The spectrum of clinical syndromes caused by coronary atherosclerosis ranges from asymptomatic disease and stable angina pectoris (SAP) to acute coronary syndrome (ACS), including unstable angina pectoris (UAP). ACS develops as a series of nonlinear events in an otherwise slowly progressive process. The nonlinearity has been attributed to a combination of factors, of which plaque rupture and superimposed thrombosis are considered the most important. Although the pathways leading to plaque rupture remain incompletely understood, recent research has shown that the participation of immune cells and inflammation is a key factor.

Given the vital role of leukocyte recruitment at all stages of cardiovascular disease progression, from early plaque development to plaque rupture, the chemokine family is thought to contribute significantly to the pathogenesis of cardiovascular disease. Fractalkine (FKN or CX3CL1) is particularly interesting because of its potential pathophysiological role in atherosclerosis. High-affinity adhesion is mediated by direct binding of FKN to its receptor, CX3CR1, on monocytes, T lymphocytes, and natural killer (NK) cells.

Intravascular optical coherence tomography (OCT) has recently emerged as a high-resolution imaging method of plaque characterization. OCT is an optical analog of intravascular ultrasound, with a resolution of approximately 10–20 μm. We have recently shown that OCT enables more reliable identification of plaque rupture in vivo than conventional imaging techniques such as intravascular ultrasound and coronary angiography.

A previous study has shown that the level of FKN/CX3CR1 is increased in coronary artery disease (CAD), but to the best of our knowledge, the relationship between coronary plaque morphology and FKN/CX3CR1 has not yet been investigated. The aim of this study, therefore, was to investigate whether the FKN–CX3CR1 pathway affects coronary plaque rupture at the culprit site in patients with UAP.

**Background:** Recent studies suggest that fractalkine (FKN or CX3CL1) and its cognate receptor, CX3CR1, play a role in atherogenesis, so the relationship between coronary plaque rupture, as observed by preintervention optical coherence tomography, and plasma levels of FKN and CX3CR1 was investigated in this study.

**Methods and Results:** The study population consisted of 46 patients with unstable angina pectoris (UAP), 30 patients with stable angina pectoris, and 25 healthy controls. The UAP patients underwent a preintervention optical coherence tomography study, which revealed that the number of patients with and without plaque rupture at the culprit site was 27 (rupture group) and 19 (non-rupture group), respectively. Plasma levels of soluble FKN (sFKN) and CX3CR1 were measured by enzyme-linked immunosorbent assay and flow cytometry, respectively. The plasma levels of sFKN were significantly increased in UAP patients with plaque rupture compared with patients in the other groups. Multiple logistic regression analysis showed that CD14+CD16+CX3CR1+ monocytes and CD3+CX3CR1+ lymphocytes were independent predictors of the presence of ruptured plaque.

**Conclusions:** Increases in the FKN level and the number of CX3CR1-expressing mononuclear cells might contribute to coronary plaque rupture. (Circ J 2010; 74: 337–345)

**Key Words:** Acute coronary syndrome; Chemokine; Coronary plaque rupture; Optical coherence tomography
Figure 1. Expressions of CX3CR1-positive monocytes, T lymphocytes, and natural killer (NK) cells in patients with angina pectoris. CX3CR1 surface expressions were measured by flow cytometry as described in the Methods section. The numbers of CD14+CD16+ monocytes (A), and CD3+CX3CR1+ T lymphocytes and CD3-CD16+CX3CR1+ NK cells (B) were determined on hospital admission.
Table 1. Clinical Characteristics of Patients

<table>
<thead>
<tr>
<th></th>
<th>Control (n=25)</th>
<th>SAP (n=30)</th>
<th>UAP</th>
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</thead>
<tbody>
<tr>
<td><strong>Age, years</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male (%)</td>
<td>14 (56)</td>
<td>19 (63)</td>
<td>19 (70)</td>
</tr>
<tr>
<td><strong>Risk factors</strong></td>
<td></td>
<td></td>
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<tr>
<td>Hypertension</td>
<td>11 (44)</td>
<td>15 (50)</td>
<td>16 (59)</td>
</tr>
<tr>
<td>Smoking</td>
<td>11 (44)</td>
<td>16 (59)</td>
<td>10 (53)</td>
</tr>
<tr>
<td>Cholesterol, mg/dl</td>
<td>175±30</td>
<td>111±33</td>
<td>120±36</td>
</tr>
<tr>
<td>LDL-C, mg/dl</td>
<td>96±30</td>
<td>111±33</td>
<td>112±31</td>
</tr>
<tr>
<td>HDL-C, mg/dl</td>
<td>52±12</td>
<td>49±14</td>
<td>43±15</td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>106±23</td>
<td>108±27</td>
<td>103±19</td>
</tr>
<tr>
<td><strong>Laboratory parameters on admission</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>CRP, mg/L</td>
<td>0.70±0.66</td>
<td>3.20±2.75</td>
<td>5.99±3.33*</td>
</tr>
<tr>
<td>WBC, 10^3 cells/μl</td>
<td>5.9±1.3</td>
<td>6.2±1.8</td>
<td>7.2±2.4</td>
</tr>
<tr>
<td><strong>Medications on admission</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspirin</td>
<td>–</td>
<td>20 (67)</td>
<td>17 (63)</td>
</tr>
<tr>
<td>ACEIs or ARBs</td>
<td>–</td>
<td>15 (50)</td>
<td>16 (59)</td>
</tr>
<tr>
<td>β-blockers</td>
<td>–</td>
<td>11 (37)</td>
<td>4 (15)</td>
</tr>
<tr>
<td>Lipid-lowering drugs</td>
<td>–</td>
<td>10 (33)</td>
<td>8 (30)</td>
</tr>
</tbody>
</table>

Mean±SD, or n (percentage).
*P=0.0020, Rupture vs Non-rupture; P<0.0001, Rupture vs SAP; P<0.0001, Rupture vs Control; P=0.0003, Non-rupture vs Control; P=0.0004, SAP vs Control.
SAP, stable angina pectoris; UAP, unstable angina pectoris; LDL-C, low-density lipoprotein-cholesterol; HDL-C, high-density lipoprotein-cholesterol; CRP, C-reactive protein; WBC, white blood count; ACEIs, angiotensin-converting enzyme inhibitors; ARBs, angiotensin-receptor blockers.

**Methods**

**Patient Population**
Our study included 46 consecutive UAP patients (defined as having ischemic chest pain at rest within the preceding 48 h, transient ST-T segment depression, and/or T-wave inversion but no evidence of myocardial necrosis by enzymatic criteria) who underwent OCT. OCT revealed plaque rupture at the culprit site in 27 (rupture group) and none in 19 (non-rupture group). We also included 30 patients with SAP (defined as having effort angina >3 months and a positive exercise test). The diagnosis of CAD was confirmed by coronary angiography. Culprit lesions were identified by the association of ECG signs of ischemia and angiographic findings of the lesion. For comparison, blood was collected from 25 healthy controls matched for age, sex, and smoking habits. Although asymptomatic CAD could not be totally excluded, all controls were evaluated as healthy based on clinical examination, disease history, and analysis of high-sensitivity C-reactive protein (hsCRP) and lipid parameters. Neither the patients nor the control subjects included in this study showed any evidence of known systemic inflammatory diseases, including peripheral vascular disease, significant endocrine, hepatic, renal, or inflammatory disease, and surgery or major trauma in the previous month. All patients (both UAP and SAP) underwent coronary angiography. Culprit lesions were morphologically classified as previously reported that is, they were classified by a consensus of 2 investigators (H.T. and H.K.): (1) concentric stenosis = symmetric narrowing of a coronary artery; the borders of the lesion are smooth or only slightly irregular; (2) eccentric stenosis = asymmetric narrowing of a coronary artery; 2 subgroups of eccentric lesions were categorized: (a) type I eccentric lesion is any asymmetric stenosis with smooth borders and a broad neck; (b) type II eccentric lesion is an asymmetric stenosis usually in the form of a convex intraluminal obstruction with a narrow base or neck because of 1 or more overhanging edges or borders that are very irregular or scalloped; (3) multiple irregularities = 3 or more serial and severe (>70%), closely spaced obstructions in a coronary artery. When a culprit lesion was identified as suitable for percutaneous coronary intervention (PCI) using a coronary stent, the procedure was performed.

The study complied with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of Wakayama Medical University. Written informed consent was given by all participants.

**Clinical Parameters**
The clinical parameters assessed included age, sex, and coronary risk factors (smoking, hypertension, diabetes mellitus, hyperlipidemia, and obesity). The diagnostic criteria for coronary risk factors were as follows: hypertension = blood pressure ≥140/90 mmHg, and/or a history of antihypertensive drug use; diabetes mellitus = fasting plasma glucose of ≥126 mg/dl or casual plasma glucose ≥200 mg/dl, or diabetes as shown by an oral glucose tolerance test; hyperlipidemia = serum total cholesterol level ≥220 mg/dl or serum triglyceride level ≥150 mg/dl; obesity = body mass index ≥25 kg/m².

**OCT Imaging Protocol**
Oral aspirin (162 mg) and intravenous heparin (8,000 U) were given to all patients before the coronary intervention. OCT was used to observe the culprit lesion in the coronary artery. A 0.014-inch (distal) OCT catheter (Image Wire; LightLab Imaging Inc, Westford, MA, USA) was advanced distal to the culprit lesion through an occlusion catheter (Helios; Goodman Co Ltd, Nagoya, Japan). In order to remove the blood from the imaging field, the occlusion balloon was inflated to 0.5 atm proximal to the culprit lesion, and Ringer's solution was then infused into the coronary artery from the
Figure 2. Relationships between hsCRP and sFKN or CX3CR1+ leukocytes. There was a significant positive correlation between hsCRP and sFKN (A). However, expression levels of CD14+CD16+CX3CR1+ monocytes (B), CD3+CX3CR1+ T-lymphocytes (C), and CD3-CD16+CX3CR1+ NK cells (D) did not show any significant positive correlation with hsCRP levels. hsCRP, high-sensitivity C-reactive protein; NK, natural killer; sFKN, soluble fractalkine.

Table 2. Coronary Angiographic Findings

<table>
<thead>
<tr>
<th></th>
<th>SAP (n=30)</th>
<th>UAP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of vessels with ≥50% obstruction</td>
<td>Rupture (n=27)</td>
</tr>
<tr>
<td>Distribution of CAD</td>
<td></td>
<td>1.53±0.68</td>
</tr>
<tr>
<td>1 vessel</td>
<td>1.63±0.74</td>
<td>12 (63)</td>
</tr>
<tr>
<td>2 vessels</td>
<td>1.53±0.70</td>
<td>10 (33)</td>
</tr>
<tr>
<td>3 vessels</td>
<td></td>
<td>3 (10)</td>
</tr>
<tr>
<td>Distribution of culprit vessel</td>
<td></td>
<td>1.63±0.74</td>
</tr>
<tr>
<td>Left anterior descending</td>
<td>14 (47)</td>
<td>13 (48)</td>
</tr>
<tr>
<td>Left circumflex</td>
<td>7 (23)</td>
<td>7 (26)</td>
</tr>
<tr>
<td>Right</td>
<td>9 (30)</td>
<td>7 (26)</td>
</tr>
<tr>
<td>Morphology at the culprit site</td>
<td></td>
<td>1.63±0.74</td>
</tr>
<tr>
<td>Concentric</td>
<td>6 (20)</td>
<td>4 (19)</td>
</tr>
<tr>
<td>Type I eccentric</td>
<td>13 (44)</td>
<td>10 (41)</td>
</tr>
<tr>
<td>Type II eccentric</td>
<td>7 (23)</td>
<td>8 (22)</td>
</tr>
<tr>
<td>Multiple irregularities</td>
<td>4 (13)</td>
<td>5 (19)</td>
</tr>
</tbody>
</table>

Data are mean±SD or n (%). CAD, coronary artery disease. Other abbreviations see in Table 1.
For proximal lesions, we used the flush-only (non-occlusive) technique for OCT image acquisition, which is a newly developed alternative to the balloon-occlusion technique. To flush the vessel, we infused commercially available Dextran 40 and lactated Ringer’s solution (Low Molecular Dextran L Injection, Otsuka Pharmaceutical, Tokushima, Japan) directly from the guiding catheter at a rate of 2.5–4.5 ml/s using an injector pump (Mark V, Medrad, Inc, PA, USA). The culprit lesion was subsequently imaged with an automatic pullback device moving at 1 mm/s. The OCT images were digitized and analyzed using the M2CV OCT console.

**OCT Image Analysis**
All OCT images were analyzed by 2 independent investigators (H.T. and H.K.) who were unaware of the clinical presentations. When there was discordance between observers, a consensus reading was obtained. The presence of plaque rupture, intracoronary thrombus or thin-cap fibroatheroma (TCFA) at a culprit lesion was noted. The validated criteria for plaque characterization were described previously. Briefly, plaque rupture was identified by fibrous cap discontinuity and cavity formation of the plaque. Intracoronary thrombus was identified as a mass protruding into the lumen from the surface of the vessel wall. TCFA was defined as a plaque with lipid content in ≥2 quadrants and the thinnest part of a fibrous cap measuring <60 μm.

**Cytometric Analysis**
For cytometric analysis, monoclonal antibodies against CD14 (phycoerythrin (PE), Clone M5E2, Becton Dickson Bioscience, San Jose, CA, USA), CD16 (PE-Cy5, Clone 3G8, BD Pharmingen), CD3 (a specific marker of T lymphocytes, PE, clone UCHT1, Becton Dickinson, San Jose, CA, USA), and CX3CR1 (fluorescein isothiocyanate (FITC), Clone 2A9-1, MBL, Nagoya, Japan) were used. CD16 is expressed in monocytes, as well as NK cells and granulocytes, but not in T or B lymphocytes (Technical data sheet, BD Pharmingen). A total of 100 μl of blood was incubated for 15 min at room temperature. For erythrocyte lysis and leukocyte fixation,
1 ml of lysing solution was added (Lysing solution Becton Dickinson, Germany). Dead cells (~15%) were identified with propidium iodine and gated out. Nonspecific IgG isoatypes were used as negative controls. Flow cytometry was performed using a FACSCaliber instrument with CellQuest software (Becton Dickinson Bioscience). Monocytes were first gated in a forward scatter/sideward scatter (FSC/SSC) dotplot, and then 3-color fluorescence was measured within the monocyte gate (Figure 1A). The CD14+CD16+ cells was defined as monocytes expressing CD16 and either high levels

![Figure 5. CX3CR1-expressing monocytes, T lymphocytes and natural killer (NK) cells and plaque rupture. CX3CR1 surface expression was measured by flow cytometry as described in the Methods section. The numbers of CD14+CD16+CX3CR1+ monocytes (A), CD3+CX3CR1+ T lymphocytes (B), and CD3−CD16+CX3CR1+ NK cells (C) in 46 patients with unstable angina pectoris (UAP) and plaque rupture (27 patients) or no rupture (19 patients), 30 patients with stable angina pectoris (SAP), and 25 healthy controls were determined on hospital admission.]
of CD14 (CD14brightCD16−) (Figure 1A, upper right quadrant) or lower levels of CD14 (CD14dimCD16+) (Figure 1A, upper left quadrant). Thus, CD14brightCD16+ and CD14dimCD16+ were not analyzed separately, based on a previous study. Thereafter, CD14CD16CX3CR1+ cells were determined. Similarly, lymphocytes were first gated in an FSC/SSC dotplot and then both CD3CX3CR1+ T lymphocytes and CD3CD16CX3CR1+ NK cells were determined (Figure 1B). List mode files were collected in 100,000 cells from each sample.

**Blood Sampling and Analysis**

Peripheral blood samples were collected from all subjects upon admission. Plasma samples were collected in EDTA anticoagulant tubes and stored at −80°C until assay.

Plasma levels of sFKN were measured with an enzyme-linked immunosorbent assay kit (no. DY365 DuoSet System for human fractalkine/CX3CL1, R&D Systems, Minneapolis, MN, USA). hsCRP was analyzed using a commercially available testing kit (N-Latex CRP II, Dade Behring, Marburg, Germany).

**Statistical Analysis**

If not stated otherwise, continuous variables are expressed as mean±SD. Baseline characteristics were analyzed using the χ² test, Fisher test or 1-way ANOVA, as appropriate. When data were not normally distributed (according to the Shapiro-Wilks test), they are expressed as median and range, and were analyzed with nonparametric methods. When more than 2 groups of individuals were compared, the nonparametric Kruskal-Wallis test was used. If a significant difference was found, pairwise comparisons using the Bonferroni test were made. To assess correlations between 2 parameters, simple linear regressions were calculated using the least-squares method. Multiple logistic regression analysis was used to identify independent predictors of coronary plaque rupture. The multiple regression model adjusted for hsCRP was performed including sFKN or CX3CR1-positive cells, which had a significant association with plaque rupture by univariate analysis. The crude and adjusted odds ratios (ORs) and their 95% confidence intervals (CIs) were calculated. P values <0.05 were considered significant. All statistical analyses were performed with the statistical software package SPSS 11.0 (SPSS Inc, Chicago, IL, USA).

**Results**

**Patient Characteristics and Coronary Angiographic Findings**

The patients’ characteristics are summarized in Table 1. There were no differences in terms of age, sex, risk factors, white blood cell count, number of diseased vessels or medications on admission. However, in line with previous studies, the levels of hsCRP were significantly higher in UAP patients with plaque rupture than in the other patient groups (Table 1). The crude OR of hsCRP for plaque rupture was 1.375 (95%CI: 1.057–1.788). We also found a significant positive correlation between hsCRP and sFKN (Figure 2A), but did not find any significant relationships between hsCRP and CD14CD16CX3CR1+ monocytes, CD3CX3CR1+ T lymphocytes or CD3CD16CX3CR1+ NK cells (Figures 2B–D, respectively). The coronary angiography findings for all 76 patients with SAP and UAP are listed in Table 2. The incidence of 1-, 2-, and 3-vessel disease, and the distribution of culprit vessels were not significantly different among groups (Table 2). In addition, morphologic findings on coronary angiography were not significantly different among groups (Table 2).

**OCT Findings**

We used OCT to analyze the culprit lesions in 46 UAP patients and they were successfully observed in all patients without any serious procedural complications. Plaque rupture at the culprit site was observed in 27 UAP patients (59%; rupture group) and no plaque rupture was found in 19 UAP patients (41%; non-rupture group). Representative cases are shown in Figure 3. OCT findings of the culprit lesions in the rupture group revealed that 21 (78%) patients presented with thrombus, 12 (44%) with TCFA, and 17 (63%) with a break at the plaque shoulder.

**Expressions of sFKN in AP**

As shown in Figure 4, CAD patients had significantly increased plasma levels of sFKN compared with healthy controls. It is interesting that the levels in UAP patients with plaque rupture were significantly higher than those in the UAP patients without plaque rupture and the SAP patients (Figure 4).

**Effect of CX3CR1-Expressing Monocytes, T Lymphocytes and NK Cells on Plaque Rupture**

The numbers of CD14CD16CX3CR1+ monocytes, CD3CX3CR1+ T lymphocytes, and CD3CD16CX3CR1+ NK cells were all significantly higher in UAP patients with plaque rupture than in the other patient groups (Figures 5A–C). After adjustment for hsCRP, multiple logistic regression analysis showed that CD14CD16CX3CR1+ monocytes and CD3CX3CR1+ T lymphocytes were independent predictors of the presence of ruptured plaque (Table 3).

**Discussion**

Accumulating evidence suggests that the destabilization of coronary plaque is associated with systemic immune activation. However, it remains unclear whether plaque rupture is triggered by inflammation. In this regard, the present study has shown for the first time that upregulation of FKN and
CX3CR1-expressing mononuclear cells, including monocytes, T lymphocytes, and NK cells, is associated with plaque rupture in UAP patients.

There is growing evidence to suggest that the FKN–CX3CR1 pathway may be involved in atherosclerosis and cardiovascular pathophysiology.28 Lesnik et al23 and Combadiere et al24 reported that FKN expression is upregulated in atherosclerotic lesions of apolipoprotein E-deficient (apoE−/−) mice and that crossing CX3CR1−/− into the apoE−/− background results in decreased atherosclerotic lesion formation. In addition, gene polymorphisms at amino acids 249 and 280 of CX3CR1 in humans are reported to be a genetic risk factor for CAD.25,26 CX3CR1-V249I/T280M heterozygosity is associated with a markedly reduced risk of acute coronary events. This protective effect could be explained by the decreased ability of CX3CR1-expressing monocytes, T lymphocytes, and NK cells to invade the vascular wall. On the other hand, ACS develops if inflammation of the atherosclerotic plaque leads to plaque rupture, subsequent thrombosis, and myocardial ischemia.27,28 In the present study the levels of hsCRP, FKN, and CX3CR1 in the UAP (non-rupture) group were similar to those in the SAP group, which suggests that coronary plaque rupture itself might be related not only to inflammation but also to FKN/CX3CR1. The precise mechanisms behind these results remain unresolved. The risk of plaque rupture depends on the number and activation status of macrophages, rather than on plaque size.30 One possible mechanism is that the increase in CX3CR1-expressing monocytes may result in upregulation of monocytes within the inflamed plaque. Another possibility is an effect of CX3CR1-expressing T lymphocytes and NK cells on macrophage activation at the culprit lesion. Liuzzo et al showed that monocytes from UAP patients have a molecular fingerprint of recent interferon (IFN)-γ signaling, which suggests that monocytes are activated, at least in part, by IFN-γ probably derived from stimulated T lymphocytes and NK cells.31 Taken together, these findings suggest that an increase in CX3CR1-expressing monocytes, T lymphocytes, and NK cells might result in a transient burst of macrophage activation, leading to plaque rupture. In line with this, Caligiuri et al showed that in culprit lesions of ACS, the percentage of activated T lymphocytes is significantly increased and that atherectomy specimens from UAP patients but not SAP patients induce lymphocyte proliferation.32 In addition, Clerc and Rouz demonstrated that patients with severe atherosclerotic disease have higher circulating levels of NK cells.33 Furthermore, immunohistochemical analysis of autopsy specimens revealed that NK cells exist more in the shoulder regions than in the necrotic core.34 Several studies have found that elevated hsCRP levels may be related to the presence of ruptured plaque, and those conducted in the setting of AMI suggest that elevated hsCRP levels may reflect the inflammatory activity of plaque rupture.20,21 In the present study, we confirmed that elevated hsCRP levels in UAP patients are significantly associated with plaque rupture.

Study Limitations

First, the study was conducted at a single center with a small sample size. Larger cohort studies will be necessary to confirm our findings. Second, our study was observational and does not provide a mechanistic explanation for the significant relationship between plaque rupture at the culprit site and the levels of expression of CX3CR1-expressing mononuclear cells. Specifically, we could not determine the trigger activating the recruited T lymphocytes and NK cells to release macrophage-stimulating mediators. Further studies are therefore needed to unravel the mechanisms of ACS, especially those related to coronary plaque rupture.

Conclusions

This study identified for the first time that increases in sFKN and CX3CR1-expressing monocytes, T lymphocytes, and NK cells are significantly related to plaque rupture, which may provide new conceptual and therapeutic approaches for treating ACS. In addition, CD14+CD16+CX3CR1+ monocytes and CD3+CX3CR1+ T lymphocytes were independent predictors of coronary plaque rupture by multiple logistic regression analysis. These findings also highlight the importance of characterizing specific functions of the chemokine network to enable therapeutic intervention.

Acknowledgment

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Disclosure

Conflict of interest and financial disclosure: none.

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