Widespread Myocardial Inflammation and Infarct-Related Artery Patency

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Background—Diffuse coronary vascular inflammation is associated with acute coronary syndromes. However, it is unknown whether inflammation also occurs within the myocardium. Therefore, this study was aimed at assessing the presence of activated cells in unaffected remote myocardium of patients with acute myocardial infarction (AMI), in comparison to the peri-infarct region from the same cases, and in comparison to myocardial specimens from control hearts.

Methods and Results—Sixteen patients dying 1 to 12 weeks after AMI and 16 control subjects were selected at autopsy. Myocardial specimens were taken at remote unaffected viable regions and at peri-infarct regions in cases with AMI. Confocal microscopy was performed to measure the number of activated cells (DR+/H11001), T-lymphocytes (CD3+/H11001), and activated T-lymphocytes (CD3+/DR+/H11001). Activated cells and activated T-lymphocytes were found in remote unaffected regions in 11 of 16 cases (69%), in peri-infarct zone in all cases (100%), and in none of the control hearts (0%, P<0.001 versus others). A greater myocardial inflammatory burden in remote regions but not in peri-infarct regions was associated with persistent infarct-related artery occlusion (P<0.05).

Conclusions—This study for the first time shows the presence of activated T-lymphocytes in remote unaffected myocardial regions in approximately two thirds of patients with recent AMI. Because these cells are associated with persistent infarct-related artery occlusion, our data may suggest that an antigenic stimulus present also in the myocardium triggers an immune response that may be critical to precipitate artery occlusion. (Circulation. 2004;110:46-50.)

Key Words: inflammation ■ myocardial infarction ■ lymphocytes ■ immune system

C oronary vascular inflammation plays a pivotal role in most cases of acute coronary syndromes (ACS).1 Inflammatory phenomena within vulnerable plaques might explain plaque rupture (or erosion) and superimposed thrombosis, which lead to vessel closure and ensuing myocardial ischemic damage.1 Recent data have suggested that in ACS, inflammation is not confined to a single plaque but is widespread to the entire coronary circulation.2–3 Diffuse inflammatory activation of the coronary tree may explain complex clinical scenarios such as those characterized by multiple ruptured plaques,4 altered coronary flow and abnormal myocardial perfusion related to nonculprit vessels,5–6 and high rate of clinical recurrence despite optimal medical and interventional treatment.7 Perivascular and extravascular activation in the ischemic and nonischemic myocardium of patients with troponin-negative unstable angina has been recently reported,8 whereas the presence of inflammation within the myocardial tissue has not been thoroughly investigated. Therefore, to ascertain whether a widespread myocardial inflammation occurs in ACS, we have evaluated the presence of activated cells in unaffected viable myocardium of patients with recent myocardial infarction, in comparison to the peri-infarct region from the same cases and in comparison to myocardial samples from control hearts.

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Methods

Study Groups
Subjects were enrolled from a series of consecutive postmortem examinations. Sixteen cases were selected according to the following inclusion and exclusion criteria: Recent myocardial infarction (1 to 12 weeks before death) and identifiable corresponding infarct lesion at pathology were the inclusion criteria, whereas very recent or ongoing myocardial necrosis (within 6 days) was an exclusion criterion.

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Sixteen consecutive control subjects were enrolled among the cases of death in the absence of any evident cardiac disease. Causes of death were upper and lower gastrointestinal bleeding (5 cases), sepsis (5 cases, of which 4 had acute infective/inflammatory pulmonary disease), stroke (2 cases), trauma (2 cases), and anaphylaxis (1 case). The presence of chronic systemic inflammatory disease or advanced cancer and delay between death and autopsy (>30 hours) were exclusion criteria for both groups.

**Pathology**

Gross examination of the hearts was performed to define the infarct area and the infarct-related artery (IRA) and the non-IRAs. Arteries were defined as occluded if failure to demonstrate residual lumen at gross examination and at cross-sectional microscopic analysis, caused by the presence of atheroma and/or thrombosis, occurred. Tissue specimens were obtained at peri-infarct regions, where viable myocardium was prevalent and reparative fibrosis was only marginal, and in regions of the left ventricle remote from the site of recent AMI to define the extent of myocardial scar in cases of multivessel disease, apparently normal at gross pathology and associated with a non-totally occluded related coronary artery (in cases of multivessel disease). The anterior left ventricular wall was sampled in control hearts. We used standard van Gieson–hematoxylin staining. The presence of activated cells in the myocardium was determined by immunohistochemical analysis with conventional fluorescent microscopy and confocal microscopy. We used a mouse monoclonal antibody anti-human HLA DR (Dako, 1:50 dilution) (for the major histocompatibility class II molecules (DR)), and fluorescence was obtained with a streptavidin–Texas red fluorescent conjugate. We used a mouse monoclonal antibody anti-T-lymphocyte CD3 (Dako, 1:100 dilution) to identify the presence of T-lymphocytes. In this case, fluorescence was obtained by incubating the sections with a streptavidin–fluorescein conjugate (fluorescein isothiocyanate). Double staining for DR and CD3 was performed to identify the percentage of activated (DR+) T-lymphocytes by confocal microscopy. Sections were first incubated with monoclonal antibodies to CD3, rinsed, and then incubated with biotinylated mouse immunoglobulin G. They were then rinsed again and incubated with antibodies conjugated to streptavidin peroxidase. To enhance the signal, sections were incubated using the Tyramide signal amplification biotin system (NEN Life Science Products). Fluorescence was obtained by incubating the sections with a streptavidin–fluorescein conjugate (fluorescein isothiocyanate). After the first reaction, a second reaction with primary antibodies to DR was induced, and fluorescence was obtained with a streptavidin–Texas red fluorescent conjugate. Control sections were incubated with a mixture of irrelevant monoclonal reagents with a similar isotype.

Images were acquired by means of the Noran confocal microscope with a ×60/1.4 NA immersion oil lens. Three-dimensional stacks were acquired at a resolution of 0.1 μm in the x, y, and z axes. The number of DR+ cells and of the subgroup of activated lymphocytes (DR+/CD3) were expressed as the number of cells per millimeter squared. Cell count was performed by two pathologists unaware of clinical characteristics of the patients and control subjects. Activated T-lymphocytes were considered perivascular if vascular endothelium was present in a ×40 magnification field or interstitial if lymphocytes were sparse between muscular fibers in absence of vascular structures.

**Statistical Analysis**

The χ² and Fisher exact tests were used to compare discrete variables, when appropriate. SPSS 11.0 for Windows was used. Quantitative results were expressed as median and interquartile range. Nonparametric tests were used to compare DR+ cells among different regions of each subject (Wilcoxon test for paired data) and among different subjects (Mann-Whitney U test for nonpaired data).

**Results**

**Clinical and Pathological Characteristics**

Clinical data regarding medical history and clinical course of disease from AMI to death were available in all cases (Table 1). Ten patients had had symptoms or signs of heart failure before death. Each patient had at least one associated medical condition that variably contributed to death: 6 had sepsis, of whom 5 had severe pneumonia; 6 had concomitant respiratory insufficiency; 4 had renal failure; 4 had severe upper or lower gastrointestinal bleeding; 2 had ischemic strokes; 1 had a cerebral hemorrhage; and 1 had motor vehicle trauma. All patients were receiving aspirin (100 to 300 mg daily).

Control cases had similar age (70 [68 to 87] years) and male-to-female ratio (9/7). Comorbidities at time of death were not significantly different, comparing cases with control cases.

**Widespread Myocardial Inflammation**

Activated (DR+) cells and activated T-lymphocytes (CD3+/DR+) were found in remote unaffected viable myocardial regions in 11 of 16 cases (69%), in peri-infarct zone in all cases (100%, P=0.043 versus remote regions), and in none of the control hearts (0%, P<0.001 versus remote and peri-infarct regions). Virtually all T-lymphocytes were found to be activated at double staining for CD3 and DR, representing >70% of all DR+ cells. A high percentage of activated T-lymphocytes was interstitial, up to 80% in peri-infarct regions and up to 40% in the remote regions. Figure 1 shows the presence of activated T-lymphocytes in a region remote from the infarct area in the perivascul caregiver compartment as well as in the interstitium.

The total number of DR+ cells as well as the number of activated T-lymphocytes was significantly lower in remote regions (79 [58 to 154]/mm² and 50 [25 to 88]/mm², respectively) when compared with the peri-infarct zone (181 [126 to 301]/mm², P=0.002, and 131 [66 to 182]/mm², P=0.006,
respectively), although significantly higher than in control hearts (0 and 0, \( P < 0.001 \) for all comparisons) (Figure 2). Reparative fibrosis was present in peri-infarct regions and absent in remote regions in all AMI cases. Signs of ongoing or very recent necrotic cell death were absent in all cases.

Myocardial Inflammation and Persistent IRA Occlusion
The total number of activated cells in the remote unaffected regions was significantly higher in cases with persistent IRA occlusion when compared with cases with nonoccluded IRA (92 [38 to 156] versus 33 [0 to 58]/mm², \( P = 0.028 \)). Similarly, persistent IRA occlusion was associated also with higher number of activated T-lymphocytes (68 [17 to 92] versus 13 [0 to 25]/mm², \( P = 0.047 \)) (Figure 3). Persistent IRA occlusion was found in 100% of cases with number of activated T-lymphocytes above median (50 cells/mm²) and in 36% of the remaining cases (\( P = 0.034 \)). Interestingly, the median values of both all activated cells and activated T-lymphocytes in the 3 cases with spontaneous recanalization of the IRA after AMI were 0 and 0, respectively, whereas the highest values were found among the 3 cases of permanent IRA occlusion despite fibrinolytic treatment at time of AMI (median, 96/mm² and 95/mm², respectively). No significant difference was found in comparing the number of activated cells or of activated T-lymphocytes at the peri-infarct site in cases with or without permanent IRA occlusion (200 [127 to 421] versus 163 [96 to 279]/mm², \( P = 0.46 \), and 142 [94 to 254] versus 75 [50 to 142]/mm², \( P = 0.10 \), respectively).

Myocardial Inflammation and Recurrent AMI
Subjects who had had multiple AMI before death compared with cases with single AMI had a significantly more intense infiltrate in the unaffected remote region, showing a nearly 3-fold higher number of all activated cells (96 [56 to 169] versus 38 [0 to 75]/mm², \( P = 0.033 \)) and a 6-fold higher number of activated T-lymphocytes (67 [27 to 117] versus 13 [0 to 50]/mm², \( P = 0.050 \)). Similarly, cases with multiple AMI had a significantly greater number of activated T-lymphocytes at peri-infarct sites, although they had a nonsignificantly higher total number of activated cells (183 [133 to 596] versus 75 [50 to 142]/mm², \( P = 0.027 \)).
Myocardial Inflammation and Other Relevant Clinical Variables

The total numbers of activated cells and of activated T-lymphocytes in viable myocardium were not different in comparing patients according to the number of coronary vessels affected (single-vessel versus multivessel coronary artery disease, 37 [0 to 125] versus 63 [0 to 92]/mm², \( P = 0.83 \), and 25 [0 to 92] versus 33 [0 to 67]/mm², \( P = 1.00 \), respectively) or according to the presence or absence of signs or symptoms of heart failure (60 [0 to 83] versus 65 [9 to 139]/mm², \( P = 0.72 \), and 27 [0 to 74] versus 35 [6 to 77]/mm², \( P = 0.94 \), respectively). Furthermore, the prevalence of activated T-lymphocytes in the remote region was similar in cases with and without heart failure (67% and 70%), being significantly higher than in control cases (\( P < 0.01 \)). The numbers of all DR+ cells and of DR+ T-lymphocytes in remote or peri-infarct regions were not correlated to the infarct extension evaluated on a 4-grade scale by 2 independent pathologists. Moreover, the numbers of activated cells and activated T-lymphocytes in the remote region or peri-infarct regions were independent from the time from AMI to death (\( R = -0.19 \), \( P = 0.47 \), and \( R = -0.038 \), \( P = 0.89 \), respectively). No difference was found in numbers of activated cells and activated T-lymphocytes in comparing cases with (6 cases) versus those without (10 cases) evidence of sepsis at time of death (82 [0 to 112] versus 78 [0 to 138]/mm², \( P = 0.88 \), and 56 [0 to 69] versus 48 [0 to 87]/mm², \( P = 0.98 \), respectively).

Discussion

This study, for the first time, to the best of our knowledge, shows the presence of an active inflammatory infiltrate in unaffected viable myocardium in patients with recent AMI. We report the presence of lymphocytes expressing HLA-DR, an accepted marker of T-lymphocyte activation,\(^9,10\) in peri-infarct regions in all patients and in remote myocardium in two thirds of patients, in contrast with lack of inflammation in control hearts.

An inflammatory infiltrate in peri-infarct area in the days and weeks after AMI had already been described both in human AMI and animal AMI models,\(^11,12\) whereas the presence of activated cells, in particular T-lymphocytes in regions remote from the infarct area, is novel. A number of studies suggest a recent immune activation within the culprit plaques of patients with unstable angina.\(^13\) These plaques are characterized by a markedly higher number of T cells, macrophages, and smooth muscle cells expressing DR molecules than that found in patients with stable angina.\(^13\) Moreover, T cells of culprit plaques express CD25 on their membranes, suggesting that these cells are equipped to mount an immune response in vivo.\(^14\) More importantly, Buffon et al\(^1\) have recently shown that in patients with unstable angina, leukocyte activation through the coronary circulation is not confined to the culprit vessel,\(^2\) and Spagnoli et al\(^3\) have confirmed that in patients with AMI, activated T-lymphocytes are found in the coronary arterial wall both in the culprit and nonculprit epicardial coronary arteries.\(^3\) Furthermore, it has been recently found that in unstable angina, widespread vascular inflammation is not confined to epicardial vessels because it also involves small intramural vessels.\(^6\) We now describe diffuse myocardial inflammation in cases with recent AMI.

The causes of inflammation associated with acute coronary syndromes are still poorly known. The presence of activated T-lymphocytes with a skewed T-cell repertoire in the arterial wall suggests an antigenic stimulus, which triggers adaptive immunity.\(^15,16\) Human and experimental studies have shown that antigens are formed into plaques during LDL oxidation and degradation. Indeed, T-cell clones from human atherosclerotic plaques respond to oxidized LDL by proliferation and IFN-\(\gamma\) secretion, and circulating T cells from unstable but not from stable patients or normal control patients proliferate in vitro in response to oxidized LDL and/or autologous proteins obtained from coronary culprit lesions.\(^17,18\) Another candidate antigen is represented by Chlamydia subspecies antigens.\(^19,20\) Conversely, in animal models in which AMI is caused by coronary artery ligation, activated T cells are consistently found in the infarct region but not in the remote myocardium.\(^12\) The demonstration in our study of interstitial activated T cells expressing HLA-DR (also CD25, the IL-2 membrane receptor; data not shown) in the remote myocardium of patients with recent AMI suggests that the antigenic stimuli associated with coronary instability are also present in the extravascular compartment. Taken together, these findings suggest that the search for the triggers of the immune response associated with acute coronary syndromes should take into account antigens that are present also within the myocardium. At present, it cannot be ruled out that the presence of activated T-lymphocytes might be a mere conse-
quence of myocardial necrosis, resulting in the release of segregated antigens. This may explain the presence of activated T cells in peri-infarct regions observed in all patients. However, it is not an obvious explanation for activated cells in remote myocardial regions observed in a subset of patients only. Because these cells are associated with persistent IRA occlusion, our data rather suggest that an antigenic stimulus present also in the myocardium triggers an immune response that may be critical to precipitate coronary artery occlusion.

The lack of activated cells in remote myocardium in approximately one third of patients might be due to weaker antigenic stimuli or to a different pathogenesis of AMI.

Nonetheless, additional studies are warranted to elucidate how serum C-reactive protein (CRP) and interleukin-1 receptor antagonist (IL-1Ra) levels are elevated in approximately one third of patients might be due to weaker antigenic stimuli or to a different pathogenesis of AMI. Notably, reported prevalence of systemically detectable inflammatory markers in ACS varies. Serum CRP and proinflammatory cytokines such as interleukin-6 are elevated in ≈70% of patients with severe unstable angina on admission, in 50% of such patients at discharge, and in 45% of such patients at 6 months of follow-up.2 Therefore, the triggers of coronary thrombosis and vasoconstriction are not necessarily the same in all patients with ACS.

The association between widespread myocardial inflammation and persistent IRA occlusion is in keeping with the observation that among patients with an ACS, systemic inflammation is associated with a worse short- and mid-term outcome2,21–23 and with reperfusion failure in patients with ST-elevation AMI undergoing fibrinolysis.24 Interestingly, the inflammatory burden in the peri-infarct region was not associated with persistent IRA occlusion. This is not surprising because the number of activated T-lymphocytes in this region is probably amplified by the inflammatory response to myocardial necrosis.

In conclusion, the present study, albeit limited by its observational postmortem design, describes the presence of a widespread inflammatory response in recent AMI involving the myocardium. Although the source of these cells and the stimuli for activation are still underestimated, these findings may open the way to further studies for the search of the cause of acute coronary syndromes beyond classic risk factors and established pathophysiological mechanisms.25 Nonetheless, additional studies are warranted to elucidate cause-and-effect links and diagnostic and/or therapeutic implications.

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