Atherosclerosis is a chronic inflammatory–fibroproliferative disease of the vessel wall. Apart from the local process in the vessel wall, there are also systemic signs of an inflammatory reaction associated with lesion development. Recently, serum markers of inflammation were found to predict a future risk of coronary events in initially healthy persons, as well as in patients with connective tissue disease.

Methods and Results
The study group comprised 73 consecutive patients with connective tissue disease who were admitted to the Department of Cardiovascular Medicine between April 2000 and March 2003. Of the 73 patients, 38 (19 men, 19 women) were diagnosed as having an ischemic heart disease (7 patients acute coronary syndrome, 19 patients coronary spastic angina, 12 patients stable exertional angina). In the present study, 19 (50.0%) of the 38 patients of ischemic heart disease were diagnosed as having coronary spastic angina. In the same study period, 151 (38.7%) of 390 patients with ischemic heart disease (without connective tissue disease) were diagnosed as having coronary spastic angina. The frequency of the patients with coronary spastic angina tended to be higher in patients with connective tissue disease than in patients without connective tissue disease. Among the study patients, serum CRP concentrations (mg/dl) were higher in patients with acute coronary syndrome (1.50±1.19, n=7) and those with coronary spastic angina (1.06±1.78, n=19) than in those with non-ischemia (0.35±0.40, n=35, p<0.05).

Conclusions
Coronary spastic angina is a frequent complication in patients with connective tissue disease and the inflammatory condition is associated with coronary spastic angina and unstable angina in patients with connective tissue disease.

Key Words: Connective tissue disease; Inflammation; Vasospasm

We recently reported that there is significant inflammation in patients with coronary spastic angina as well as in those with unstable angina. Coronary spasm is implicated in the pathogenesis of not only variant angina, but also unstable angina and acute myocardial infarction. Recently, there was the first report that young women with a history of Kawasaki disease had acetylcholine-induced coronary spasm. To the best of our knowledge, there has not been a report that coronary spasm is associated with IHD in patients with connective tissue disease, so we examined whether there is a relationship between connective tissue disease and coronary spastic angina, and whether the inflammatory condition is associated with IHD in patients with connective tissue disease.

Methods

Study Population
We studied 73 consecutive patients with connective tissue disease who admitted to the Department between April 2000 and March 2003. Each diagnosis of connective tissue disease was made by a medical specialist before admission and 38 patients were diagnosed with IHD.

Patients With IHD
Acute coronary syndrome was defined as chest pain at rest with documented transient ST segment depression or ST segment elevation of 0.1 mV in at least 2 continuous electrocardiographic (ECG) leads. There were 7 patients (5 men, 2 women; mean age 68±11 years).
years) with acute coronary syndrome in this study and in each of them the last spontaneous attack at rest had occurred within 24 h of admission. Five of the 7 patients had new Q wave development and an increase in creatine kinase concentration of more than twice the normal upper limit. We confirmed by coronary arteriography that all patients with acute coronary syndrome had significant coronary artery stenosis and no coronary spasm. Nineteen patients (10 men, 9 women; mean age 66±11 years) were defined as the coronary spastic angina group. All patients in this group had spontaneous attacks at rest associated with ST-segment depression >1.0 mm on an exercise test, ≥90% narrowing of the major coronary arteries, and no acetylcholine, as previously reported.12 Acetylcholine, 20–100 mg, was injected into the left and right coronary arteries separately in all patients. The sensitivity and specificity of this method for provoking coronary spasm have been validated by Okumura et al.13 The stable exertional angina group comprised 12 patients (4 men, 8 women; mean age 62±14 years) who had typical exertional chest discomfort associated with horizontal or down-sloping ST-segment depression >1.0 mm on an exercise test, >90% narrowing of the major coronary arteries, and no acetylcholine-induced coronary spasm. However, the patients in this group had no acute events or worsening of symptoms during the previous 6 months and no anginal episodes within the week preceding enrollment. Thirty-five patients (7 men, 28 women; mean age 61±12 years) with heart disease other than IHD comprised the non-IHD group. Informed consent was obtained from each patient. The study was in agreement with the guidelines approved by the institutional ethics committee.

Blood Samples

Blood samples were obtained from all patients while in the recumbent position using a 21-gauge needle for clean venipuncture of an antecubital vein. The first 3 ml of blood was used for biochemical assessment and the subsequent 3 ml blood was collected sequentially into evacuated tubes containing 0.3 ml of sodium heparin for cytometric analysis of cytokine production.

Flow Cytometric Analysis

The 3 ml blood samples were mixed with 3 ml of either non-activating medium [10% fetal calf serum-supplemented RPMI1640 with 40 mg/ml Brefeldin A (Sigma, St Louis, MO, USA)] or activating medium [non-activating medium with 40 ng/ml phorbol myristate acetate (Calbiochem, La Jolla, CA, USA) and 4 mg/ml ionomycin (Sigma)] and then incubated for 4 h at 37°C and 5% CO2. After washing with ice-cold phosphate buffered saline, cells were recovered by centrifugation, resuspended in 3 ml of cytokine production buffer (containing 0.3 ml of sodium heparin for cytometric analysis and 3 ml blood), and permiabilization of cells were both performed using IntraPrep tool (Beckman Coulter, High Wycombe, UK) according to the manufacturer’s instructions and intracellular cytokines were stained with fluorescein isothiocyanate (FITC)-labeled anti-human interferon (IFN)-γ and phycoerythrin (PE)-labeled anti-human interleukin (IL)-4 monoclonal antibodies (Beckman Coulter). The IL-4 and IFN-γ-producing CD4+ T cells were analyzed using a FACScan™ instrument (Becton Dickinson, San Jose, CA, USA). Non-specific staining with the isotype-matched control monoclonal antibody was <1%.

Statistical Analysis

The data are given as mean±SD. The comparisons of continuous data among the 4 groups were performed with

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Table 1  Classification of Patients With Ischemic Heart Disease

<table>
<thead>
<tr>
<th></th>
<th>Acute coronary syndrome (n=7)</th>
<th>Coronary spastic angina (n=19)</th>
<th>Stable exertional angina (n=12)</th>
<th>Non-ischemia (n=35)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Mean</td>
<td>68±11</td>
<td>66±11</td>
<td>62±14</td>
<td>61±12</td>
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<td>Range</td>
<td>54–84</td>
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<td>24–85</td>
<td>31–85</td>
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<td>M/F</td>
<td>5/2</td>
<td>10/9</td>
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<tr>
<td>Hypertension</td>
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<td>12</td>
</tr>
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<td>Smoking</td>
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<td>2</td>
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<td>Diabetes mellitus</td>
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<tr>
<td>Obesity</td>
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<td>4</td>
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</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>193±31</td>
<td>208±53</td>
<td>192±38</td>
<td>191±68</td>
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<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>43±20</td>
<td>60±19</td>
<td>47±12</td>
<td>52±14</td>
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<tr>
<td>Triglyceride (mg/dl)</td>
<td>141±32*</td>
<td>103±53</td>
<td>161±83*</td>
<td>112±48</td>
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<tr>
<td>No. of coronary arteries narrowed &gt;75%</td>
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<td>1</td>
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Medications

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<td>Steroids</td>
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<td>Angiotensin-converting enzyme inhibitors</td>
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<td>Calcium antagonists</td>
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<td>6</td>
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<tr>
<td>Warfarin</td>
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<td>3</td>
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</tbody>
</table>

HDL, high-density lipoprotein. Values are expressed as mean±SD or number.

*p<0.05 vs non-ischemia group.
frequency data among the 4 groups were compared by the one-way ANOVA followed by the Bonferroni method. The statistically significant.

**Results**

This study included consecutive 73 patients with connective tissue disease (26 men, 47 women, mean age ± SD 65±12 years), 38 of whom were diagnosed with IHD: acute coronary syndrome (7 patients), coronary spastic angina (19 patients), and stable exertional angina (12 patients) (Table 1). In patients with IHD, the rate of diagnosis of coronary spastic angina was high in the patients with rheumatoid arthritis (10 patients among 15 patients), polymyalgia rheumatica (3 among 4) and Sjögren syndrome (2 among 2). In the present study, 19 (50.0%) of the 38 patients were diagnosed as having coronary spastic angina. In the same study period, 151 (38.7%) of 390 patients with IHD (without connective tissue disease) were diagnosed as having coronary spastic angina. The frequency of the patients with coronary spastic angina seemed to be higher in patients with connective tissue disease than in patients without connective tissue disease. There were no significant differences in coronary risk factors (except for concentrations of triglyceride) among the 4 study groups. The triglyceride concentrations were higher in the stable exertional angina group than in the coronary spastic angina and non-IHD groups. There were 38 patients (19 men [50%] and 19 women [50%]) with IHD; however, 35 patients with non-IHD (7 men [20%] and 28 women [80%]) were included in the present study. The rate of patients who were suffering from IHD was higher in men than in women among the patients with connective tissue disease (p<0.05).

**Assessment of C-Reactive Protein (CRP) Concentrations**

Serum CRP concentrations (mg/dl) were higher in patients with acute coronary syndrome (1.50±1.19) and those with coronary spastic angina (1.06±1.78) than in those with non-IHD (0.35±0.40, p<0.05, Fig 1, Left). There were no differences in the serum CRP concentrations between the acute coronary syndrome and coronary spastic angina groups. Serum CRP concentrations were higher in patients receiving steroid therapy (n=26) than in patients not receiving that therapy (n=47, Fig 1, Right). The other medica-

**Assessment of Frequencies of IFN-γ-Producing T Cells and IL-4-Producing T Cells in the 4 Patient Groups**

The frequency of peripheral CD4+ T cells staining for IFN-γ was 27.8±13.4% in 10 consecutive patients with connective tissue disease and 15.5±5.8% in normals (n=21). The frequency of IFN-γ-producing T cells was found to be significantly higher in the connective tissue disease group than in the normal subjects (p<0.05). The frequency of peripheral CD4+ T cells staining for IL-4 was 3.7±2.2% in patients with connective tissue disease and 3.1±1.9% in the normal group. The differences in frequency between the 2 groups were not significant.

**Discussion**

Atherosclerosis is an inflammatory process involving various immune cells,

particulary T cells and macrophages in the plaque.

Patients with connective tissue disease also have a chronic inflammatory process and connective tissue disease has been recognized as a risk factor for IHD. Manzi et al showed that the relative risk for a myocardial infarction in women with lupus aged 35–44 years was 52.3-fold the risk for women without lupus. Del Rincon et al showed that the incidence of cardiovascular events in rheumatoid arthritis patients is extremely high in their cohort and that rheumatoid arthritis was independent of traditional cardiovascular risk factors. In the present study, more than 50% of rheumatoid arthritis patients who were admitted to the Department of Cardiovascular Medicine had IHD. Another study has shown that activated T cells are related to plaque instability. Nanki et al reported that CX3CR1 expression by peripheral CD4+ and CD8+ T cells was up-regulated in rheumatoid arthritis patients. The peripheral CD4+ and CD8+ T cells expressing CX3CR1 predominantly produced IFN-γ and tumor necrosis factor TNF-α. Those data are compatible with our result that the frequency of IFN-γ-producing T cells was significantly higher in the connective tissue disease group than in normal subjects. We have recently reported a significant increase in activated T cells in patients with not only unstable angina but also coronary spastic angina. In the present study, there was no difference in the frequency of IL-4-producing T cells between the patients with connective

Coronary Spasm and Connective Tissue Disease
tissue disease and those without it, which suggests that T cell activation in patients with connective tissue disease is similar to that in patients with unstable angina and coronary spastic angina. Furthermore, in the present study 67% of the cases of IHD were caused by coronary spasm in patients with rheumatoid arthritis. These data indicate that coronary spastic angina is also associated with inflammation. Coronary spasm is implicated in the pathogenesis of unstable angina and acute myocardial infarction. There is a possibility that coronary spasm caused the myocardial infarction in some extent in the patients described earlier. In a prospective study, Petri et al found that the duration of prednisone therapy, and the presence of hypertension, hyperlipidemia and obesity were risk factors for later coronary artery disease. In the present study, serum CRP concentrations were higher in patients receiving steroid therapy than in patients not receiving that treatment, which suggests that the currently recommended steroid therapy is not controlling the inflammation in these patients. Furthermore, serum CRP concentrations were higher in patients with acute coronary syndrome and those with coronary spastic angina than in those with non-IHD. It has been reported that serum CRP concentrations are higher in patients with acute coronary syndrome among patients without connective tissue disease than about the increased concentration of CRP in patients with coronary spastic angina. The CRP concentration can be used to predict major adverse cardiac events in patients with coronary artery disease. Furthermore, it has been reported that healthy Japanese with more coronary risk factors had higher concentrations of CRP. CRP increases the expression of cell adhesion molecules and reduces nitric oxide production by endothelial cells. These findings suggest that patients with connective tissue disease who are receiving steroid therapy have such an active inflammatory condition that they have high CRP concentrations that lead to cardiac events occurring.

In conclusion, this is the first report of coronary spastic angina in patients with connective tissue disease. Furthermore, the increased inflammatory condition in patients with connective tissue disease is associated with coronary spastic angina and acute coronary syndrome.

Acknowledgments

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