Diffuse and Active Inflammation Occurs in Both Vulnerable and Stable Plaques of the Entire Coronary Tree: A Histopathologic Study of Patients Dying of Acute Myocardial Infarction
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Diffuse and Active Inflammation Occurs in Both Vulnerable and Stable Plaques of the Entire Coronary Tree

A Histopathologic Study of Patients Dying of Acute Myocardial Infarction

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Rome, Italy; and Minneapolis, Minnesota

OBJECTIVES

This study was undertaken to define and compare geographic coronary artery inflammation in patients who were dying of acute myocardial infarction (AMI), chronic stable angina (SA), and noncardiac causes (CTRL).

BACKGROUND

Biochemical markers and flow cytometry provide indirect evidence of diffuse coronary inflammation in patients dying of acute coronary syndromes. Yet no histopathologic studies have corroborated these findings. A key unanswered question is whether the inflammatory burden involves the entire coronary tree or is limited to a few plaques.

METHODS

We examined 544 coronary artery segments from 16 patients with AMI, 109 segments from 5 patients with SA, and 304 coronary segments from 9 patients with CTRL.

RESULTS

An average of 6.8 ± 0.5 vulnerable segments per patient were found in the AMI group (in addition to culprit lesions) compared with an average of 0.8 ± 0.3 and 1.4 ± 0.3 vulnerable lesions/patient in the SA and CTRL groups, respectively. The AMI group, independent of the type of plaque observed, showed significantly more inflammatory infiltrates compared with the SA and CTRL groups (121.6 ± 12.4 cell/mm² vs. 37.3 ± 11.9 cell/mm² vs. 26.6 ± 6.8 cell/mm², p = 0.0001). In AMI patients, active inflammation was not only evident within the culprit lesion and vulnerable plaques but also involved stable plaques. These showed a three- to four-fold higher inflammation than vulnerable and stable plaques from the SA and CTRL groups, respectively.

CONCLUSIONS

This histopathologic study found that both vulnerable and stable coronary plaques of patients dying of AMI are diffusely infiltrated by inflammatory cells. (J Am Coll Cardiol 2005;45:1585–93) © 2005 by the American College of Cardiology Foundation

Recent clinical and histopathologic data suggest that inflammation plays a key role in coronary artery plaque instability and subsequent occlusive thrombosis (1–3). Inflammation in acute coronary syndromes is suggested by numerous clinical studies showing increased acute-phase reactants in the serum of patients with unstable angina and those at risk of future myocardial infarction (4,5). Although topographic correlation has been shown between the presence of an inflammatory infiltrate and the site of plaque rupture and thrombosis (3), acute myocardial infarction (AMI) may reflect global coronary artery inflammatory activity, with multifocal involvement rather than a localized pathophysiologic process. Buffon et al. (6) recently showed that unstable angina was associated with widespread inflammation in the coronary vasculature by measuring neutrophil myeloperoxidase activity. We reported similar results using flow cytometry, suggesting multifocal inflammatory cell activation in the coronary arteries of patients dying of AMI (7). Diffuse plaque instability is further supported by recent angiographic findings of multiple complex coronary plaques in patients with unstable angina (8) and transmural myocardial infarction (9).

There are no histopathologic data regarding the distribution of inflammatory infiltrates in acute coronary syndromes, derived from serial, complete sections of the whole coronary tree. In particular, a key unanswered question is whether the inflammatory burden involves the entire coronary tree or is limited to a few focal plaque sites in such patients. This study was thus undertaken to define and compare geographic coronary artery inflammation in patients dying of AMI and those dying of noncardiac causes with or without a positive clinical history of SA. We hypothesized that widespread inflammation of the coronary tree in patients dying of AMI is correlated not only with the presence of multiple vulnerable plaques, rich in inflamma-
ed at our institution from January 2002. Patients were divided into three groups (Table 1): 1) 16 patients dying of AMI (AMI group, 10 male/6 female, mean age 72.1 ± 1.5 years); 2) 5 age-matched individuals dying of non-cardiac causes but with SA (SA group, 3 male/2 female, mean age 74.8 ± 3.4 years), and 3) 9 age-matched control patients dying of non-cardiac causes and who did not have a clinical cardiac history (CTRL group, 5 male/4 female, mean age 72.9 ± 2.7 years). In the AMI group, the time from symptom onset to death was ≤72 h for all cases. Clinical history and standard electrocardiographic findings defined AMI.

Patients with chronic inflammatory diseases or tumors were excluded from the study to avoid bias caused by immunologic changes. No differences were observed among

### MATERIALS AND METHODS

**Patient population.** Our study was designed to assess a consecutive series of 30 autopsy cases from patients who

### Table 1. Clinical, Morphologic, and Morphometric Data

<table>
<thead>
<tr>
<th>Cases</th>
<th>Age (yrs)</th>
<th>Gender</th>
<th>Risk Factors</th>
<th>IEL Area (mm²)</th>
<th>Plaque Area (mm²)</th>
<th>Percent Cross-Sectional Narrowing</th>
<th>Amount of Vulnerable Plaques</th>
<th>Inflammatory Infiltrate* (cell × mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTRL group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>1</td>
<td>73</td>
<td>M</td>
<td>H, S</td>
<td>14.9 ± 1.7</td>
<td>7.2 ± 0.6</td>
<td>48.3 ± 2.2</td>
<td>2</td>
<td>54.5 ± 11.7</td>
</tr>
<tr>
<td>2</td>
<td>67</td>
<td>M</td>
<td>H</td>
<td>8.0 ± 1.4</td>
<td>4.6 ± 0.4</td>
<td>57.0 ± 2.1</td>
<td>1</td>
<td>1.0 ± 0.4</td>
</tr>
<tr>
<td>3</td>
<td>76</td>
<td>F</td>
<td>L, S</td>
<td>14.1 ± 1.8</td>
<td>8.4 ± 0.9</td>
<td>59.4 ± 3.4</td>
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<td>51.5 ± 12.3</td>
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<tr>
<td>4</td>
<td>65</td>
<td>M</td>
<td>H, S</td>
<td>11.0 ± 1.6</td>
<td>5.9 ± 0.2</td>
<td>53.0 ± 0.6</td>
<td>2</td>
<td>37.6 ± 10.9</td>
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<tr>
<td>5</td>
<td>79</td>
<td>M</td>
<td>D, S</td>
<td>9.1 ± 1.8</td>
<td>4.0 ± 0.4</td>
<td>43.9 ± 1.8</td>
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<td>40.2 ± 8.5</td>
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<tr>
<td>6</td>
<td>82</td>
<td>F</td>
<td>H, D, L</td>
<td>13.6 ± 1.7</td>
<td>7.5 ± 0.7</td>
<td>55.3 ± 3.0</td>
<td>1</td>
<td>2.1 ± 1.1</td>
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<tr>
<td>7</td>
<td>58</td>
<td>F</td>
<td>S, L</td>
<td>12.7 ± 1.7</td>
<td>8.3 ± 0.8</td>
<td>65.2 ± 2.9</td>
<td>1</td>
<td>24.5 ± 8.5</td>
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<tr>
<td>8</td>
<td>76</td>
<td>M</td>
<td>H, S</td>
<td>14.3 ± 1.7</td>
<td>7.2 ± 0.6</td>
<td>50.5 ± 2.2</td>
<td>0</td>
<td>12.9 ± 4.1</td>
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<tr>
<td>9</td>
<td>80</td>
<td>F</td>
<td>H, S</td>
<td>12.2 ± 1.6</td>
<td>6.1 ± 0.5</td>
<td>49.9 ± 2.5</td>
<td>1</td>
<td>14.8 ± 5.8</td>
</tr>
<tr>
<td>Mean ± SEM</td>
<td>72.9 ± 2.7</td>
<td>12.2 ± 0.8</td>
<td>6.9 ± 0.6</td>
<td>53.6 ± 2.1</td>
<td>1.4 ± 0.3</td>
<td>26.6 ± 6.8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| SA group | | | | | | | | |
| 1 | 80 | F | H, S | 9.1 ± 1.5 | 4.42 ± 0.3 | 48.4 ± 2.2 | 0 | 63.6 ± 12.1 |
| 2 | 64 | M | D, L | 8.2 ± 1.7 | 6.52 ± 0.5 | 79.2 ± 2.3 | 0 | 9.9 ± 2.7 |
| 3 | 78 | M | S, L | 12.2 ± 1.2 | 3.74 ± 0.6 | 30.6 ± 0.8 | 1 | 68.0 ± 9.0 |
| 4 | 82 | F | H, S | 11.6 ± 1.0 | 3.57 ± 0.6 | 30.6 ± 0.7 | 1 | 23.5 ± 7.3 |
| 5 | 70 | M | H | 12.5 ± 1.0 | 4.58 ± 0.7 | 36.7 ± 1.9 | 2 | 21.42 ± 5.1 |
| Mean ± SEM | 74.8 ± 3.4 | 10.7 ± 0.9 | 4.6 ± 0.5 | 45.1 ± 9.1 | 0.8 ± 0.3 | 37.3 ± 11.9 |

| AMI group | | | | | | | | |
| 1 | 68 | F | H, D, L | 8.5 ± 0.7 | 5.0 ± 0.6 | 58.5 ± 0.9 | 7 | 127.2 ± 22.0 |
| 2 | 82 | M | H, S | 11.0 ± 1.6 | 8.8 ± 0.5 | 80.3 ± 2.9 | 11 | 45.3 ± 7.5 |
| 3 | 62 | M | H, S, L | 14.8 ± 1.4 | 7.4 ± 0.5 | 49.7 ± 2.4 | 5 | 91.1 ± 10.8 |
| 4 | 75 | M | D, S, L | 13.3 ± 1.9 | 9.5 ± 0.9 | 70.9 ± 2.5 | 5 | 115.7 ± 12.7 |
| 5 | 80 | F | H | 12.9 ± 1.5 | 9.6 ± 1.5 | 74.1 ± 3.7 | 5 | 185.4 ± 15.7 |
| 6 | 84 | M | H, D | 14.0 ± 1.8 | 9.9 ± 0.7 | 70.8 ± 1.9 | 5 | 138.1 ± 11.8 |
| 7 | 59 | M | S, L | 14.2 ± 1.3 | 10.1 ± 0.8 | 71.3 ± 1.9 | 7 | 73.7 ± 14.1 |
| 8 | 68 | F | H, S | 14.9 ± 1.5 | 11.0 ± 0.6 | 73.7 ± 1.9 | 10 | 147.9 ± 15.3 |
| 9 | 61 | M | H, S | 8.0 ± 1.5 | 5.3 ± 0.3 | 66.6 ± 2.2 | 6 | 42.0 ± 2.5 |
| 10 | 76 | F | S, L | 8.5 ± 1.1 | 7.3 ± 0.1 | 86.1 ± 2.1 | 10 | 154.9 ± 12.0 |
| 11 | 83 | M | H, S | 9.2 ± 1.2 | 7.7 ± 0.2 | 83.1 ± 2.0 | 9 | 213.6 ± 25.5 |
| 12 | 65 | M | H, S | 10.7 ± 1.6 | 7.3 ± 0.9 | 68.4 ± 1.6 | 5 | 66.9 ± 7.6 |
| 13 | 74 | F | H | 11.2 ± 1.9 | 6.3 ± 0.7 | 56.4 ± 2.3 | 6 | 119.0 ± 7.5 |
| 14 | 79 | M | D, L | 12.8 ± 2.0 | 9.3 ± 0.5 | 72.5 ± 2.0 | 5 | 166.7 ± 6.4 |
| 15 | 62 | F | H, S | 13.2 ± 2.1 | 9.2 ± 0.8 | 69.4 ± 1.5 | 4 | 158.6 ± 12.8 |
| 16 | 76 | M | H, S, L | 11.8 ± 2.2 | 8.3 ± 0.4 | 70.4 ± 1.9 | 9 | 99.1 ± 3.2 |
| Mean ± SEM | 72.1 ± 1.5 | 11.8 ± 0.6 | 8.2 ± 0.4 | 70.1 ± 2.3 | 6.8 ± 0.5 | 121.6 ± 12.4 |

**Statistical analysis (p)**

<table>
<thead>
<tr>
<th></th>
<th>CTRL vs. AMI</th>
<th>SA vs. AMI</th>
<th>CTRL vs. SA</th>
</tr>
</thead>
<tbody>
<tr>
<td>n.s.</td>
<td>1.00</td>
<td>0.05</td>
<td>0.004</td>
</tr>
<tr>
<td>n.s.</td>
<td>1.00</td>
<td>0.001</td>
<td>0.001</td>
</tr>
</tbody>
</table>

*CD68+ = CD3-positive cells in the plaque cap and shoulder.
AMI = acute myocardial infarction; CTRL = control; D = diabetes; H = hypertension; L = hyperlipidemia; n.s. = no significant differences were observed among the three groups in the distribution of the major risk factors; S = smoke; SA = chronic stable angina; SEM = standard error of the mean.*

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the three groups in the distribution of the major risk factors (hypertension, hyperlipidemia, smoke, diabetes) (Table 1). All autopsies were performed within 12 to 24 h of death.

**Tissue handling and processing.** The hearts were weighed and perfusion-fixed by infusing the coronary arteries with 10% neutral-buffered formalin. The four major epicardial coronary arteries (left main, left anterior descending, left circumflex, and right coronary arteries) and their major branches were carefully dissected from the heart, cut transversely at 5-mm intervals, and decalcified if necessary.

In particular, the left main segments were obtained from the ostium of the left coronary tree to the bifurcation of the left anterior descending and left circumflex coronary arteries. Coronary segments from the left anterior descending artery were collected from the ostium of the vessel to the origin of the second diagonal branch and segments from the left circumflex artery from the ostium of the vessel to the origin of the second obtuse marginal branch. Segments from the right coronary artery were obtained from the ostium of the vessel to the origin of the posterior descending artery in case of right dominance.

A total of 957 arterial segments were embedded in paraffin and serially sectioned: 544 from AMI, 109 from SA, and 304 from CTRL groups, respectively. Coronary segments from patients dying of AMI were subdivided in two additional groups: 1) infarct-related coronary arteries (IRA), and 2) non–infarct-related coronary arteries (non-IRA). For histopathologic examination, arterial sections were stained with hematoxylin and eosin and Movat pentachrome stain.

Myocardial sections were then made transversely at 1.0-cm intervals from apex to base. The myocardium was macroscopically examined for the presence and extent of an infarcted area. In all cases, at least one complete transverse heart section was processed for histopathologic examination, and the infarction was confirmed by light microscopy. Cases were selected for study only if the infarct was in a single coronary artery distribution, and when the acute infarct region correlated with the coronary artery containing thrombus.

**Histopathologic and morphometric studies.** All coronary plaques from the three groups were evaluated by light microscopic examination. In each coronary segment, the following histologic variables were recorded: 1) presence of acute antemortem thrombus; 2) cap rupture; 3) cap erosion; 4) lumen area (L); 5) internal elastic lamina (IEL) area; 6) plaque area calculated as (IEL – L); 7) cross-sectional narrowing defined as (plaque area/IEL area × 100) (10); 8) minimum cap thickness; 9) cross-sectional area of lipid-necrotic core; and 10) cross-sectional area of calcific deposits if present and quantifiable.

The cross-sectional images were acquired by a charge-coupled device on three separate arrays camera connected to a computer. Histologic areas were calculated using the program Scion Image 4.0.2 (Scion Corp., Frederick, Maryland) manually tracing the perimeter of the different vessel components. All histopathologic variables were independently graded by two pathologists blinded to the clinical history.

Plaques were classified according to the modified American Heart Association atherosclerosis classification (11) and to the recent consensus document of the American Heart Association (12) into three categories: culprit plaques, characterized by the presence of an acute thrombus associated with plaque rupture or plaque erosion; and vulnerable plaques, including: 1) thin fibrous cap atheromata, characterized by a lesion composed of a lipid-rich core covered by a <65-μm-thick fibrous cap containing many lipid-laden macrophage foam cells (>25 per high-power magnification) (11), 2) plaques with stenosis >90%, and 3) superficial calcified nodules. The remaining plaques were classified as stable plaques.

**Immunohistochemical studies.** Immunohistochemistry was performed on all arterial segments to characterize and quantify cell types within the plaque. Serial 3-μm-thick sections were cut from paraffin blocks and mounted on slides previously treated with a poly-L-lysine solution (Sigma Chemical Co., St. Louis, Missouri). The following primary monoclonal antibodies were used: alpha-smooth actin, CD68 (human macrophage) (Dakopatts, Glostrup, Denmark), CD3 (human T-cells) (Dakopatts), and human leukocyte antigen-DR (Dakopatts).

The number of immunohistochemically positive cells for each antibody was evaluated at a magnification of ×40 in non-contiguous fields (with an area of 0.22 mm²) in the cap and shoulder region of the plaque (near the lipid necrotic core). A mean of five fields per region were analyzed, and the fields were read until the value of the standard error of the mean of total positive inflammatory cells was <5%.

Double-labeled immunohistochemistry for CD3 (alkaline phosphatase/anti-alkaline phosphatase) and human leukocyte antigen-DR (diaminobenzidine-detected streptavidin/biotin immunoperoxidase) was used to detect activated T-lymphocytes.

**Statistical analysis.** Data were reported as mean ± standard error of the mean and analyzed by SPSS 6.0 (Statistical Package for the Social Sciences) software (SPSS Inc., Chicago, Illinois). For statistical analysis, only one average value for each patient was used. One-way analysis of variance test using the Bonferroni correction for simultaneous tests (N = 3) was used to assess the differences in IEL, lumen and plaque area, percentage of cross-sectional narrowing, number of vulnerable plaques, cap thickness, area of necrotic lipid core, and plaque inflammation between the experimental groups. The Pearson chi-square test was used to assess differences in the distribution of risk factors and various types of plaques among the experimental groups. Partial correlation coefficients describing the relationship between AMI and coronary inflammation while adjusting for the effects of plaque area, percentage of cross-sectional narrowing, percentage of cross-sectional area of lipidic-necrotic core, and that of cap thickness were also calculated. Multiple linear regression was also used to
identify a subset of independent variables (group of patients, plaque area, percentage of cross-sectional narrowing, histo-
cytologic components of the plaque), which significantly correlated with coronary inflammation. A p value of <0.05
was considered statistically significant.

RESULTS

Myocardial pathology. Myocardial histopathologic exam-
ination confirmed AMI as the cause of death in 16 cases.
The infarct was transmural and from a single coronary artery
distribution in all cases. Infarct timing was 24 h or less in 11
cases (68.7%) and between 24 and 72 h in 5 cases (31.3%).
Myocardium from CTRL patients showed neither infarct
nor necrosis in all cases. The cause of death in patients with
SA was bronchopneumonia in two patients, bowel infar-
ction in one patient, and pulmonary embolism in two
patients. In the CTRL group, the cause of death was
bronchopneumonia in four patients, bowel infarction in two
patients, pulmonary embolism in two patients, and intrace-
rebral hemorrhage in one patient.

Morphologic examination of coronary artery seg-
ments. Morphometric analysis showed in the AMI group
comparred with the SA and CTRL groups a significantly
greater plaque area (p = 0.001 and 0.05, respectively) and
cross-sectional narrowing (Table 1). The IEL area was
similar between groups.

In all 16 AMI cases, a coronary thrombus was found in
the artery supplying the infarcted myocardium. Lumen
thrombosis was associated with cap rupture in 14 cases
(87.5%) (Fig. 1). In the remaining two cases (12.5%),
thrombus was attached to a superficial erosion.

The culprit plaques showed the following histologic
features: 1) a necrotic lipidic-core with an average extent
of 58.55 ± 4.94% of the plaque area and with a minimum
value of 30%; 2) a thin fibrous capsule with an average
thickness of 48.31 ± 10.34 μm and with a maximum value
of 65 μm; 3) a large inflammatory infiltrate of the cap,
consisting of both macrophagic foamy cells, CD68 positive,
and T-lymphocytes, CD3 positive. In all cases at least 25
inflammatory cells per high-power field were present. In
non-IRA vessels (AMI group) neither erosion, rupture, nor
thrombosis was detected, which was also true in all coronary
segments from the SA and CTRL groups.

In addition to plaques with cap rupture or erosion,
multiple vulnerable plaques were observed in the coronary
trees of patients dying of AMI (Table 1). In particular, 109
vulnerable plaques (6.8 ± 0.5 per patient, corresponding to
20.0% of the coronary segments examined) have been
observed, consisting respectively of 31 thin fibrous cap
atheromatosa, 31 superficial calcified nodules, and 47 plaques
with stenosis >90% (Table 2). Thirty-six of the 109
vulnerable plaques were located in the IRA coronary seg-
ments (in 16.6% of the segments analyzed), whereas the
other 73 were situated in the non-IRA segments (in 22.3%
of the segments analyzed) (p = not significant) (Table 3).

In the control group, only 13 vulnerable plaques were
observed: 3 thin fibrous cap atheromatosa, 8 superficial calci-
fied nodules, and 2 plaques with stenosis >90%, with a
mean of 1.4 ± 0.3 vulnerable plaques per patient (Table 1),
corresponding to 4.3% of the coronary segments examined.
In the SA group, only four vulnerable plaques were present
(0.8 ± 0.3 vulnerable plaques per patient, 3.7% of the
segments analyzed), all of them consisting of superficial
calciﬁed nodules.

Plaque inflammation. A higher number of inflammatory
cells was observed in the coronary trees of patients with
AMI compared with the SA and CTRL groups, indepen-
dent of the type of plaque observed (121.6 ± 12.4 cells ×
mm² vs. 37.3 ± 11.9 cells × mm² vs. 26.6 ± 6.8 cells ×
mm², p = 0.001; respectively) (Table 1, Fig. 2). No
significant differences were observed in the plaque inflam-
matary infiltrate between vulnerable and stable plaques from
SA and CTRL patients. The significant correlation between
AMI and coronary inflammation remained highly signifi-
cant (p = 0.001) after the adjustment for other plaque
characteristics related to instability, such as plaque area,
luminal narrowing, area of lipidic necrotic core, and cap
thickness. In addition, the association between AMI and
inflammation persisted after multivariate adjustment, enter-
ing simultaneously in a multiple linear regression model all
of the different histopathologic variable confounders.

Patients dying of AMI showed diffuse inflammatory
infiltrates in the entire coronary tree (Fig. 1). In fact, no
difference was observed in the number of inflammatory cells
between the IRA and non-IRA coronary arteries (128.7 ±
13.0 cells × mm² vs. 119.7 ± 14.6 cells × mm², p = 0.41)
(Table 3, Fig. 2).

Quantitative immunohistochemical analysis showed that
38.2 ± 3.1% of cells in the coronary plaques of AMI
patients were monocytes/macrophages (positive for CD68)
and 17.8 ± 2.2% were T-lymphocytes (positive for CD3).
Significantly fewer CD68- and CD3-positive cells were
found in the SA and CTRL groups compared with those of
AMI patients (Fig. 2). The majority of T-lymphocytes in
the AMI plaques were human leukocyte antigen-DR posi-
tive (Fig. 1), indicating an activated state, whereas fewer
activated lymphocytes occurred in the CTRL group
(Fig. 3).

No statistically significant differences were observed;
moreover, in the inflammatory infiltrate of the plaque
between patients who died fewer than 24 h from AMI onset
and patients who died more than 24 h from AMI onset.

Both ruptured and vulnerable plaques in the AMI group
showed significantly more inflammatory infiltrates, com-
pared with those observed in the stable plaques of the same
group of patients (164.1 ± 13.9 cell × mm² vs. 139.0 ±
15.7 cell × mm² vs. 109.3 ± 11.8 cell × mm², p = 0.006
and 0.04, respectively) (Fig. 2). On the contrary, no statis-
tically significant differences were observed between culprit
and vulnerable plaques (p = 0.21). However, stable plaques
in AMI patients had three-fold greater inflammation than
Figure 1. Micrographs showing the coronary tree from one patient who died of acute myocardial infarction (AMI). (A1 to A4) Infarct-related coronary artery (IRA) (left circumflex coronary). (A1) Coronary section showing cap rupture near the shoulder (arrow) associated with an acute thrombus (T) (hematoxylin-eosin, ×2); (A2) high-power view of the fibrous cap at the rupture site showing many macrophages cells, CD68-positive (×20); (A3) in the same plaque, numerous T-lymphocytes, CD3-positive, were present in the shoulder (alkaline phosphatase, ×20); (A4) on a serial section, double immunohistochemistry showed that many T-lymphocytes were positive also for human leukocyte antigen-DR antibody, indicating an activated state (diaminobenzidine, ×20). (B and C) Coronary plaques in the non-IRA segments from the same patients. (B1) A vulnerable plaque (thin fibrous cap atheroma) in the left anterior descending coronary vessel, characterized by a large lipid-necrotic core associated with a thin inflamed fibrous cap (Movat, ×2); (B2) high-power view of the fibrous cap of the plaque represented in the insert of panel B1 showing many macrophages, CD68-positive (×10); (B3) immunohistochemistry stain against HLA-DR antigens showing a diffuse positive reaction in the cap of the plaque represented in the insert of panel B1 (×15). (C1) Micrograph showing another stenotic plaque present in the right coronary artery of the same patient (Movat, ×2); (C2) a very large number of macrophages, CD68-positive, were present in the cap (insert of panel C1, ×10), associated with numerous T-lymphocytes, CD3-positive ([C3], ×10).
the coronary plaques of SA patients, and about four-fold of that measured in the CTRL patients (p < 0.001) (Fig. 2).

DISCUSSION

The results of this autopsy series of patients affected by acute coronary syndromes, in which a detailed histopathologic analysis of the entire coronary tree was performed, clearly shows for the first time that: 1) patients dying of AMI bear a widespread inflammation not only in culprit plaques but also in other plaques along the major epicardial coronary arteries; 2) although ruptured and vulnerable plaques from patients who died of AMI had more inflammation than stable plaques, high-grade inflammatory infiltration is also present in stable plaques irrespective of IRA segments and non-IRA segments; and 3) in patients dying of AMI, the degree of inflammatory infiltration of the stable plaques in the whole coronary tree is three to four times greater than that observed in SA and CTRL patients.

The correlation between AMI and inflammation remained highly significant after adjustment for other plaque characteristics related to instability. The inflammatory infiltrate should be thus considered an independent expression of coronary disease severity in all plaques of AMI. These findings contrast with the hypothesis that more severe atherosclerosis influences the inflammatory infiltrate and support the concept that inflammation is an independent determinant of an acute, fatal coronary event.

These results are consistent with those of recent investigations by our group using flow cytometry in post-mortem specimens (7) and by Buffon et al. (6). The in vivo observation of Buffon et al. (6) may explain our finding that the diffuse and active inflammatory infiltrate is a dynamic phenomenon and not a postmortem one. Taken together, these studies challenge the current notion that coronary vulnerability responsible for acute coronary syndromes is caused by a single inflamed vulnerable plaque and strongly suggests diffuse inflammation of the entire coronary tree.

Plaque inflammation has emerged as an obligatory feature in events leading to plaque vulnerability and rupture (1,2). Plaque rupture coexists with numerous inflammatory cells, mainly macrophage foam cells (3). Additionally, T-cell and macrophage recruitment at dysfunctional endothelium sites is a common observation in early and advanced atherosclerotic lesions (1,13). Macrophages synthesize and release multiple growth factors, and also secrete metalloproteinases that weaken the fibrous cap and predispose it to rupture (14). Immunohistochemical findings in the AMI group also found significantly more T-lymphocytes, mostly activated (Fig. 1), inferring a fundamental role for this cytotype in plaque destabilization (1). Activated T-lymphocytes secrete

### Table 2. Distribution of the Various Plaque Types in the Three Groups of Patients

<table>
<thead>
<tr>
<th>Plaque Types</th>
<th>CS of Patients Without Stable Angina Who Died of Noncardiac Causes (CTRL Group)</th>
<th>CS of Patients With Stable Angina Who Died of Noncardiac Causes (SA Group)</th>
<th>CS of Patients Who Died of AMI (AMI Group)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N = 304 CS (%)</td>
<td>N = 109 CS (%)</td>
<td>N = 544 CS (%)</td>
</tr>
<tr>
<td>Culprit plaques with thrombosis</td>
<td>0</td>
<td>0</td>
<td>16 (3.0)</td>
</tr>
<tr>
<td>Associated with cap rupture</td>
<td>0</td>
<td>0</td>
<td>14 (2.6)</td>
</tr>
<tr>
<td>Associated with cap erosion</td>
<td>0</td>
<td>0</td>
<td>2 (0.4)</td>
</tr>
<tr>
<td>Vulnerable plaques</td>
<td>13 (4.3)</td>
<td>4 (3.7)</td>
<td>109 (20.0)</td>
</tr>
<tr>
<td>Thin fibrous-cap atheromata</td>
<td>3 (1.0)</td>
<td>0</td>
<td>31 (5.7)</td>
</tr>
<tr>
<td>Superficial calcified nodule</td>
<td>8 (2.6)</td>
<td>4 (3.7)</td>
<td>31 (5.7)</td>
</tr>
<tr>
<td>Plaques with stenosis &gt;90%</td>
<td>2 (0.7)</td>
<td>0</td>
<td>47 (8.6)</td>
</tr>
<tr>
<td>Stable plaques</td>
<td>291 (95.7)</td>
<td>105 (96.3)</td>
<td>419 (77.0)</td>
</tr>
</tbody>
</table>

CS = coronary segments; other abbreviations as in Table 1.

### Table 3. Distribution of the Various Plaque Types and Quantitative Evaluation of Inflammatory Infiltrate Present in the Plaques of Patients Dying of AMI

<table>
<thead>
<tr>
<th>Plaque Types</th>
<th>IRA Segments of AMI Group N = 217 CS (%)</th>
<th>Non-IRA Segments of AMI Group N = 327 CS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culprit plaques with thrombosis</td>
<td>16 (7.4)</td>
<td>0</td>
</tr>
<tr>
<td>Associated with cap rupture</td>
<td>14 (6.4)</td>
<td>0</td>
</tr>
<tr>
<td>Associated with cap erosion</td>
<td>2 (0.9)</td>
<td>0</td>
</tr>
<tr>
<td>Vulnerable plaques</td>
<td>36 (16.6)</td>
<td>73 (22.3)</td>
</tr>
<tr>
<td>Thin fibrous-cap atheromata</td>
<td>15 (6.9)</td>
<td>16 (4.9)</td>
</tr>
<tr>
<td>Superficial calcified nodule</td>
<td>8 (3.7)</td>
<td>23 (7.0)</td>
</tr>
<tr>
<td>Plaques with stenosis &gt;90%</td>
<td>13 (6.0)</td>
<td>34 (10.4)</td>
</tr>
<tr>
<td>Stable plaques</td>
<td>165 (76.0)</td>
<td>254 (77.7)</td>
</tr>
<tr>
<td>Inflammatory infiltrate* (cells x mm², mean ± SEM)</td>
<td>128.7 ± 13.0†</td>
<td>119.7 ± 14.6†</td>
</tr>
</tbody>
</table>

*CD68+ CD3-positive cells in the plaque cap and shoulder (in all plaques, independently from the histopathologic type).
†Statistical analysis: p = 0.41.
AMI = acute myocardial infarction; CS = coronary segments; IRA = infarct-related coronary artery.
cytokines that regulate plaque destabilization through macrophage activation, smooth muscle cell growth, and extracellular matrix synthesis (1,13).

Few previous studies have quantified inflammatory infiltrates in the entire coronary tree (15,16). Boyle et al. (15) studied inflammation in 351 ruptured and unruptured coronary plaques and showed inflammation in about 40% in the deeper plaque layers associated with lipid core. Extensive involvement of the coronary tree in subjects dying of AMI, independent of multiple vulnerable plaques, comes from the autopsy study by Davies and Thomas (17), who observed 115 thrombi in 74 patients dying within 6 h of AMI. Other studies used angiographic observations in patients with unstable angina and non–Q-wave myocardial infarction to show that such patients have multiple complex coronary lesions rather than a single culprit plaque (8). These findings have since been extended to patients with AMI by Goldstein et al. (9), who identified multiple complex coronary plaques in as many as 40% of AMI patients undergoing coronary angiography. Furthermore, in the present study, histopathologic examination showed an average of 6.8 ± 0.5 vulnerable plaques per patient, in addition to plaques with endoluminal thrombosis, in the coronary trees of patients dying of AMI, compared with 0.8 ± 0.3 and 1.4 ± 0.3 vulnerable lesions per patient in the SA and CTRL groups, respectively (Table 1). However, the significant increase in the degree of inflammation observed in the plaques of individuals who died of AMI, compared

Figure 2. Distribution of inflammatory cells in the coronary plaques (data are presented as means ± standard error of the mean). (A) Inflammatory cells in the acute myocardial infarction (AMI) group were significantly higher than those in the chronic stable angina (SA) and control (CTRL) groups, independently of the type of plaque observed (statistical analysis: A vs. B: p = 0.001; A vs. C: p = 0.001; B vs. C: p = not significant). (B) In the AMI group, no significant difference was observed between infarct-related coronary arteries (IRAs) and non-IRAs, independently of the type of plaque observed. (C) Both culprit and vulnerable plaques in the AMI group showed significantly more inflammatory infiltrates, compared with those observed in the stable plaques of the same group of patients (A vs. C: p = 0.006; B vs. C: p = 0.04). No statistically significant differences were observed between culprit and vulnerable plaques (p = 0.21). However, stable plaques in AMI patients had three-fold greater inflammation than stable and vulnerable coronary plaques of SA patients, and about four-fold of that measured in CTRL patients (C vs. D: p = 0.001; C vs. E: p = 0.001). (D) Immunohistochemical characterization of the various cytotypes in the coronary plaques. Significantly fewer CD68- and CD3-positive cells were found in stable and vulnerable plaques from SA and CTRL groups compared with those from AMI patients (AMI vs. SA and CTRL: actin p = 0.01; CD68: p = 0.01; CD3: p = 0.01).
with the controls, is not only attributable to the higher number of inflammatory cells–rich vulnerable plaques, but mainly to the marked increase in the degree of inflammation observed also in the stable plaques.

These observations support the concept that plaque vulnerability and adverse outcome is not only a localized vascular accident but a reflection of a more generalized pathophysiologic process of diffuse inflammatory involvement of the three major epicardial coronary arteries.

Regarding the possible causes of the presence of a diffuse inflammation in the coronary tree, several studies have focused on the antigenic role of the oxidized lipoproteins (18) and on probable infectious agents such as Chlamydia pneumoniae (Cp) (19, 20), although recent trials of antibiotic usage failed to show significant benefit (21, 22).

The mechanisms linking generalized inflammation in multiple coronary plaques to fatal clinical outcomes lies outside of the goals of our study. Gibson et al. (23),
however, recently showed that adverse clinical outcomes in AMI were associated with global coronary flow reduction both in culprit and non-culprit coronary arteries. Thus, it is possible to hypothesize that delayed coronary flow in non-IRA segments may result from reduced coronary vasodilata-
tion in remote territories (24) mediated by coronary artery inflammation and vasoactive substance release (25).

CONCLUSIONS

Fatal AMI is characterized by diffuse coronary instability and is not associated with a single vulnerable plaque. Moreover, isolated vulnerable plaques are also present in control patients with either a positive or a negative clinical history of stable angina, but are not associated with a widespread inflammation of the entire coronary tree.

A future challenge is to identify patients at high risk of acute cardiovascular events before clinical syndromes de-
velop. At present, aside from imaging modalities such as ultrasonography, magnetic resonance, and local temperature probes that could help to identify vulnerable plaques (26,27), highly sensitive inflammatory circulating markers such as hsCRP, cytokines, pregnancy-associated plasma protein-A, and Cp-HSP60 are currently the best candidates for diffuse active plaque detection (4,5,28–30). In this setting, the present study establishes the importance of a functional feature such as a generalized inflammatory state of the coronary tree as major determinant of the propensity to cause fatal complications, providing new potential ther-
apeutic targets and novel avenues to risk assessment.

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Diffuse and Active Inflammation Occurs in Both Vulnerable and Stable Plaques of the Entire Coronary Tree: A Histopathologic Study of Patients Dying of Acute Myocardial Infarction

Alessandro Mauriello, Giuseppe Sangiorgi, Stefano Fratoni, Giampiero Palmieri, Elena Bonanno, Lucia Anemona, Robert S. Schwartz, and Luigi Giusto Spagnoli

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