Original Article

Hyperglycemia and Inflammatory Property of Circulating Monocytes are Associated with Inflammatory Property of Carotid Plaques in Patients Undergoing Carotid Endarterectomy

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Aim: This study aims to determine the association between glucose metabolism and proinflammatory/anti-inflammatory properties of circulating monocytes or those of carotid plaques in patients who underwent carotid endarterectomy.
Methods: Clinical characteristics and expression levels of proinflammatory/anti-inflammatory markers in circulating monocytes/carotid plaques were examined in 12 patients with diabetes and 12 patients without diabetes.
Results: Circulating monocytes from patients with diabetes revealed higher tumor necrosis factor (TNF)-α and lower interleukin (IL)-10 expression levels compared with those from patients without diabetes, which was also observed in carotid plaques from patients with diabetes. Hyperglycemia revealed positive and negative correlations with the ratios of IL-6$^+$ and IL-10$^+$ cells in carotid plaques, respectively. Moreover, we determined a positive correlation between circulating monocytes and carotid plaques with respect to TNF-α and IL-6 expressions.
Conclusions: The inflammatory property of circulating monocytes was associated with that of carotid plaques. Hyperglycemia increased inflammatory properties and decreased anti-inflammatory properties of carotid plaques.

Key words: Monocytes, Hyperglycemia, Carotid plaque, Diabetes, Atherosclerosis

Introduction

Type 2 diabetes contributes to an increased risk of developing atherosclerotic diseases such as coronary heart disease and stroke[1, 2]. The Hisayama study demonstrated that diabetes is an independent risk factor for ischemic stroke in the Japanese population[3]. Therefore, it is of clinical importance to predict and evaluate the onset of stroke in patients with diabetes. The monocyte/macrophage (Mϕ) system plays critical roles in the pathogenesis of inflammatory diseases, including atherosclerosis. This system demonstrates at least two distinct phenotypes of differentiation: proinflammatory (M1) and anti-inflammatory (M2)[4, 5]. M1 Mϕ in atherosclerotic plaques secrete inflammatory cytokines, such as tumor necrosis factor-α (TNF-α), and promote plaque development and rupture, thereby contributing to the formation of atherothrombosis[6, 7]. Regarding the properties of circulating monocytes, this and other studies previously reported the unfavorable inflammatory balance of M1/M2-like phenotypes of circulating monocytes in obese patients with and without diabetes[4, 8]. This study was designed to elucidate the association of glucose metabolism and inflammatory/anti-inflammatory properties of circulating monocytes with those of carotid plaques in

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patients who underwent carotid endarterectomy (CEA).

Methods

Subjects

A total of 24 male Japanese patients with advanced carotid plaques who underwent CEA were recruited from the Department of Neurosurgery of our hospital. The study protocol was approved by the Ethics Committee for Human Research at the Kyoto Medical Center (ID: 11-06). Written informed consent was obtained from all participants. This study is registered in the University Hospital Medical Information Network Clinical Trial Registry (UMIN-CTR) System (ID: UMIN000019115).

Data Collection and Laboratory Methods

Anthropometric and metabolic parameters were measured as previously described[5, 9, 10]. The HbA1c levels were measured before the meal on the day prior to CEA. The insulin resistance index was assessed using the homeostasis model assessment of insulin resistance (HOMA-IR). Human circulating monocytes were obtained from blood samples and were analyzed as previously described[5, 9, 10]. In brief, peripheral blood mononuclear cells were collected from heparinized blood samples by density-gradient centrifugation with lymphocyte separation solution (Nacalai Tesque, Kyoto, Japan) before the meal on the day prior to CEA. Circulating monocytes were then isolated by magnetic-activated cell sorting with anti-human CD14 immunomagnetic beads (Miltenyi Biotec, Bergisch Gladbach, Germany).

The expression levels of genes of interest were analyzed using quantitative RT-PCR and immunohistochemistry using standard procedures[5, 9-11]. The primers used in the assays were purchased from Sigma-Aldrich (Tokyo, Japan), and the primer sequences are listed in **Supplementary Table 1**. The primary antibodies for immunohistochemistry were as follows: anti-TNF-α (R&D Systems, Minneapolis, MN, USA), anti-interleukin (IL)-6 (R&D Systems), anti-IL-10 (R&D Systems), anti-major histocompatibility complex class Ⅱ (MHC-Ⅱ) (Abcam plc, Cambridge, UK), anti-CD86 (Abcam plc), anti-mannose receptor CD206 (Abcam), and anti-CD3 (Abcam). For quantitative analysis in immunohistochemistry, two areas were randomly selected from each serial section of carotid plaques. The number of immunohistochemically positive cells of interest was divided by the total number of cells obtained by counterstaining with hematoxylin, and the ratio of each positive cell was obtained.

Statistical Analysis

If normally distributed, continuous data were reported as mean ± standard deviation, and if not normally distributed, they were reported as median and interquartile range (IQR). Student's t-test was used for comparisons of the means between the two groups. If data were not normally distributed, the Mann–Whitney U test was used. For comparisons of the median among the ratios of immunohistochemically positive cells, the Wilcoxon signed-rank test was used. Spearman's rank correlation coefficient (ρ) was used to investigate the correlations between proinflammatory/anti-inflammatory parameters (expression levels in circulating monocytes and carotid plaques and percentage of positive cells in the plaque) and metabolic parameters. Moreover, Spearman's partial rank correlation coefficients (ρ_{pa}) were obtained by employing medications (calcium antagonist, ACE/ARB, and statins) as control variables. A P value of < 0.05 was considered significant. All statistical analyses were performed with SPSS v.22.0 for Windows (IBM Japan, Ltd., Tokyo, Japan).

Results

Of all 24 patients who underwent CEA (12 with diabetes and 12 without diabetes; mean age, 72.3 ± 8.2 years), the serum FPG and HbA1c levels were significantly higher in patients with diabetes than in patients without diabetes ($P < 0.001$) (**Table 1**). To elucidate the impact of diabetes on proinflammatory/anti-inflammatory properties of circulating monocytes and carotid plaques, we examined TNF-α, IL-6, and IL-10 expression levels in these cell types and the ratios of cells expressing the cytokines in the plaques (**Table 2** and **Fig. 1**). As shown in **Table 2**, we found that the relative mRNA level of TNF-α [median, 1.7 (IQR, 1.0–5.6)] was significantly higher and that of IL-10 [0.4 (0.2–0.9)] was significantly lower in circulating monocytes of patients with diabetes than in those of patients without diabetes [TNF-α, 1.0 (0.8–1.7), $P = 0.049$; IL-10, 1.0 (0.3–1.4), $P = 0.025$]. With respect to carotid plaques, we found that both the relative mRNA levels of TNF-α [1.7 (1.1–3.3)] and IL-6 [1.8 (1.0–4.1)] were significantly higher in the carotid plaques of patients with diabetes than in those of patients without diabetes [TNF-α, 1.0 (0.7–2.0), $P = 0.042$; IL-6, 1.0 (0.5–1.1), $P = 0.038$]. In addition, the percentage of IL-6⁺ cells [63.2 (40.8–71.1)] was significantly higher and that of IL-10⁺ cells [58.0 (37.6–65.2)] was significantly lower in carotid plaques of patients with diabetes than in those of patients with diabetes [IL-6⁺, 42.9 (38.7–54.9), $P = 0.040$; IL-10⁺, 70.9 (59.3–83.7), $P = 0.019$].

Table 1. Clinical characteristics and metabolic parameters of nondiabetic and diabetic patients

	Nondiabetic patients	Diabetic patients	*P*-value*
n (male)	12	12	
Age (years)	74.2 ± 8.9	70.4 ± 7.2	0.270
Duration of diabetes (years)	-	6.5 [2.3–17.5]	-
Body weight (kg)	60.6 ± 9.3	63.7 ± 8.4	0.392
BMI (kg/m^2)	23.5 ± 3.0	23.8 ± 3.1	0.772
WC (cm)	84.9 ± 6.7	84.6 ± 9.4	0.931
SBP (mmHg)	133.4 ± 17.3	127.3 ± 14.8	0.364
DBP (mmHg)	69.4 ± 13.1	71.2 ± 13.8	0.753
FPG (mmol/L)	5.5 ± 0.9	7.5 ± 1.4	<0.001
IRI (pmol/L)	39.0 [20.3–51.5]	43.5 [27.8–78.3]	0.453
HbA1c (%)	5.6 ± 0.3	7.0 ± 0.6	<0.001
HOMA-IR	1.9 ± 1.5	4.1 ± 4.5	0.131
TC (mmol/L)	4.4 ± 1.1	4.1 ± 1.0	0.481
TG (mmol/L)	1.4 [1.1–2.1]	1.2 [0.8–1.4]	0.073
HDL-C (mmol/L)	1.4 ± 0.4	1.2 ± 0.3	0.278
LDL-C (mmol/L)	2.5 ± 1.0	2.4 ± 0.7	0.779
hsCRP (μg/mL)	1.3 [0.2–4.4]	2.3 [0.3–3.9]	0.686
Proportion of patients with/taking:			
hypertension (%)	83.3	100.0	0.478
dyslipidemia (%)	83.3	83.3	>0.999
calcium antagonist (%)	66.7	33.3	0.220
ACE/ARB (%)	41.7	91.7	0.027
statins (%)	66.7	66.7	>0.999
antidiabetic medications (%)	0.0	83.3	-

Data are shown as the mean ± SD or median [IQR]. BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; IRI, immunoreactive insulin; HbA1c, hemoglobin A1c; HOMA-IR, homeostasis model assessment of insulin resistance; TC, total cholesterol; TG, triglycerides; HDL-C, HDL-cholesterol; LDL-C, LDL-cholesterol; hsCRP, high-sensitive C-reactive protein; ACE, angiotensin-converting enzyme; and ARB, angiotensin II type 1 receptor blocker. *nondiabetic *versus* diabetic patients

To further examine the properties of circulating monocytes and carotid plaques, we analyzed the expression levels of cell-surface markers for proinflammatory/anti-inflammatory properties of circulating monocytes/Mφ. Circulating monocytes are classified by the expression levels of markers, such as CD14, CD16, and CD68, thereby reflecting different inflammatory states[12, 13]. Furthermore, MHC-II, a marker of circulating monocytes/Mφ, and CD86 are upregulated in M1 Mφ, whereas CD206 and Dectin-1 are highly expressed in M2 Mφ[14-16]. As shown in **Table 2** and **Fig. 1b**, we found no significant difference between patients with and without diabetes with respect to the expression levels of these markers in circulating monocytes and carotid plaques. This differed from that observed for proinflammatory/anti-inflammatory cytokine levels (**Table 2** and **Fig. 1a**). Furthermore, we found that the ratio of cells expressing CD3, a T-cell marker, in plaques was not significantly different between patients with and without diabetes (**Table 2**). Furthermore, our data revealed that the MHC-II$^+$ ratio was significantly higher than the CD3$^+$ ratio in carotid plaques of both patient without diabetes [75.4 (66.4–78.8) and 10.3 (8.6–14.0), *P*=0.002] and those with diabetes [83.1 (73.7–88.1) and 13.0 (8.9–15.0), *P*=0.002], thereby suggesting that the Mφ ratio was higher than the T-cell ratio in carotid plaques of both patients.

Next, we examined the correlation between glucose metabolism and inflammatory properties of carotid plaques in all patients who underwent CEA. As shown in **Table 3** and **Fig. 2**, we found that FPG and HbA1c levels displayed significant negative correlations with the IL-10$^+$ ratio in carotid plaques [FPG, $\rho = -0.471$, *P*=0.020 (**Fig. 2a**); HbA1c, $\rho = -0.428$, P=0.037 (**Fig. 2b**)]. Furthermore, IRI and HOMA-IR revealed significant negative correlations with the IL-10$^+$ ratio in carotid plaques (IRI, $\rho = -0.543$, *P*=0.006; HOMA-IR, $\rho = -0.640$, *P*<0.001) (**Table 3**). Moreover, as shown in **Tables 3** and **4** and **Fig. 3**,
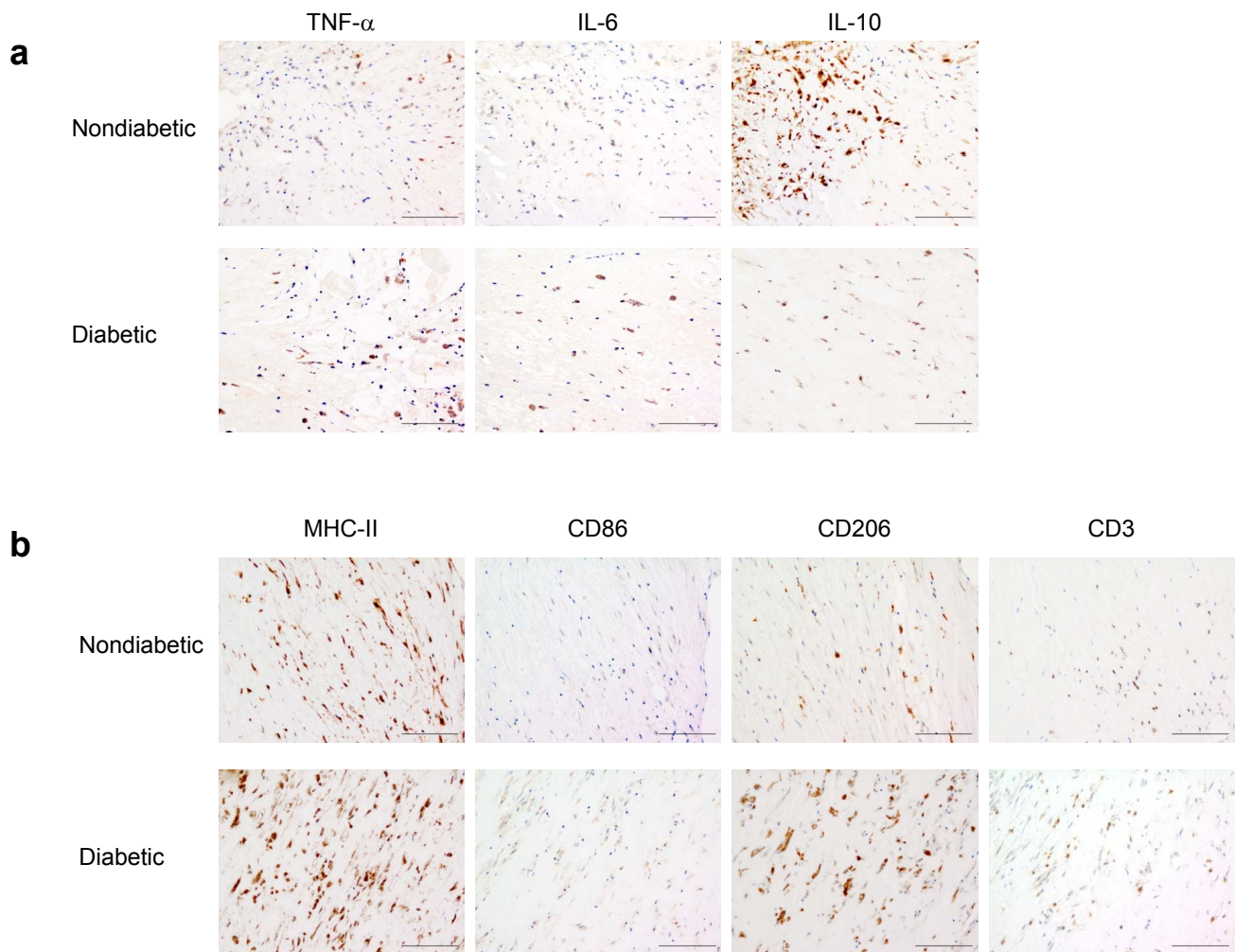
Fig. 1. Immunohistochemical analyses of cells expressing proinflammatory/anti-inflammatory cytokines (a), M1/M2 markers, or T-cell markers (b) in carotid plaques of patients without diabetes (upper panels) and thos with diabetes (lower panels) who underwent CEA

Positive cells expressing the cytokine/cell-surface marker of interest were immunohistochemically detected using a specific antibody and a 3, 3′-diaminobenzidine tetrahydrochloride solution, followed by counterstaining with hematoxylin. Magnification, ×200 and scale bars, 100 μm

HbA1c levels revealed significant positive correlations with the IL-6$^+$ ratio ($\rho = 0.466$, $P = 0.022$) (**Table 3** and **Fig. 3a**) and TNF-α expression levels ($\rho = 0.527$, $P = 0.01$) (**Table 4** and **Fig. 3b**) in carotid plaques. The HbA1c level was not significantly correlated with the expression levels of cell-surface markers, such as MHC-II, CD86, CD206, and Dectin-1, and the ratios of MHC-II$^+$, CD86$^+$, CD206$^+$, and CD3$^+$ cells in carotid plaques. In partial rank correlation analyses employing medications as control variables, we observed significant negative correlations between glucose metabolism and the IL-10$^+$ ratio in carotid plaques (FPG, $\rho_{\mathrm{pa}} = -0.488$, $P = 0.034$; IRI, $\rho_{\mathrm{pa}} = -0.540$, $P = 0.017$; HbA1c, $\rho_{\mathrm{pa}} = -0.452$, $P = 0.048$; and HOMA-IR, $\rho_{\mathrm{pa}} = -0.629$, $P = 0.004$), which is similar

to the case of single correlation analyses in **Table 3** and **Fig. 2**. Furthermore, we found a significant positive correlation between HbA1c levels and the IL-6$^+$ ratio in carotid plaques ($\rho_{\mathrm{pa}} = 0.606$, $P = 0.006$).

We further addressed the association between circulating monocytes and carotid plaques with regard to the proinflammatory/anti-inflammatory cytokine levels in all patients who underwent CEA (**Tables 3** and **4**). We observed that the TNF-α expression level in circulating monocytes was positively correlated with the TNF-α^+ ratio ($\rho = 0.444$, $P = 0.034$) (**Table 3**) and TNF-α expression level ($\rho = 0.459$, $P = 0.027$) (**Table 4**) in carotid plaques. In addition, the IL-6 expression level in circulating monocytes revealed a significant positive correlation with that in carotid plaques (ρ

Table 2. Inflammatory/anti-inflammatory properties of monocytes and carotid plaque of nondiabetic and diabetic patients

	Non-diabetic patients	Diabetic patients	*P*-value*
n	12	12	-
mRNA level in monocytes			
TNF-α	1.0 [0.8–1.7]	1.7 [1.0–5.6]	0.049
IL-6	1.0 [0.6–1.7]	0.7 [0.3–1.5]	0.525
IL-10	1.0 [0.3–1.4]	0.4 [0.2–0.9]	0.025
CD14	1.0 [0.7–1.4]	1.5 [1.1–1.8]	0.078
CD16	1.0 [0.8–1.4]	0.8 [0.7–1.5]	0.862
CD68	1.0 [0.8–1.1]	0.9 [0.8–0.9]	0.166
mRNA level in plaque			
TNF-α	1.0 [0.7–2.0]	1.7 [1.1–3.3]	0.042
IL-6	1.0 [0.5–1.1]	1.8 [1.0–4.1]	0.038
IL-10	1.0 [0.5–1.4]	1.4 [0.9–2.0]	0.149
MHC-II	1.0 [0.5–1.6]	0.9 [0.3–1.9]	0.603
CD86	1.0 [0.8–1.8]	1.0 [0.6–1.6]	0.908
CD206	1.0 [0.6–1.4]	0.8 [0.4–2.1]	0.644
Dectin-1	1.0 [0.4–2.1]	1.8 [0.5–6.4]	0.326
Percent of positive cells in plaque			
TNF-α (%)	54.2 [44.8–63.9]	65.5 [52.1–76.1]	0.193
IL-6 (%)	42.9 [38.7–54.9]	63.2 [40.8–71.1]	0.040
IL-10 (%)	70.9 [59.3–83.7]	58.0 [37.6–65.2]	0.019
MHC-II (%)	75.4 [66.4–78.8]	83.1 [73.7–88.1]	0.094
CD86 (%)	14.7 [11.9–23.5]	17.5 [11.4–22.7]	0.729
CD206 (%)	32.2 [25.5–42.5]	37.4 [26.6–40.4]	0.817
CD3 (%)	10.3 [8.6–14.0]	13.0 [8.9–15.0]	0.299

Data are shown as the median [IQR]. The mRNA expression levels of nondiabetic patients are set at 1.0 and relative values are shown. *nondiabetic *versus* diabetic patients

Table 3. Correlations of the positive ratios of cells expressing proinflammatory/anti-inflammatory cytokines in carotid plaque with glu cose metabolism or proinflammatory/anti-inflammatory properties of circulating monocytes

	Percent of positive cells in plaque					
	TNF-α		IL-6		IL-10	
	ρ	*P*-value	ρ	*P*-value	ρ	*P*-value
FPG	0.191	0.371	0.346	0.098	− 0.471	0.020
IRI	− 0.066	0.759	− 0.030	0.891	− 0.543	0.006
HbA1c	0.246	0.247	0.466	0.022	− 0.428	0.037
HOMA-IR	− 0.014	0.947	0.087	0.686	− 0.640	<0.001*
mRNA level in monocytes						
TNF-α	0.444	0.034	0.406	0.054	0.212	0.330
IL-6	0.309	0.142	0.148	0.491	− 0.130	0.544
IL-10	− 0.251	0.248	− 0.362	0.090	0.145	0.508

FPG, fasting plasma glucose; IRI, immunoreactive insulin; HbA1c, hemoglobin A1c; HOMA-IR, homeostasis model assessment of insulin resistance. *$P<$ =0.0008. ρ, Spearman's rank correlation coefficient

=0.427, *P*=0.037) (**Table 4**) but not with the IL-6$^+$ ratio in carotid plaques (**Table 3**). Conversely, no significant correlation was observed with respect to the IL-10 expression level between circulating monocytes and carotid plaques (**Tables 3** and **4**). Significant correlations between circulating monocytes and carotid

a

$\rho = -0.471$
$P = 0.020$

IL-10$^+$ (positive ratio in plaque) vs FPG (mmol/L)

b

$\rho = -0.428$
$P = 0.037$

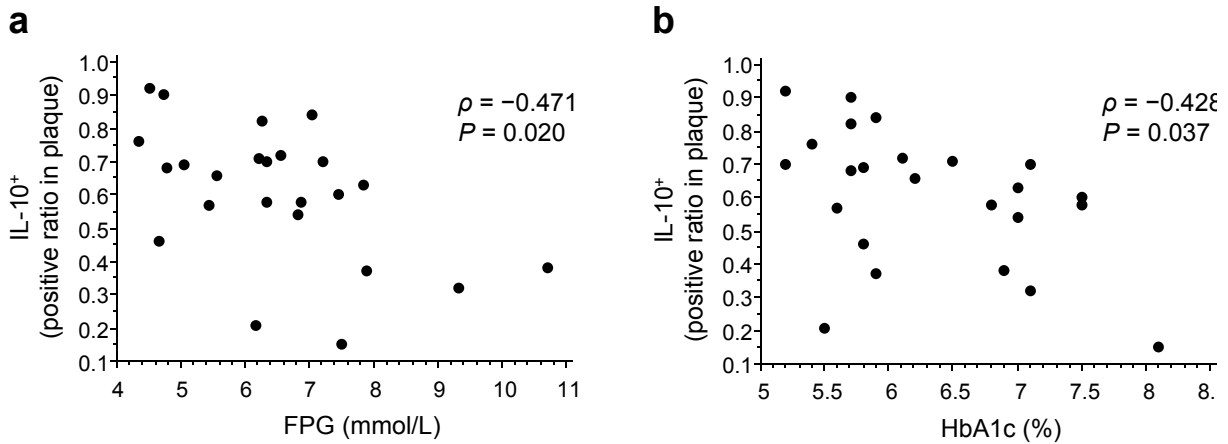IL-10$^+$ (positive ratio in plaque) vs HbA1c (%)

Fig. 2. Correlation between glucose metabolism and the IL-10$^+$ ratio in carotid plaques

Scatter plots highlighting the correlation between the IL-10$^+$ ratio in carotid plaques and both FPG (a) and HbA1c (b) levels. ρ, Spearman's rank correlation coefficient

Table 4. Correlations between the expression levels of proinflammatory/anti-inflammatory cytokines in carotid plaque and glucose metabolism or proinflammatory/anti-inflammatory phenotypes of circulating monocytes

| | mRNA level in plaque | | | | | |
| | TNF-α | | IL-6 | | IL-10 | |
	ρ	P-value	ρ	P-value	ρ	P-value
FPG	0.185	0.399	0.219	0.304	0.259	0.222
IRI	−0.041	0.854	−0.256	0.228	0.241	0.256
HbA1c	0.527	0.010	0.359	0.085	0.101	0.640
HOMA-IR	−0.027	0.904	−0.110	0.607	0.238	0.264
mRNA level in monocytes						
TNF-α	0.459	0.027	0.332	0.122	−0.053	0.809
IL-6	0.091	0.680	0.427	0.037	−0.102	0.636
IL-10	−0.360	0.100	−0.322	0.134	−0.091	0.678

FPG, fasting plasma glucose; IRI, immunoreactive insulin; HbA1c, hemoglobin A1c; HOMA-IR, homeostasis model assessment of insulin resistance. ρ, Spearman's rank correlation coefficients
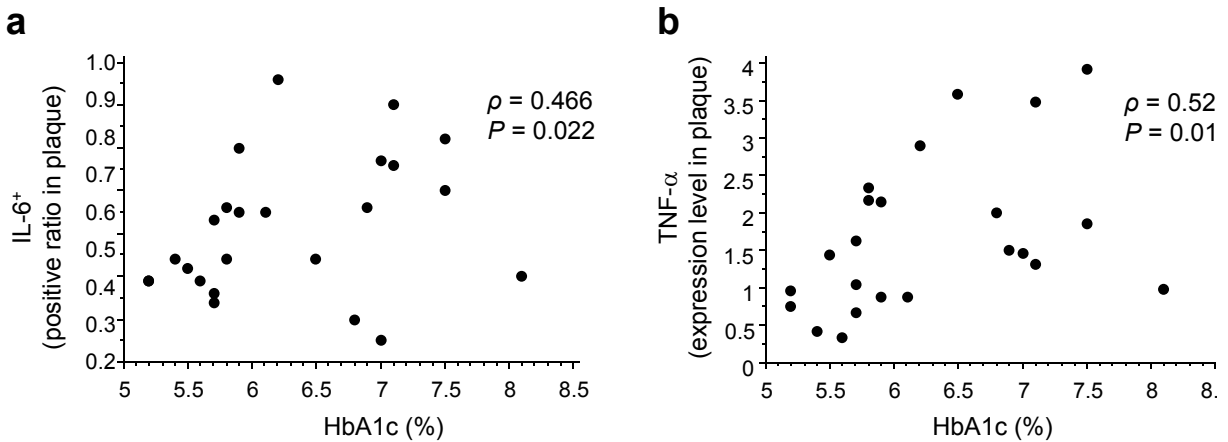
a

$\rho = 0.466$
$P = 0.022$

IL-6$^+$ (positive ratio in plaque) vs HbA1c (%)

b

$\rho = 0.52$
$P = 0.01$

TNF-α (expression level in plaque) vs HbA1c (%)

Fig. 3. Correlation between HbA1c level with the IL-6$^+$ ratio and TNF-α expression level in carotid plaques

Scatter plots highlighting the correlation between HbA1c level and the IL-6$^+$ ratio (a) or TNF-α expression level (b) in carotid plaques. ρ, Spearman's rank correlation coefficient

plaques regarding inflammatory properties were also obtained by partial rank correlation analyses, wherein medications were control variables.

Finally, we investigated the association between clinical settings and proinflammatory/anti-inflammatory properties of circulating monocytes and carotid plaques. We found that the properties of circulating monocytes and carotid plaques did not significantly correlate with the duration of diabetes (**Supplementary Table 2**). One of the 12 patients with diabetes had a transient weakness, but all the patients had no paralysis and were able to walk. Regarding the severity of diabetes, we found that the TNF-α expression level was significantly higher in circulating monocytes of patients with diabetes having neuropathy [8 patients, 4.0 (1.8–8.2)] than in those of patients with diabetes not having neuropathy [4 patients, 1.0 (0.9–1.2), $P=0.014$]. In addition, the IL-10 expression level was significantly lower in carotid plaques of patients with diabetes having a stroke [6 patients, 0.4 (0.2–0.8)] than in those of patients with diabetes not having a stroke [6 patients, 1.0 (0.7–1.2), $P=0.036$]. Consequently, we suggest that inflammatory properties of circulating monocytes and carotid plaques are exacerbated in patients with advanced diabetes.

Discussion

This study is the first to demonstrate that carotid plaques from patients with diabetes have a higher ratio of inflammatory cells and a lower ratio of anti-inflammatory cells compared with those from patients without diabetes. Furthermore, anti-inflammatory properties of carotid plaques were decreased by hyperglycemia. Moreover, we observed a positive correlation between circulating monocytes and carotid plaques, particularly with regard to inflammatory properties. These findings suggest that hyperglycemia and inflammatory properties of circulating monocytes are closely associated with inflammatory properties of carotid plaques in patients who underwent CEA.

Hyperglycemia causes endothelial cell damage, triggering monocyte recruitment[17]. In addition, circulating monocytes with inflammatory properties display an intrinsically higher transendothelial migratory capacity than those with anti-inflammatory properties[13]. Therefore, our findings suggest that circulating monocytes with inflammatory properties infiltrate carotid plaques and differentiate into M1 Mϕ more efficiently relative to circulating monocytes with anti-inflammatory properties in patients with hyperglycemia. Alternatively, hyperglycemia may have deleterious impact on IL-10 production in Mϕ in carotid plaques. Sato T *et al.* reported that IL-10 production

by Mϕ in atherothrombotic plaques is impaired in patients with acute coronary syndrome having diabetes and insulin resistance[18]. Therefore, hyperglycemia may be involved in the reduced anti-inflammatory properties of Mϕ in carotid plaques.

A recent epidemiological study, the Malmo Diet and Cancer study, reported that elevated CD14^{high}CD16$^-$ monocytes in a heterogeneous monocyte population can predict cardiovascular events[19]. On the other hand, Fadini *et al.*[13] reported that the functions of inflammatory and anti-inflammatory monocytes rather than traditional cell-surface markers are associated with microangiopathy in patients with diabetes[13]. In addition, Neele *et al.*[20] reported that surface markers expressed by Mϕ do not reveal the activation status of Mϕ[20]. Similarly, in this study, no significant difference was observed between patients with and without diabetes with regard to the expression levels of cell-surface markers for inflammatory/anti-inflammatory properties in circulating monocytes and carotid plaques. However, data regarding the proinflammatory/anti-inflammatory cytokine expression levels revealed that inflammatory properties of circulating monocytes and carotid plaques were more exacerbated in patients with diabetes than in those without diabetes. Furthermore, we found that these inflammatory properties were aggravated in patients with advanced diabetes. Accordingly, our data reported for the first time that inflammatory properties of circulating monocytes/Mϕ were exacerbated in the absence of remarkable changes of cell-surface inflammatory/anti-inflammatory markers in patients with diabetes. Further studies are required to clarify the mechanisms by which the cytokine expression is influenced by diabetes in the absence of concomitant changes of cell-surface markers.

Carotid ultrasonography may be a useful tool for predicting the atherosclerotic status of coronary arteries[21]; however, the association between circulating monocytes and carotid plaques pertaining inflammatory/anti-inflammatory properties remains unclear. On the basis of the proinflammatory/anti-inflammatory cytokine expression levels, we demonstrated for the first time that inflammatory properties of circulating monocytes are positively correlated with those of carotid plaques. In a previous study involving histological and gene expression analyses of carotid plaques, a higher gene expression level of inflammatory cytokines in carotid plaques was correlated with the morphological features of a more advanced or an unstable condition[22]. Moreover, the balance between proinflammatory/pro-thrombotic and anti-inflammatory mediators in mononuclear cells of carotid plaques may modulate plaque progression[23]. Therefore, our find-

ings suggest that inflammatory properties of circulating monocytes are useful as potential novel markers for evaluating atherosclerosis progression and predicting cardiovascular risk, thereby leading to novel therapeutic targets.

This study had some limitations. This study included a cohort comprising 24 males. A study with a larger sample size would further clarify the association between glucose metabolism and proinflammatory/anti-inflammatory properties of circulating monocytes and carotid plaques. Furthermore, a cohort study comprising females would be informative. In this study, the expression of the genes of interest in carotid plaques was analyzed using plaque tissues. Because carotid plaques contain multiple cell types, the difference in mRNA levels, such as IL-10 levels, in carotid plaques between patients with and without diabetes may be vague. Furthermore, Saraiva et al.[24] reported several mechanisms of posttranscriptional regulation with an impact on IL-10 mRNA half-life and on the amount of IL-10 secretion in Mϕ[24]. Hence, the inconsistency between immunohistochemical and plaque mRNA data may be attributable to these mechanisms. In addition, a longitudinal study would help to provide novel insights in the correlation between proinflammatory/anti-inflammatory properties of circulating monocytes and/or carotid plaques and prognosis of patients who underwent CEA.

Finally, further investigation in larger cohorts with more extensive phenotypic analyses of circulating monocytes would be expected to elucidate the critical types of circulating monocytes, thereby providing further insights into the diagnostic significance of circulating monocytes in evaluating atherosclerosis and cardiovascular disease progression.

Conclusion

Hyperglycemia and inflammatory properties of peripheral blood monocytes demonstrated positive correlations with inflammatory properties of carotid plaques in patients who underwent CEA. Inflammatory properties of circulating monocytes may be potential novel markers for evaluating atherosclerosis progression.

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Conflict of Interest

The authors declare that there is no duality of interest associated with this manuscript.

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Supplementary Table 1. Primer sequences for quantitative real-time PCR

Gene	Forward primer (5′–3′)	Reverse primer (5′–3′)	Reference
GAPDH	TGCACCACCAACTGCTTAGC	GGATGCAGGGATGATGTTCTG	1
TNF-α	CGCTCTTCTGCCTGCTGCACTT	TCGGGGTTCGAGAAGATGATCTGAC	This study
IL-6	AAATGCCAGCCTGCTGACGAAG	AACAACAATCTGAGGTGCCCATGCTAC	2
IL-10	GTGATGCCCCAAGCTGAGA	CACGGCCTTGCTCTTGTTTT	RTPrimer DB ID: 8230
CD14	CAGTATGCTGACACGGTCAAGG	ATCTCGGAGCGCTAGGGTTTA	This study
CD16	CGGTGCAGCTAGAAGTCCAT	GGTTGACACTGCCAAACCTTG	This study
CD68	CATCCCCACCTGCTTCTCTC	GAGAATGTCCACTGTGCTGC	This study
MHC-Ⅱ	GCATGGTGTGTCTGAAGCTC	CAGCTCCAAGAAACGTGGTC	3
CD86	AGCACAGACACACGGATGAG	AGAGGAGCAGCACCAGAGAG	This study
CD206	CTTTGGACGGATGGACGAGG	GGATTAGTCAAGGAAGGGTCGGA	This study
Dectin-1	TGTGCTGCATCTCCTCCTTG	GGCTGGAAAGAACCCCTGTG	This study

GAPDH, glyceraldehyde-3-phosphate dehydrogenase; TNF-α, tumor necrosis factor-a; IL-6, interleukin-6; IL-10, interleukin-10; MHC-Ⅱ, major histocompatibility complex, class Ⅱ.

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Supplementary Table 2. Correlations between the proinflammatory/anti-inflammatory phenotypes of circulating monocytes or carotid plaque and the duration of diabetes in diabetic patients ($n=12$)

	Duration of diabetes	
	ρ	P-value
mRNA level in monocytes		
TNF-α	−0.279	0.407
IL-6	−0.060	0.854
IL-10	−0.046	0.894
CD14	−0.298	0.346
CD16	0.144	0.656
CD68	−0.025	0.940
mRNA level in plaque		
TNF-α	−0.324	0.331
IL-6	−0.249	0.435
IL-10	0.208	0.516
MHC-Ⅱ	−0.225	0.483
CD86	0.242	0.448
CD206	−0.474	0.120
Dectin-1	0.021	0.948
Percent of positive cells in plaque		
TNF-α	0.126	0.696
IL-6	0.175	0.585
IL-10	−0.488	0.108
MHC-Ⅱ	−0.228	0.476
CD86	0.042	0.897
CD206	0.119	0.712
CD3	0.151	0.640

ρ, Spearman's rank correlation coefficient