Macrophage Infiltration in Acute Coronary Syndromes
Implications for Plaque Rupture

Pedro R. Moreno, MD; Erling Falk, MD; Igor F. Palacios, MD; John B. Newell, BA; Valentin Fuster, MD, PhD; John T. Fallon, MD, PhD

**Background** Rupture of atherosclerotic plaques is probably the most important mechanism underlying the sudden onset of acute coronary syndromes. Macrophages may release lytic enzymes that degrade the fibrous cap and therefore produce rupture of the atherosclerotic plaque. This study was designed to quantify macrophage content in coronary plaque tissue from patients with stable and unstable coronary syndromes.

**Methods and Results** Hematoxylin and eosin and immunostaining with anti-human macrophage monoclonal antibody (PG-M1) were performed. Computerized planimetry was used to analyze 26 atherectomy specimens comprising 524 pieces of tissue from 8 patients with chronic stable angina, 8 patients with unstable angina, and 10 patients with non-Q-wave myocardial infarction. Total plaque area was 417±87 mm²×10⁻² in patients with stable angina, 601±157 mm²×10⁻² in patients with unstable angina, and 499±87 mm²×10⁻² in patients with non-Q-wave myocardial infarction (P=NS). The macrophage-rich area was larger in plaques from patients with unstable angina (61±18 mm²×10⁻²) and non-Q-wave myocardial infarction (87±32 mm²×10⁻²) than in plaques from patients with stable angina (14±5 mm²×10⁻²) (P=.024). The percentage of the total plaque area occupied by macrophages was also larger in patients with unstable angina (13±5.6%) and non-Q-wave myocardial infarction (14.6±4.6%) than in patients with stable angina (3.14±1%) (P=.018). Macrophage-rich sclerotic tissue was largest in patients with non-Q-wave myocardial infarction (67±30 mm²×10⁻²) and unstable angina (55±19 mm²×10⁻²) than in patients with stable angina (11.5±4.1 mm²×10⁻²) (P=.046). Macrophage-rich atheromatous gruel was also largest in patients with non-Q-wave myocardial infarction (15±4 mm²×10⁻²) than in patients with unstable angina (3.3±1.7 mm²×10⁻²) or stable angina (2.4±1.2 mm²×10⁻²) (P=.026).

**Conclusions** Macrophage-rich areas are more frequently found in patients with unstable angina and non-Q-wave myocardial infarction. This suggests that macrophages are a marker of unstable atherosclerotic plaques and may play a significant role in the pathophysiology of acute coronary syndromes. (Circulation. 1994;90:775-778.)

**Key Words** plaques • myocardial infarction • angina • macrophages

**Methods**

**Patient Population**

From 140 consecutive directional atherectomy procedures performed between March 1 and December 31, 1992, at the Massachusetts General Hospital, coronary specimens were obtained from 26 patients who met the following clinical inclusion criteria: (1) chronic stable angina; Canadian Cardiovascular Society classification I through IV (3 patients), (2) unstable angina: angina pectoris at rest <20 minutes' duration and with ECG changes consistent with ischemia without cardiac enzyme elevation (8 patients), and (3) non-Q-wave myocardial infarction: angina pectoris at rest >20 minutes' duration with cardiac enzyme elevation and ECG changes consistent with ischemia in the absence of Q waves (10 patients).

The rest of the atherectomy population was excluded because of other types of unstable angina (recent onset, accelerated, rest angina with no ECG changes, rest angina with left bundle branch block, rest angina with previous pacemaker implantation, and postinfarction angina) (65 patients); restenosis (24 patients); inadequacy of tissue sample (total sample area <1.5 mm²) (11 patients); rescue atherectomy for failed percutaneous transluminal coronary angioplasty (4 patients); acute myocardial infarction (4 patients); and miscellaneous others (silent ischemia, ischemic arrhythmia, congestive heart failure) (6 patients).

**Atherectomy Specimens**

Multiple pieces of tissue were obtained from each lesion and were immediately immersed in 10% formalin. Tissue was then
Macrophage Infiltration in Coronary Tissue From Patients With Acute Coronary Syndromes

<table>
<thead>
<tr>
<th></th>
<th>Stable Angina</th>
<th>Unstable Angina</th>
<th>Non-Q MI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>TA</td>
<td>417±87</td>
<td>601±157</td>
<td>499±79</td>
<td>NS</td>
</tr>
<tr>
<td>MRA</td>
<td>14±5†</td>
<td>61±18</td>
<td>87±32</td>
<td>0.024</td>
</tr>
<tr>
<td>MRST</td>
<td>11.5±4.1†</td>
<td>55±19</td>
<td>67±30</td>
<td>0.046</td>
</tr>
<tr>
<td>MRAG</td>
<td>2.4±1.2*</td>
<td>3.3±1.7‡</td>
<td>15±4</td>
<td>0.026</td>
</tr>
</tbody>
</table>

Non-Q MI indicates non-Q-wave myocardial infarction; TA, total area; MRA, macrophage-rich area; MRST, macrophage-rich sclerotic tissue; and MRAG, macrophage-rich atheromatous gruel. Area quantification is expressed as mm²x10⁻².

*P<.05 between stable angina and non-Q-wave myocardial infarction.
†P<.01 between stable angina and unstable angina.
‡P<.001 between unstable angina and non-Q-wave myocardial infarction.

routinely processed for paraffin embedding according to conventional techniques. Sections were serially cut at 5 μm, mounted on lysine-coated slides, and stained with hematoxylin and eosin and by the trichrome method.

Immunocytochemistry

Human macrophage antibody staining was performed using 5-μm-thick sections deparaffinized and hydrated to distilled water. Slides were placed in phosphate-buffered saline (PBS) for 2 minutes, digested in freshly prepared 0.05% trypsin solution at 37°C for 30 minutes, and rinsed well in PBS. Sections were then stained with an anti-human panmacrophage antibody (CD68, PG-M1-Dako) using an avidin-biotin complex technique and developed with alkaline phosphatase. Positive control staining was performed using a tenacin antibody.

Total and Segmental Area Quantification

Macrophage content was quantified by computer-aided planimetry. Each sample was outlined manually from the PG-M1–stained section, and the area occupied by stained macrophages within each histologically defined component was measured. Total area (mm²x10⁻²) and the percentage of the total area occupied by macrophages are reported.

Statistical Analysis

Results are expressed as mean±SEM; probability values <.05 were considered statistically significant. ANOVA was used for comparison of the three groups. For comparison of discrete variables, a Fisher test was used. For comparison of two linear populations, a two-tailed Student’s t test was used. For nonlinear comparison, the two-tailed Student’s t test was performed with the logarithmic number of each individual value.

Results

The demographic characteristics of the population were similar for the three groups of patients. Age, male/female ratio, and total plasma cholesterol levels were 65±12 years, 8:0, and 191±41 mg/dL in patients with chronic stable angina; 66±11 years, 6:2, and 185±65 mg/dL in patients with unstable angina; and 64±7 years, 9:1, and 195±37 mg/dL in patients with non-Q-wave myocardial infarction, respectively (P=NS). Low-density lipoprotein and high-density lipoprotein fractions were 129±35 and 34±11 mg/dL in patients with chronic stable angina, 124±62 and 42±4 mg/dL in patients with unstable angina, and 118±42 and 40±22 mg/dL in patients with non-Q-wave myocardial infarction, respectively (P=NS). The incidence of other risk factors for coronary artery disease including hypertension, diabetes, cigarette smoking, and family history of coronary artery disease was also similar in the patient population.

The site of the culprit lesion at coronary angiography followed the same pattern distribution. The left anterior descending coronary artery was the culprit for the clinical syndrome in 30% of patients with stable angina, 30% of patients with unstable angina, and 20% of patients with non-Q-wave myocardial infarction (P=NS). The right coronary artery was the culprit in 30% of patients with stable angina, 20% of patients with unstable angina, and 40% of patients with non-Q-wave myocardial infarction (P=NS). The left circumflex coronary artery was the culprit in 20% of patients with stable angina, 20% of patients with unstable angina, and 10% of patients with non-Q-wave myocardial infarction (P=NS). Finally, saphenous vein graft disease was the culprit for coronary syndrome in 10% of patients with unstable angina and 20% of patients with non-Q-wave myocardial infarction (P=NS).

A total of 524 pieces of tissue, 20±2 from each lesion, were stained and quantified. The total plaque and macrophage area measurements are given in the Table. The percentage of the total plaque area occupied by macrophages was larger in plaque tissue from patients with unstable angina (13.3±5.6%) and non-Q-wave myocardial infarction (14.6±4.6%) than in plaque tissue from patients with stable angina (3.1±1%) (P=0.018).

The Figure illustrates an example of the immunostaining of macrophage-rich regions in atherectomy tissue from a patient with stable angina and a patient with non-Q-wave myocardial infarction.

PG-M1 staining identified macrophages in three histologically defined components of the plaque: atheromatous gruel, sclerotic tissue, and thrombus. Macrophages were predominately found in the sclerotic component of the plaque in all three groups. There was a significantly larger amount of macrophage-rich sclerotic tissue in plaques from patients with unstable angina (13±6%) and non-Q-wave myocardial infarction (12±3.8%) versus plaque tissue from patients with stable angina (2.5±0.8%). In addition, the percentage of macrophage-rich atheromatous gruel was significantly greater in plaque tissue from patients with non-Q-wave myocardial infarction (2.7±0.9%) than in plaque tissue from either stable (0.6±0.3%) or unstable (0.4±0.2%) angina patients. The area of macrophage-rich thrombus was also larger in tissue from patients with non-Q-wave myocardial infarction (5.1±5.1%) and unstable angina (2.8±2.8%) than in tissue from
patients with chronic stable angina (0.2±0.2%), but this difference did not reach statistical significance.

**Discussion**

This study was designed to identify and quantify the macrophage content of human coronary plaque tissue from patients with precisely defined ischemic coronary syndromes. The results reveal that macrophage-rich areas are more frequently found in plaque tissue from patients with the acute coronary syndromes of unstable angina and non-Q-wave myocardial infarction than in plaque tissue from patients with chronic stable angina.

The most striking difference among the three groups was the magnitude of the total macrophage infiltration area. This area, constituted by three different components of the plaque, was significantly larger in plaque tissue from patients with acute coronary syndromes.

The distribution of macrophages at different sites within the plaque followed the same pattern. Macrophage-rich sclerotic tissue was larger in plaque tissue from patients with unstable angina and non-Q-wave myocardial infarction, and macrophage-rich atheromatous gruel was particularly prevalent in plaque tissue from patients with non-Q-wave myocardial infarction. Therefore, the extent and distribution of macrophages within plaque tissue may be very important to the clinical presentation of coronary artery disease.

Several pathophysiological mechanisms may play a significant role in the process of plaque ulceration, including inflammation, rheological factors, circumferential wall stress, circadian variation, and vasoconstriction. Macrophages are the predominant inflammatory cell in atherosclerotic plaques, and their role in the pathogenesis of coronary plaque ulceration and rupture has recently received increasing attention.
Davies et al described an increased density of macrophages in ruptured aortic plaques in comparison with intact plaques, and Richardson et al described macrophages at the site of plaque rupture in coronary atherosclerotic lesions in patients dying from coronary thrombosis.6-8 Recently, van der Wal et al9 studied the cellular characteristics of coronary plaques in 20 patients who died from acute myocardial infarction. Macrophages were the predominant cell at the immediate site of either rupture or superficial erosion of the fibrous cap. They also suggested that a local inflammatory reaction, as evidenced by the presence of HLA-DR antigen expression on intimal plaque cells, was occurring adjacent to the luminal lesions using the same methodology previously reported from the same group.9

Human atherosclerotic plaques express endothelial adhesion and chemotactic molecules for monocytes.10,11 It is also known that macrophages can release metalloproteinases such as interstitial collagenase, gelatinase, and stromelysin with subsequent degradation of collagen, elastin, and proteoglycans.12-15 Shah et al16 have documented an increase in collagen breakdown when monocyte-derived macrophages were incubated with human aortic plaques, implying that macrophages could be responsible for plaque rupture.

The fact that intact aortic plaques and coronary plaques from patients with stable angina contain macrophages suggests that inflammation is a constitutive component of the atherosclerotic plaque and does not always induce instability, but further studies are needed to resolve this issue.9

The results of this study support the hypothesis that macrophage content of coronary plaque tissue is significantly increased in coronary atherosclerotic plaques of patients with acute coronary syndromes. Macrophage-rich areas within plaque tissue are significantly more extensive in patients with unstable angina and non-Q-wave myocardial infarction. Despite potential sampling limitations,17 the results of the present study suggest that macrophages play a crucial role in the inflammatory component of the acute coronary syndromes.

Acknowledgments

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