggregates was identified in single parameter histograms of anti-CD41-PE fluorescence displaying events from the monocyte gate (Fig. 1B). The positive region was determined using a PE-conjugated IgG isotypic control (Fig. 1B). All antibodies were purchased from Beckman Coulter, Inc. The level of P-selectin expressed on platelets was determined as described previously (21).

Ultrasonography

Ultrasonographic scanning of the carotid artery was performed using an ultrasonic phase-locked echotracking system, which was equipped with a high-resolution real-time 10-MHz liner scanner (SSD 650 CL, Aloka Co Ltd). In brief, each subject was examined in the supine position, and the examination included the carotid bulb and 4 cm of the right common carotid artery. Both the near and far walls of these arterial segments were scanned longitudinally and transversely to assess the occurrence of plaques, which were defined as localized echo structures that encroached into the vessel lumen and for which the distance between the media-adventitia interface and the lesion surface facing the lumen was ≥ 1 mm (21, 28).

Biological analyses

Serum levels of creatinine, total cholesterol, and HDL-cholesterol were measured by enzymatic methods adapted to an autoanalyzer (Hitachi 7470; Hitachi). Non-HDL-cholesterol was calculated by subtracting HDL-cho-

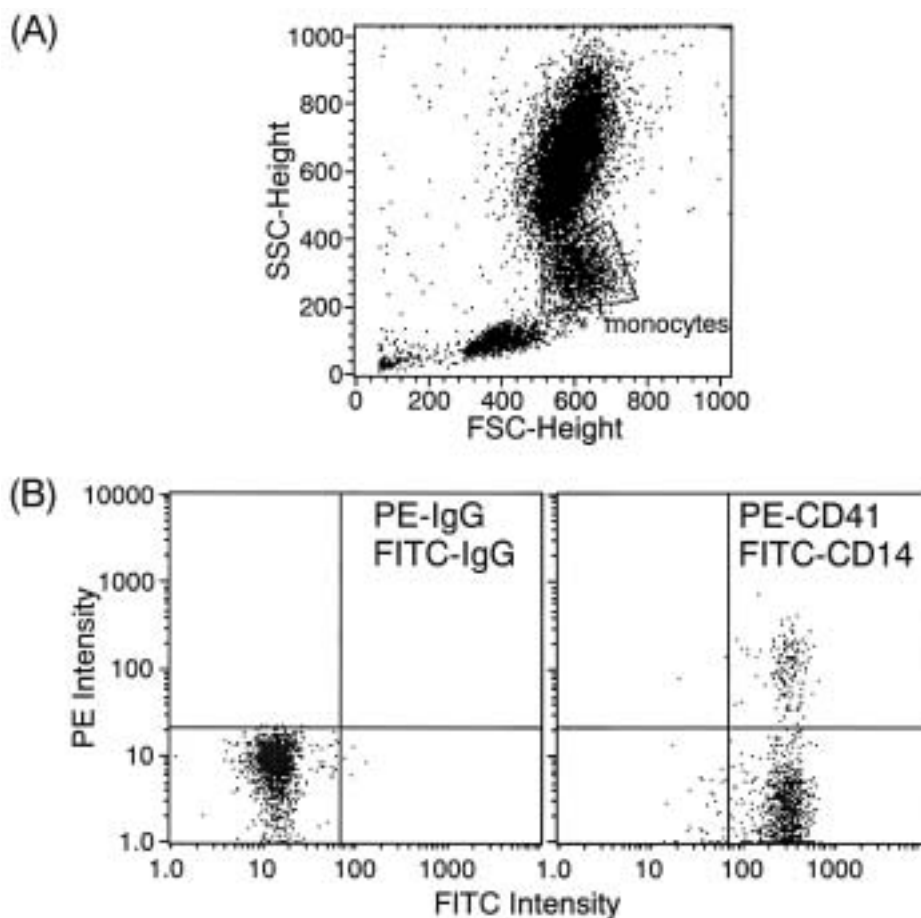


Fig. 1. Flow cytometric analyses of platelet-monocyte aggregates. Blood samples were double-stained with fluorescein isothiocyanate (FITC)-labeled anti-CD14 (monocyte marker) and phycoerythrin (PE)-labeled anti-CD41 (platelet marker), or FITC-labeled IgG1 and PE-labeled IgG1 as controls. Monocytes were identified by their characteristic light scattering properties (Fig. 1A), and cells in the window were analyzed for positivity of CD14 and CD41 (Fig. 1B). FSC: forward scatter, SSC: side scatter.

lesterol from total cholesterol. Plasma glucose levels were measured by the glucose oxidation method and glycohemoglobin (HbA_{1c}) by high pressure liquid chromatography (normal range, 4.0% to 5.8%).

Statistical analyses

All data are expressed as the mean \pm standard deviation (SD) unless otherwise indicated. Statistical analyses were performed with the use of StatView V software (SAS Institute). Student's *t*-test or chi-square test was used to compare the data between the groups. To evaluate the relation between the percentage of platelet-monocyte aggregates and other factors, simple or multiple regression analyses were performed. Predictive variables including platelet-monocyte aggregates for the occurrence

of carotid plaques were analyzed by logistic regression analysis. $P < 0.05$ was considered significant.

Results

Circulating platelet-monocyte aggregates are increased in diabetic subjects

We first examined the levels of platelet-monocyte aggregates in patients with diabetes and compared them with those in non-diabetic subjects. Type 2 diabetic subjects ($7.74 \pm 4.04\%$, mean \pm SD) showed significantly ($p = 0.0020$) higher percentages of platelet-monocyte aggregates than non-diabetic subjects ($6.03 \pm 4.38\%$). There was no significant difference ($p = 0.8897$) between the male ($6.84 \pm 4.06\%$) and female ($6.92 \pm 4.52\%$) subjects.

Table 1. Clinical characteristics of the subjects

	All	type 2 DM	non DM
No.	240	120	120
Age (years)	58.0 ± 7.5	58.5 ± 8.2	57.5 ± 6.7
Gender (M/F)	120/120	60/60	60/60
Body mass index (kg/m ²)	24.4 ± 3.8	25.3 ± 4.1*	23.5 ± 3.3
Systolic blood pressure (mm Hg)	129.7 ± 18.9	134.4 ± 21.4*	125.0 ± 14.7
Diastolic blood pressure (mm Hg)	78.3 ± 10.7	78.5 ± 11.7	78.2 ± 9.6
Smoking index (cigarette-years)	354 ± 671	432 ± 814	276 ± 480
Fasting plasma glucose (mg/dl)	120.1 ± 35.8	140.6 ± 40.5*	99.5 ± 8.9
HbA _{1c} (%)	6.4 ± 1.9	7.9 ± 1.8*	5.0 ± 0.4
Non-HDL-cholesterol (mg/dl)	157.1 ± 39.2	154.3 ± 39.1	160.0 ± 39.1
HDL-cholesterol (mg/dl)	56.1 ± 17.6	50.9 ± 15.6*	61.4 ± 17.9
Platelet-monocyte aggregate (%)	6.88 ± 4.29	7.73 ± 4.04*	6.03 ± 4.38
Hypertension	78 (32.5%)	49 (40.8%)*	29 (24.2%)
Hyperlipidemia	88 (36.7%)	39 (32.5%)*	49 (40.8%)
Presence of carotid plaques	35 (14.6%)	26 (21.7%)*	9 (7.5%)

Data are shown as the mean ± SD. HDL: high density lipoprotein. *, $p < 0.05$, Student's *t*-test or chi-square test

Table 2. Simple regression analyses of the factors associated with platelet-monocyte aggregates

	All	type 2 DM	non DM
Age	-0.080	-0.312**	0.149
Body mass index	0.232**	0.114	0.289**
Systolic blood pressure	0.036	-0.108	0.109
Diastolic blood pressure	0.024	-0.039	0.093
Smoking index	0.059	-0.001	0.093
Fasting plasma glucose	0.126	0.051	-0.123
HbA _{1c}	0.290**	0.222*	0.401**
Non-HDL-cholesterol	-0.027	-0.069	0.145
HDL-cholesterol	-0.217**	-0.121	-0.207*
Platelet P-selectin	0.155*	0.148	0.128

** $p < 0.01$, * $p < 0.05$, Student's *t*-test or chi-square test. HDL: high density lipoprotein.

Gender-associated differences in platelet-monocyte aggregates were not observed in the diabetic or non-diabetic group.

Circulating platelet-monocyte aggregates are associated with status of glycemic control

Table 2 shows the results of simple regression analyses of the relation between platelet-monocyte aggregates and clinical factors in all, diabetic and non-diabetic subjects. In all subjects, platelet-monocyte aggregates correlated positively with body mass index, HbA_{1c} and platelet P-selectin, and negatively with HDL-cholesterol. Of interest, the level of platelet-monocyte aggregates

showed only a weak, but significant, correlation with the percentage of P-selectin-positive platelets, suggesting that these two parameters do not necessarily represent the same platelet status. In diabetic subjects, platelet-monocyte aggregates correlated positively with HbA_{1c}, and negatively with age. In non-diabetic subjects, platelet-monocyte aggregates correlated positively with body mass index and HbA_{1c}, and negatively with HDL-cholesterol. Thus, platelet-monocyte aggregates are significantly associated with glycemic status both in diabetic and in non-diabetic subjects. Moreover, the other factors associated with the aggregates appear not to be the same for diabetic and non-diabetic subjects. Table 3

Table 3. Multiple regression analyses of clinical factors affecting platelet-monocyte aggregates

	All	type 2 DM	non DM
Age	-0.105	-0.333*	0.086
Gender (F = 0, M = 1)	-0.050	-0.096	0.090
Smoking index	0.032	-0.002	0.061
Body mass index	0.101	-0.013	0.076
Systolic blood pressure	0.003	0.027	-0.066
Non-HDL-cholesterol	0.012	-0.106	0.086
HDL-cholesterol	-0.099	-0.126	-0.087
HbA _{1c}	0.268*	0.247*	0.461*
R ²	0.138*	0.179*	0.284*

Standard regression coefficients are shown. R²: coefficient of determination. *: $p < 0.01$. HDL: high density lipoprotein.

shows the results of multiple regression analyses of the factors associated with platelet-monocyte aggregates. In a model including age, gender, smoking index, body mass index, systolic blood pressure, non-HDL-cholesterol, HDL-cholesterol and HbA_{1c} as variables, HbA_{1c} was identified as a strong factor independently associated with platelet-monocyte aggregates in all, diabetic and non-diabetic subjects. Age was identified as a factor independently associated with platelet-monocyte aggregates only in diabetic subjects. Thus, glycemic status is one of the major factors associated with platelet-monocyte aggregates.

Platelet-monocyte aggregates are associated with occurrence of carotid plaque in diabetic subjects.

Carotid plaques occurred significantly more frequently in the diabetic group than non-diabetic control (Table 1). To examine the potential role of platelet-monocyte aggregates in atherogenesis in diabetes, we compared the percentage of platelet-monocyte aggregates between subjects with and without carotid plaques. As shown in Table 4, the subjects with carotid plaques showed significantly higher percentages of platelet-monocyte aggregates than those without plaques in all subjects and non-diabetic subjects. In the diabetic group, level of platelet-monocyte aggregates tended to be higher in subjects with carotid plaques than those without then, but the difference was not statistically significant. HbA_{1c} was higher in subjects with carotid plaques than those without plaques among all subjects and non-diabetic subjects. This relation, however, was not observed in diabetic subjects. Finally, we performed multiple logistic regression analyses of the factors associated with the presence of carotid plaques in all, diabetic and non-diabetic subjects (Table 5). In a model including age, gender, smoking index, systolic blood pressure, non-HDL-cholesterol, HDL-

cholesterol, HbA_{1c}, and platelet-monocyte aggregates as independent variables, a significant association between the occurrence of carotid plaques and platelet-monocyte aggregates was observed with an adjusted OR of 1.152 (95% CI, 1.053 to 1.260) for all subjects. Of note, the association with platelet-monocyte aggregates remained significant even among diabetic subjects with an adjusted OR of 1.231 (95% CI, 1.069 to 1.417), but not in the non-diabetic population. These results suggest that the percentage of platelet-monocyte aggregates is an independent factor associated with the occurrence of carotid plaques, especially in type 2 diabetic patients.

Discussion

In the present study, we examined platelet-monocyte aggregates in 120 patients with type 2 diabetes and 120 age- and gender-matched non-diabetic subjects. To the best of our knowledge, this is the first report to analyze the factors associated with platelet-monocyte aggregates in patients with type 2 diabetes. We found that the percentage of platelet-monocyte aggregates was significantly associated with metabolic status, particularly with the status of glycemic control. Moreover, we showed for the first time in diabetic subjects that the level of platelet-monocyte aggregates was significantly associated with the presence of plaques in the carotid artery, with the association independent of other clinical risk factors.

Platelet-monocyte aggregation is increased in patients with type 2 diabetes

People with type 2 diabetes have an elevated risk of arterial thrombotic diseases including myocardial infarction and stroke (1, 2). Platelet hyperreactivity could play a key role in the pathogenesis of cardiovascular events. Individuals with diabetes have been shown to have platelets with basal or agonists-stimulated activity (4, 5). A

Table 4. Presence of carotid plaque and clinical parameters

	Carotid plaque (-)	Carotid plaque (+)	P value
All subjects			
Numbers	203	35	
Age (years)	57.3 ± 7.5	61.6 ± 6.2	0.0018
Gender (M/F)	(98, 106)	(22, 14)	0.1494
Smoking (cigarette-years)	318 ± 512	583 ± 1244	0.0330
Systolic blood pressure (mmHg)	128.6 ± 18.7	136.5 ± 19.1	0.0219
Non-HDL-cholesterol (mg/dl)	157.4 ± 34.9	156.2 ± 59.7	0.8655
HDL-cholesterol (mg/dl)	56.7 ± 18.0	53.3 ± 15.2	0.2943
HbA _{1c} (%)	6.3 ± 1.8	7.4 ± 2.2	0.0014
Platelet-monocyte aggregate (%)	6.54 ± 4.23	8.97 ± 4.17	0.0018
DM			
Numbers	94	26	
Age (years)	57.7 ± 8.561.0	± 6.8 0.0736	
Gender (M/F)	(42, 52)	(18, 8)	0.0267
Smoking (cigarette-years)	391 ± 573	596 ± 1389	0.2704
Systolic blood pressure (mmHg)	133.8 ± 21.5	137.2 ± 21.4	0.4793
Non-HDL-cholesterol (mg/dl)	152.4 ± 30.7	162.0 ± 61.0	0.2714
HDL-cholesterol (mg/dl)	51.2 ± 16.2	49.8 ± 14.3	0.6857
HbA _{1c} (%)	7.8 ± 1.7	8.1 ± 2.1	0.4463
Platelet-monocyte aggregate (%)	7.43 ± 3.81	9.03 ± 4.56	0.0734
Non-DM			
Numbers	110	10	
Age (years)	57.0 ± 6.6	63.3 ± 3.9	0.0059
Gender (M/F)	(56, 54)	(4, 6)	0.5129
Smoking (cigarette-years)	256 ± 447	550 ± 772	0.0778
Systolic blood pressure (mmHg)	124.2 ± 14.8	134.8 ± 10.8	0.0390
Non-HDL-cholesterol (mg/dl)	161.7 ± 37.7	139.4 ± 55.7	0.1042
HDL-cholesterol (mg/dl)	61.3 ± 18.4	63.3 ± 13.5	0.7428
HbA _{1c} (%)	5.0 ± 0.4	5.3 ± 0.2	0.0185
Platelet-monocyte aggregate (%)	5.78 ± 4.42	8.82 ± 2.97	0.0454

Data are shown as the mean ± SD. P values were analyzed by Student's *t*-test or chi-square test.
HDL: high density lipoprotein.

recent report by Vericel *et al.* (29) showed that in diabetic patients, basal platelet activation occurs without vascular complications, suggesting that the altered platelet function is not simply a result of vascular damage.

Conventionally available platelet function tests such as platelet aggregation induced by various agonists using turbidimetry or the measurement of plasma platelet-specific proteins, β -thromboglobulin and platelet factor IV, have problems that include low sensitivity and poor specificity. Recently, increasing numbers of published studies have attempted to use whole-blood flow cytometry to test platelet function in the clinical setting. Whole-blood

flow cytometry has the advantage of allowing the direct analysis of individual platelets in their native milieu with a high degree of sensitivity and with minimal artificial activation (30, 31). Activation-dependent changes in multiple surface receptors can be detected using monoclonal antibodies directed against them. Some reports including ours have shown an increased percentage of P-selectin-positive platelets in diabetic patients (19–21), which has been considered a good marker of platelet activation. It has been shown, however, that degranulated platelets circulating *in vivo* rapidly lose their surface P-selectin yet continue to circulate and function (22, 23).

Table 5. Multiple logistic regression analyses of factors affecting the presence of carotid plaques

variables	All OR (95% CI)	type 2 DM OR (95% CI)	non DM OR (95% CI)
Age	1.089 (1.024–1.158)**	1.089 (1.007–1.177)*	1.169 (0.981–1.394)
Gender	1.681 (0.688–4.109)	5.195 (1.481–18.215)*	0.096 (0.006–1.443)
Smoking index	1.000 (1.000–1.001)	1.000 (1.000–1.001)	1.002 (1.000–1.004)
Systolic blood pressure	1.010 (0.990–1.031)	0.997 (0.972–1.022)	1.044 (0.984–1.108)
Non-HDL-cholesterol	1.048 (0.708–1.552)	1.660 (1.018–2.706)*	0.577 (0.222–1.500)
HDL-cholesterol	1.308 (0.462–3.704)	2.391 (0.565–10.120)	1.079 (0.119–9.778)
HbA _{1c}	1.207 (0.989–1.473)	0.971 (0.735–1.283)	3.107 (0.272–35.533)
Platelet-monocyte aggregate	1.152 (1.053–1.260)**	1.231 (1.069–1.417)**	1.147 (0.920–1.430)
	R ² = 0.159, <i>p</i> < 0.0001	R ² = 0.163, <i>p</i> < 0.0115	R ² = 0.346, <i>p</i> = 0.0050

*; *p* < 0.05, **; *p* < 0.01. OR: odds ratio, CI: confidence interval. HDL: high density lipoprotein.

Indeed, our current data show only a weak correlation between platelet-monocyte aggregates and platelet P-selectin expression. Thus, platelet-monocyte aggregates may be a better marker of platelet activation.

Only one previous paper has reported higher levels of platelet-monocyte aggregates in diabetic patients than healthy subjects (32). Two limitations of their findings, however, are the small numbers of subjects and much younger population in the healthy groups. The present data, based on a relatively large number of diabetic subjects with age- and gender-matched non-diabetic controls, clearly shows that the percentage of platelet-aggregated monocytes is elevated in type 2 diabetic subjects. In all subjects, body mass index and glycemic control were associated positively, and HDL-cholesterol was associated negatively, with the levels of platelet-monocyte aggregates, suggesting that platelet activation status could be determined by metabolic status. With or without diabetes, glycemic status appears to be a strong determinant of platelet-monocyte aggregates, and their significant relation is independent of other clinical risk factors. We also noticed in this study that factors associated with platelet-monocyte aggregates are not identical for diabetic and non-diabetic subjects. In diabetic subjects, age appears to be a determinant, while in non-diabetic subjects, body mass index and HDL-cholesterol are associated with platelet-monocyte aggregates. Of note, only 13.8% of platelet-monocyte aggregates levels were explained by the common risk factors used as variables in our multiple regression models, indicating the existence of an unknown factor or factors yet to be identified.

Platelet-monocyte aggregates and occurrence of carotid plaque

The recruitment of peripheral monocytes to the site of vascular damage is one of the first steps in atherogen-

esis and inflammation. The powerful adhesive interactions that are required for monocytes to withstand local blood flow at the vessel wall can be described as a multistep process mediated by different adhesion molecules (33). It is becoming clear that endothelial P-selectin is essential to this process (34). Besides the role of endothelial P-selectin in the recruitment of monocytes to the atherosclerotic lesion, recent observations have unveiled the involvement of platelet P-selectin in progression of atherosclerosis (25, 26). Huo *et al* (25) reported that circulating activated platelets and platelet-leukocyte/monocyte aggregates promote the formation of atherosclerotic lesions. These animal experiments show the potential role of activated platelets in the progression of spontaneous atherosclerosis. Indeed, we have recently shown that the expression of P-selectin on platelets is associated with carotid atherosclerosis (21). However, it is not clear whether this holds true for diabetic patients.

In this study, we showed in all, diabetic and non-diabetic subjects that levels of circulating platelet-monocyte aggregates were higher in subjects with carotid plaques than those without them. Multiple logistic regression analyses revealed that platelet-monocyte aggregates are a significant and independent determinant of the presence of carotid plaques in diabetic subjects. In non-diabetic subjects, glycemic control, platelet-monocyte aggregates and the occurrence of carotid plaques are mutually associated, resulting in the lack of an independent association among these 3 parameters in multiple logistic regression analyses. Of note, this mutual relation appears to be disrupted in diabetic subjects; no relation exists between the status of glycemic control and carotid atherosclerosis. These observations may be consistent with the notion that strict glycemic control is not necessarily sufficient to prevent cardiovascular events in type 2 diabetic patients (35, 36). Finally, the present results do not exclude the possibility that increased num-

bers of platelet-monocyte aggregates in diabetic subjects are simply the result of accelerated atherosclerosis. We will have to wait for the results of a prospective study to confirm whether increased platelet activation or platelet-monocyte aggregation is indeed associated with the progression of atherosclerosis.

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