Association of Toll-Like Receptor 4 on Human Monocyte Subsets and Vulnerability Characteristics of Coronary Plaque as Assessed by 64-Slice Multidetector Computed Tomography

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Background: Although Toll-like receptor 4 (TLR-4) is involved in monocyte activation in patients with accelerated forms of atherosclerosis, the relationship between the expression of TLR-4 on circulating monocytes and coronary plaque vulnerability has not previously been evaluated. We investigated this relationship using 64-slice multidetector computed tomography (MDCT) in patients with stable angina pectoris (SAP).

Methods and Results: We enrolled 65 patients with SAP who underwent MDCT. Three monocyte subsets (CD14⁺⁺CD16⁻, CD14⁺⁺CD16⁺, and CD14⁺CD16⁺) and expression of TLR-4 were measured by flow cytometry. Intracoronary plaques were assessed by 64-slice MDCT. We defined vulnerability of intracoronary plaques according to the presence of positive remodeling (remodeling index >1.05) and/or low CT attenuation (<35 HU). The circulating CD14⁺⁺CD16⁺ monocytes more frequently expressed TLR-4 than CD14⁺⁺CD16⁻ and CD14⁺CD16⁺ monocytes ($P<0.001$). The relative proportion of the expression of TLR-4 on CD14⁺⁺CD16⁺ monocytes was significantly greater in patients with vulnerable plaque compared with those without (10.4 [4.1–14.5] % vs. 4.5 [2.8–7.8] %, $P=0.012$). In addition, the relative proportion of TLR-4 expression on CD14⁺⁺CD16⁺ monocytes positively correlated with the remodeling index ($r=0.28$, $P=0.025$) and negatively correlated with CT attenuation value ($r=-0.31$, $P=0.013$).

Conclusions: Upregulation of TLR-4 on CD14⁺⁺CD16⁺ monocytes might be associated with coronary plaque vulnerability in patients with SAP.

Key Words: Monocytes; Multidetector computed tomography (MDCT); Plaque vulnerability; Toll-like receptor 4

Circulating monocytes in human peripheral blood are heterogeneous,^{1–3} but fall into 2 subsets typically identified by the expressions of CD14 and CD16: CD14⁺CD16⁻ monocytes expressing C-C motif chemokine receptor 2 (CCR2) and CD14⁺CD16⁺ monocytes expressing C-X3-C motif chemokine receptor 1 (CX3CR1). The discovery that monocytes comprise distinct subsets in humans suggests a specialization of function and has stimulated interest in approaches that discriminate between “harmful” and “beneficial” subsets.^{4–7} In terms of the relationship between monocyte subsets and coronary plaque instability, we previously showed by optical coherence tomography that upregulation of CD14⁺CD16⁺ monocytes was associated with both plaque fibrous cap thickness⁸ and plaque rupture⁹ in patients with unstable angina pectoris. These

findings highlight the importance of characterizing different monocyte subsets, which differentially contribute to plaque infiltration and vulnerability.

Toll-like receptor 4 (TLR-4) is the signaling receptor for lipopolysaccharide (LPS)¹⁰ and also interacts with heat-shock proteins,¹¹ fibrinogen¹² and minimally modified low-density lipoprotein (LDL).¹³ The expression of TLR-4 has recently been described on the macrophages and endothelial cells in lipid-rich atherosclerotic plaque.^{14,15} TLR-4 is also implicated in monocyte activation of patients with accelerated forms of atherosclerosis. In particular, acute myocardial infarction (AMI) is associated with enhanced expression of TLR-4 in circulating monocytes.¹⁶ Moreover, the expression of TLR-4 is increased at the local site of acute coronary syndrome (ACS),¹⁷ and TLR-4 plays a key role

Received July 8, 2016; revised manuscript received January 25, 2017; accepted February 12, 2017; released online March 24, 2017. Time for primary review: 20 days

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Figure 1. Fluorescence-activated cell sorting analysis. **(A)** Monocytes were first gated in a forward scatter height/sideward scatter height (FSC-H/SSC-H) dot-plot, and 2-color fluorescence was then measured within the monocyte gate. **(B)** Monocytes were divided into 3 subsets that were defined as monocytes expressing CD14 but not CD16 (lower right quadrant: CD14⁺CD16⁻), CD16 and high levels of CD14 (upper right quadrant: CD14⁺CD16⁺), and CD16 and low levels of CD14 (upper left quadrant: CD14⁻CD16⁺), respectively. **(C–F)** For determination of CD14⁺CD16⁻TLR-4⁺, CD14⁺CD16⁺TLR-4⁺, and CD14⁻CD16⁺TLR-4⁺ monocytes, 3-color fluorescence (PE-conjugated CD14 antibody, APC-conjugated CD16 antibody, and FITC-conjugated TLR-4 antibody) was performed after gating of CD14⁺CD16⁻, CD14⁺CD16⁺, and CD14⁻CD16⁺ cells, and then CD14⁺CD16⁻TLR-4⁺, CD14⁺CD16⁺TLR-4⁺, and CD14⁻CD16⁺TLR-4⁺ cells were measured, respectively. APC, allophycocyanin; FITC, fluorescein isothiocyanate; PE, phycoerythrin; TLR-4, Toll-like receptor 4.

in the activation of the inflammatory pathway of ACS.^{18,19}

Multidetector computed tomography (MDCT) allows noninvasive assessment of coronary artery stenosis and plaque characterization.^{20–23} Recent advances in MDCT, particularly 64-slice MDCT, make it a reliable noninvasive imaging modality that allows not only visualization of the coronary artery lumen but also high diagnostic accuracy for the detection and quantification of vulnerable plaque properties; that is, positive remodeling (PR), low CT attenuation plaque (LAP), and plaque rupture.^{24–27}

The assessment of TLR-4 expression and plaque vulnerability characteristics is important from the perspective of prevention. To the best of our knowledge, there are no reports examining the relationship between the expression of TLR-4 on circulating monocyte and coronary plaques, especially plaque composition and morphology, in patients with stable angina pectoris (SAP). We used 64-slice MDCT to investigate this relationship in patients with SAP.

Methods

Patient Population

We included 156 SAP patients who were scheduled for coronary angiography at Wakayama Medical University Hospital. We excluded participants who had atrial fibrillation (n=17) and renal insufficiency (serum creatinine

>1.5mg/dL) (n=19), because they could not undergo MDCT. The remaining patients underwent MDCT, and we then excluded participants who had the following: (1) history of recent (<12 weeks) ACS (n=8); (2) culprit lesion in the left main coronary artery (n=2); (3) poor MDCT images for plaque analysis and inadequate MDCT image because of heavily calcified lesions by visual estimation (n=34); (4) malignant disease (n=2); and (5) systemic inflammatory conditions, including peripheral vascular disease, autoimmune disease, advanced liver disease, and inflammatory disease (n=9). Ultimately, we analyzed 65 patients for this study. This study was in compliance with the Declaration of Helsinki with regard to investigation in humans, and the study protocol was approved by the Institutional Ethics Committee of Wakayama Medical University. We also obtained written informed consent from all the participants.

Clinical Parameters

Clinical parameters assessed included age, sex, body mass index, and coronary risk factors, which included hypertension (blood pressure ≥140/90mmHg and/or a history of antihypertensive medication), diabetes mellitus (fasting plasma glucose ≥126mg/dL, casual plasma glucose ≥200mg/dL, or a diabetic pattern in 75-g oral glucose tolerance test), hyperlipidemia (serum total cholesterol

≥ 220 mg/dL or serum triglyceride ≥ 150 mg/dL), smoking status, obesity (body mass index ≥ 25 kg/m²), and family history.

Cytometric Analysis

For cytometric analysis, monoclonal antibodies against CD14 (phycoerythrin (PE)-conjugated, Clone M5E2, BD Biosciences, San Jose, CA, USA), CD16 (allophycocyanin (APC)-conjugated, clone B73.1, BD Biosciences) and TLR-4 (fluorescein isothiocyanate (FITC)-conjugated, clone HTA125, BD Biosciences) were used as described previously.^{17,28} A total of 100 μ L of blood was incubated in the dark for 30 min at room temperature. For erythrocyte lysis and leukocyte fixation, 1 mL of lysis solution was added (BD FACS Lyse, Lysing Solution; Becton Dickinson, Germany).

Cytometric analysis was performed in a flow cytometer (BD FACS AriaTM, Becton Dickinson) using BD FACSDiva software. Monocytes were first gated in a forward scatter/sideward scatter dot-plot, and 2-color fluorescence was then measured within the monocyte gate. Monocytes were divided into 3 subsets that were defined as monocytes expressing CD14 but not CD16, CD16 and high levels of CD14, and CD16 and low levels of CD14, respectively.^{29,30} For determination of CD14⁺⁺CD16⁻TLR-4⁺, CD14⁺⁺CD16⁺TLR-4⁺, and CD14⁺CD16⁺TLR-4⁺ monocytes, 3-color fluorescence (PE-conjugated CD14 antibody, APC-conjugated CD16 antibody, and FITC-conjugated TLR-4 antibody) was performed after gating of CD14⁺⁺CD16⁻, CD14⁺⁺CD16⁺, and CD14⁺CD16⁺ cells, and then CD14⁺⁺CD16⁻TLR-4⁺, CD14⁺⁺CD16⁺TLR-4⁺, and CD14⁺CD16⁺TLR-4⁺ cells were measured, respectively, as described previously²⁸ (Figure 1).

Blood Sampling and Analysis

Peripheral blood samples were collected from all subjects on admission in preparation for planned percutaneous coronary intervention. Plasma samples were collected in ethylenediaminetetraacetic acid anticoagulant tubes and stored at -80°C until assayed. Matrix metalloproteinase 9 (MMP-9) was analyzed with a commercially available kit (Human MMP-9 Quantikine ELISA Kit DMP900; R&D systems, Minneapolis, MN, USA). High-sensitivity C-reactive protein (hs-CRP) was analyzed with a commercially available kit (N-Latex CRP II; Dade Behring, Marburg, Germany).

Scanning and Imaging Protocol of MDCT

MDCT was performed with a 64-slice detector CT (BrillianceCT64, Philips Electronics, Netherlands). All patients with a heart rate >70 beats/min received a β -adrenergic receptor blocker (20–40 mg oral metoprolol or intravenous 1–2 mg propranolol) before the CT scan to attain the target heart rate (<70 beats/min). A bolus of 65 mL of contrast (Iopamilone 370, Bayer Schering Pharma Co., Ltd., Osaka, Japan) was injected intravenously at a flow rate of 3.5–4.5 mL/s followed by a 30-mL saline injection at the same flow rate.

Scans were obtained with the following: collimation of 0.625 mm per detector row; table feed of 8.0 mm/rotation; tube current of 400–500 mA, depending on patient body weight; tube voltage of 120 kV; and gantry rotation speed of 420 ms. The estimated mean effective radiation dose was 7–10 mSv. Transaxial images were reconstructed using an XCB convolution kernel (Cardiac Standard) with an image matrix of 512 $\times$ 512 pixels, slice thickness of 0.8 mm, and an

Figure 2. Representative case of coronary plaque. (A,B) MDCT revealed coronary plaque in the distal portion of the right coronary artery (yellow square). (C) Cross-sectional image of coronary plaque. CT attenuation value of plaque was measured in at least 3 cross-sectional images along the plaque by 5-pixel regions of interest (yellow points) at multiple sites in the plaque and averaged. (D) Corresponding angiographic finding at the lesion with coronary plaque (white arrow) are shown. MDCT, multidetector computed tomography.

increment of 0.4 mm using an ECG-gated helical scan algorithm with a resulting maximum temporal resolution of 43 ms in the center of rotation. Images were initially reconstructed at 40% or 70% of the cardiac cycle.

Image Analysis of Coronary Arteries by MDCT

The analysis of 64-slice MDCT image data was performed by 2 experienced readers (A. Taruya and S.H.). We used the analysis system (AZE VirtualPlace, AZE, Ltd., Japan) for CT analysis. Quantitative measurements were performed under concordance of 2 observers. The corresponding images on angiograms and MDCT were identified by the distances from 2 landmarks such as side branches or ostium.

Maximum intensity projections were used to identify coronary lesions, and multiplanar reconstructions in 2 orthogonal longitudinal axes across the coronary lumen were utilized to classify lesions as significant stenosis, which was defined as a diameter reduction $>50\%$.

A noncalcified coronary plaque (NCP) was defined as a low-density mass >1 mm² in size, located within the vessel wall, and clearly distinguishable from the contrast-enhanced coronary lumen and surrounding pericardial tissue. We evaluated NCP characteristics on CT by determining the vascular remodeling index (RI) and minimum CT density. The outer vessel area and arterial RI were assessed on cross-sectional images. The arterial RI was defined as the ratio between the outer vessel area at the site of maximal luminal narrowing and the mean of the proximal and distal reference sites. PR was defined as an RI >1.05 . Calcium deposition was classified as long (>3 mm), short (≤ 3 mm), or none. The evaluation of coronary plaques was performed at a width representing 200% of the mean lumen intensity and at a level representing 65% of that. The CT attenuation values of plaques were measured in multiple (at least 3 sections) cross-sectional images along the plaque by 5-pixel regions of interest at multiple sites in the plaque and then

Table 1. Characteristics of Patients With Stable Angina Pectoris

	Vulnerable plaque (+)	Vulnerable plaque (–)	P value
Patients, n	38	27	
Age (years)	70 [62–74]	67 [59–75]	0.55
Male sex	28 (74)	24 (89)	0.13
Body mass index (kg/m²)	24.4 [22.6–25.7]	23.4 [22.1–24.8]	0.41
Coronary risk factors			
Hypertension	30 (79)	21 (78)	0.91
Diabetes mellitus	13 (34)	10 (37)	0.81
Dyslipidemia	27 (71)	20 (74)	0.79
Current smoking	20 (54)	18 (69)	0.23
Family history	5 (14)	5 (19)	0.39
Obesity	11 (29)	6 (23)	0.60
Laboratory parameters on admission			
hs-CRP (mg/dL)	0.11 [0.05–0.16]	0.12 [0.05–0.61]	0.18
Total cholesterol (mg/dL)	207 [158–223]	188 [162–218]	0.78
Triglyceride (mg/dL)	137 [95–172]	123 [84–230]	0.70
LDL-C (mg/dL)	108 [86–122]	97 [77–129]	0.44
HDL-C (mg/dL)	40 [37–59]	48 [35–59]	0.90
HbA1c (%)	5.7 [5.4–6.4]	5.6 [5.4–7.0]	0.54
Medications on admission			
Aspirin	24 (63)	21 (78)	0.21
ACEI or ARB	17 (45)	11 (41)	0.75
β -blocker	9 (24)	4 (15)	0.38
CCB	18 (47)	17 (63)	0.21
Statin	22 (58)	10 (37)	0.10
Insulin	1 (3)	1 (4)	0.66

Data are presented as number (%) or median [interquartile range]. ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II type1 receptor blocker; CCB, calcium-channel blocker; HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein-cholesterol; hs-CRP, high-sensitivity C-reactive protein; LDL-C, low-density lipoprotein-cholesterol.

averaged (**Figure 2**). If the culprit plaque had any calcified components, regions of interest were positioned on the noncalcified area. Because Kashiwagi et al previously reported that thin-capped fibroatheroma showed PR and low CT attenuation value (35.1 ± 32.3),³¹ we defined vulnerable plaques as those with PR and/or LAP (<35 HU).

Statistical Analysis

Data are expressed as mean $\pm$ standard deviation values for normally distributed variables and median (interquartile range) for skewed variables. Categorical variables are presented as number (percent) and compared using the chi-square test. The nonparametric Mann-Whitney U test was used to test for differences between 2 groups. We used the nonparametric Kruskal-Wallis test to assess the difference of TLR-4 expression among the 3 monocyte subsets. Spearman's rank correlation coefficient was used to assess the correlations between 2 parameters. Multivariate linear regression was used to identify the factors associated with the expression of TLR-4 on CD14⁺⁺CD16⁺ monocytes and multivariate logistic regression was used to determine the contributors for the presence of intracoronary vulnerable plaques. Variables showing $P < 0.10$ in univariate analysis for the presence of intracoronary vulnerable plaque and clinically meaningful factors for vulnerable plaque (age, sex, coronary risk factors, hs-CRP, and medication with aspirin, angiotensin-converting enzyme inhibitor or angiotensin II type 1 receptor blocker, β -blocker, statin, or insulin)

were included in the multivariate regression analysis. $P < 0.05$ was considered statistically significant. All statistical analyses were performed with SPSS 11.0 statistical software (SPSS Inc., Chicago, IL, USA). All authors had full access to the data and take responsibility for its integrity.

Results

Patients' Characteristics

A total of 65 patients with SAP were enrolled. Of these, 38 (58%) had vulnerable coronary plaques, as defined by the presence of PR and/or LAP. The baseline clinical characteristics of the study subjects according to the presence of vulnerable plaque are listed in **Table 1**. Clinical characteristics, including hs-CRP, did not differ significantly between the 2 groups. MDCT findings, including lesion site, the number of lesions, PR and CT attenuation values, are summarized in **Table 2**.

Heterogeneous Overexpression of TLR-4

Peripheral blood samples were obtained from patients at admission and analyzed for the 3 distinct monocyte subsets (CD14⁺CD16⁺, CD14⁺⁺CD16⁺, and CD14⁺⁺CD16[–]). Circulating peripheral CD14⁺⁺CD16⁺ monocytes more frequently expressed TLR-4 than CD14⁺CD16⁺ and CD14⁺⁺CD16[–] monocytes ($P < 0.001$) (**Figure 3A**). Moreover, the expression of TLR-4 on CD14⁺⁺CD16⁺ monocytes was significantly higher in patients with vulnerable plaque

	Vulnerable plaque (+) (n=38)	Vulnerable plaque (-) (n=27)	P value
Lesion site			
LAD	18 (47)	18 (67)	0.12
LCx	10 (26)	5 (19)	0.46
RCA	16 (42)	6 (22)	0.10
Multivessel disease	6 (16)	2 (7)	0.27
Outer vessel area (mm ²)	20.2 [16.4–24.5]	16.9 [12.4–20.3]	0.02
Luminal area (mm ²)	6.1 [3.8–8.9]	6.7 [6.2–13.9]	0.06
Plaque area (%)	68.9 [49.9–83.6]	46.7 [38.1–64.8]	<0.01
RI	1.11 [1.07–1.19]	0.97 [0.95–1.01]	<0.01
Positive remodeling	35 (92)	–	–
CT attenuation value (HU)	45.6 [34.6–58.7]	63.1 [54.0–69.5]	<0.01
Calcium deposition			
Long (>3mm)	17 (45)	7 (26)	0.12
Short (≤3mm)	8 (21)	4 (15)	0.38
None	13 (34)	16 (59)	0.05

Data are presented as numbers (%) or median [interquartile range]. LAD, left anterior descending artery; LCx, left circumflex artery; MDCT, multidetector computed tomography; RCA, right coronary artery; RI, remodeling index.

Figure 3. (A) Comparison of frequencies of Toll-like receptor 4 (TLR-4) expression among 3 monocyte subsets in patients with stable angina pectoris. Circulating peripheral CD14⁺CD16⁺ monocytes more frequently expressed TLR-4 than did CD14⁺CD16⁺ and CD14⁺CD16⁻ monocytes (CD14⁺CD16⁺; 6.6 [3.3–12.5] % vs. CD14⁺CD16⁺; 2.9 [1.6–6.0] % vs. CD14⁺CD16⁻; 1.3 [0.6–2.6] %, $P<0.001$). Data are presented as box and whisker plots with median and 25–75th percentiles (boxes) and 10–90th percentiles (whiskers). (B) Comparison of frequencies of TLR-4 expression on CD14⁺CD16⁺ monocytes between patients with intracoronary vulnerable plaque and those without. TLR-4 on CD14⁺CD16⁺ monocytes was more highly expressed in patients with intracoronary vulnerable plaque than in those without (10.4 [4.1–14.5] % vs. 4.5 [2.8–7.8] %, $P=0.012$). Data are presented as box and whisker plots with median and 25–75th percentiles (boxes) and 10–90th percentiles (whiskers).

compared with those without (10.4 [4.1–14.5] % vs. 4.5 [2.8–7.8] %, $P=0.012$) (Figure 3B).

Relationship Between Vulnerable Plaque and Expression of TLR-4

Multivariate logistic regression analysis demonstrated that only the expression of TLR-4 on CD14⁺CD16⁺ monocytes was an independent contributor to intracoronary vulnerable plaques in patients with SAP ($P=0.023$, odds ratio: 1.123, 95% confidence interval: 1.016–1.241). Furthermore, the presence of intracoronary vulnerable plaques and medica-

tion with aspirin were associated with the expression of TLR-4 on CD14⁺CD16⁺ monocytes in our multivariate regression analysis (Table 3).

Relationship Between TLR-4, RI, and CT Attenuation Value

The relative proportion of the expression of TLR-4 on CD14⁺CD16⁺ monocytes positively correlated with the RI and negatively correlated with the CT attenuation value ($r=0.28$, $P=0.025$ (Figure 4A) and $r=-0.31$, $P=0.013$ (Figure 4B), respectively). On the other hand, neither RI nor CT attenuation value significantly related with the

Table 3. Multivariable Regression Analysis of TLR-4 Expression in Patients With Stable Angina Pectoris

	β coefficient	P value
Age	-0.120	0.36
Male sex	0.006	0.96
Hypertension	0.110	0.40
Diabetes mellitus	-0.129	0.32
Dyslipidemia	-0.209	0.07
Current smoking	-0.173	0.18
Obesity	-0.074	0.57
hs-CRP	-0.100	0.44
Vulnerable plaque	0.277	0.02
Aspirin	-0.310	0.01
ACEI or ARB	0.034	0.80
β -blocker	-0.157	0.23
Statin	0.048	0.71
Insulin	-0.040	0.76

TLR-4, Toll-like receptor 4. Other abbreviations as in Table 1.

hs-CRP level in our study population (data not shown). As expected, there was no significant correlation between hs-CRP and the expression of TLR-4 on CD14⁺⁺CD16⁺ monocytes (data not shown).

Relationship Between Vulnerable Plaque, TLR-4 Expression and MMP-9

The plasma levels of MMP-9 were significantly higher in patients with vulnerable plaque than in those without (42 [21–62] ng/mL vs. 27 [13–47] ng/mL, $P=0.024$) (**Figure 5A**). A scatter plot of MMP-9 and TLR-4 expression on CD14⁺⁺CD16⁺ monocytes is shown in **Figure 5B**. The plasma levels of MMP-9 positively correlated with the level of TLR4 expression on CD14⁺⁺CD16⁺ monocytes in patients with SAP ($r=0.32$, $P=0.010$).

Discussion

To evaluate the relationship between coronary plaque vulnerability and the expression of TLR-4 on circulating peripheral monocytes was the focus of this research. TLR-4 expression on CD14⁺⁺CD16⁺ monocytes was increased in patients with vulnerable plaques independent of patient characteristics including age, sex, coronary risk factors, and hs-CRP. Moreover, the expression levels of TLR-4 on CD14⁺⁺CD16⁺ monocytes significantly correlated with the values of plaque vulnerability. Taken together, the results suggested that upregulation of TLR-4 expression on CD14⁺⁺CD16⁺ monocytes might be involved in plaque vulnerability in patients with SAP.

Previous cross-sectional studies have reported that increased numbers of circulating monocytes in individuals are associated with prevalent atherosclerotic disease.^{32,33} Additionally, prospective studies suggest that the monocyte count independently predicts cardiovascular events.^{34,35} We previously reported a significant association between an increased proportion of CD14⁺⁺CD16⁺ monocytes and the severity of coronary artery disease (CAD).³⁶ These findings suggest that CD14⁺⁺CD16⁺ monocytes are closely related to CAD progression. Furthermore, CD14⁺⁺CD16⁺ monocytes more frequently expressed TLR-4 than CD14⁺CD16⁺ and CD14⁺⁺CD16⁻ monocytes, so we have focused on TLR-4 expression by CD14⁺⁺CD16⁺ monocytes. Moreover, Tsujioka et al reported that CD14⁺⁺CD16⁻ monocytes were associated with acute-phase inflammation.³⁷ In the light of these reports, we consider that CD14⁺⁺CD16⁺ monocytes and CD14⁺⁺CD16⁻ monocytes might be respectively related to chronic- and acute-phase inflammation.

TLR-4 plays an important role in CAD.³⁸ Methe et al reported that circulating levels of TLR-4-expressing monocytes were increased in patients with AMI, compared with those with SAP.³⁹ It was also reported that TLR-4 expression was increased on monocytes from coronary thrombus than from peripheral blood in patients with ACS,¹⁸ and we previously demonstrated that expression of TLR-4 on distinct monocyte subsets was associated with the pathogenesis of ACS.¹⁷ Furthermore, we previously revealed that upregulation of PSGL-1 on CD14⁺⁺CD16⁺ monocytes might play a crucial role in plaque rupture and thrombus formation, which leads to ACS event, and that the expression of TLR-4 on CD14⁺⁺CD16⁺ monocytes correlated with that of PSGL-1 on CD14⁺⁺CD16⁺ monocytes.²⁸ Thus, the expression of TLR-4 might be related to the process of plaque vulnerability. In the present study, the expression of TLR-4 on CD14⁺⁺CD16⁺ monocytes was significantly elevated in patients with vulnerable plaque compared with those without. Additionally, multivariate analysis demonstrated that only the expression of TLR-4 on CD14⁺⁺CD16⁺ monocytes was an independent factor affecting intracoronary vulnerable plaque, and both the presence of intracoronary vulnerable plaque and medication with aspirin were associated with the expression of TLR-4 on CD14⁺⁺CD16⁺ monocytes. It is likely that increased expression of TLR-4 on CD14⁺⁺CD16⁺ monocytes contributes to plaque instability in patients with SAP.

MDCT can noninvasively assess coronary artery stenosis and plaque characterization.⁴⁰ In the clinical setting, detection of vulnerable plaque is one of the most important uses of MDCT. Several studies have demonstrated the CT characteristics of plaques associated with ACS, including PR, LAP, and spotty calcification.^{41,42} Motoyama et al

reported that PR and LAPs assessed by MDCT, which portended future ACS onset, were the most important features of vulnerable plaque on MDCT.⁴³ Kashiwagi et al revealed that PR and LAP were MDCT-identified morphological features, similar to optical coherence tomography-identified vulnerable plaque.³¹ In this study, we demonstrated that the relative proportion of the expression of TLR-4 on CD14⁺⁺CD16⁺ monocytes correlated with the RI and CT attenuation value, strengthening the association between TLR-4 expression and progression of coronary plaque instability in patients with SAP. However, we could not demonstrate a causal role of TLR-4 on distinct circulating monocytes in plaque instability in patients with SAP. However, TLR-4 has been reported as involved in the pathogenesis of atherosclerosis and plaque destabilization,⁴⁴ so further investigations are required to clarify this issue.

It was reported that MMP-9 is closely related to atherosclerotic progression.⁴⁵ In the present study, the plasma MMP-9 levels had a significant correlation with the expression of TLR4 on CD14⁺⁺CD16⁺ monocytes. Our results might provide evidence that TLR-4 expression is associated with atherosclerotic progression. On the other hand, numerous studies have suggested that an elevated hs-CRP level in patients with ACS reflects inflammation of the coronary arteries.^{46,47} Necrotic debris and inflammatory mediators from coronary plaque are released into the systemic circulation, with a subsequent increase in the hepatic release of acute-phase reactants such as hs-CRP. However, the results of the present study indicated that hs-CRP levels were similar between patients with and without vulnerable plaque, and neither the RI nor CT attenuation value was significantly associated with hs-CRP levels in SAP. Naturally, we found no relation between hs-CRP level and the expression of TLR-4 on CD14⁺⁺CD16⁺ monocytes. Although our results suggested that a relative increase in the expression of TLR-4 on CD14⁺⁺CD16⁺ monocytes might be superior to hs-CRP for determining plaque instability in patients with SAP, further studies are needed for clarification.

Study Limitations

First, the study population was relatively small and our results may not be applicable to all patients with CAD because we excluded some patients with severe calcification, left main coronary lesion, renal dysfunction, or inflammatory status. Second, we did not use the Fluorescence Minus One plot as a control sample. Third, we did not investigate other inflammatory molecule expression such as CCL, CXCL, CCR, CXCR family, or other inflammatory TLR molecules. Fourth, we did not compare functional differences such as pro-inflammatory function, proliferation/migration capacity or phagocytosis, between CD14⁺⁺CD16⁺TLR-4⁺ and CD14⁺⁺CD16⁺TLR-4⁻ monocytes. Finally, correlations do not necessarily indicate causal relationships with plaque morphology, and further mechanistic studies are required to clarify the role of TLR-4 on distinct monocyte subsets in coronary plaque vulnerability.

Conclusions

The present results showed a significant association between overexpression of TLR-4 on CD14⁺⁺CD16⁺ monocytes and vulnerability of intracoronary plaque assessed by MDCT in patients with SAP. These findings suggested that upregulation of TLR-4 on CD14⁺⁺CD16⁺ monocytes might be

associated with coronary plaque vulnerability.

Acknowledgments

This work was supported in part by 2015 Wakayama Medical Award for Young Researchers, Grant-in-Aid for Young Scientists (B), Japan (No. 16K19420), and Grant-in-Aid for Scientific Research (C), Japan (No. 26461114).

Disclosures

The authors have no conflict of interest to declare.

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