OBJECTIVES
We investigated whether the source of the acute phase response in unstable angina (UA) lay within the culprit coronary plaque or distal myocardium.

BACKGROUND
An inflammatory response is an important component of the acute coronary syndromes. However, its origin and mechanism remain unclear.

METHODS
In 94 stable patients undergoing coronary angiography, the relationship between systemic levels of tumor necrosis factor (TNF)-alpha, interleukin-6 (IL-6) and C-reactive protein (CRP) and extent of atherosclerosis was studied. The temporal relationship between these markers and troponin T (TnT) was determined in 91 patients with UA. Cytokine levels were measured in the aortic root and coronary sinus of 36 unstable patients.

RESULTS
There was no relationship found between stable coronary atherosclerosis and inflammatory marker levels. Compared with this group, admission levels of IL-6 (3.6 ± 0.3 ng/ml vs. 10.7 ± 1.7 ng/ml, p < 0.05) and CRP (2.3 ± 0.1 mg/l vs. 4.6 ± 0.6 mg/l, p < 0.05) were elevated in patients with UA. In this group, IL-6 and CRP remained elevated in those who subsequently experienced major adverse cardiac events. This inflammatory response occurred in parallel to the appearance of TnT. Both TNF-alpha (19.2 ± 3.4 ng/ml vs. 17.1 ± 3.3 ng/ml, p < 0.001) and IL-6 (10.3 ± 1.4 ng/ml vs. 7.7 ± 1.1 ng/ml, p < 0.01) were elevated in the coronary sinus compared with aortic root in patients with UA. This was principally observed in those who were TnT positive. There was no cytokine gradient across the culprit plaque.

CONCLUSIONS
There is an intracardiac inflammatory response in UA that appears to be the result of low-grade myocardial necrosis. The ruptured plaque does not appear to contribute to the acute phase response. (J Am Coll Cardiol 2002;39:1917–23) © 2002 by the American College of Cardiology Foundation

A systemic inflammatory response often accompanies acute coronary syndromes, and its presence has been widely recognized as an index of further events (1). Accumulating evidence suggests that inflammation within the atherosclerotic plaque contributes to its destabilization and subsequent disruption (2–4). The widely held view is that systemic inflammation in unstable angina (UA) originates from inflammatory processes within the arterial wall after plaque disruption.

Histologic study of coronary atherectomy material has shown considerable overlap between inflammatory infiltrates in plaques harvested from patients with stable and unstable angina (5). However, the systemic levels of inflammatory markers in patients with stable angina are somewhat lower than those found in the acute coronary syndromes (6).

An alternative explanation to reconcile these observations is that the focus of inflammation does not reside within the vasculature itself but rather in injured myocardium distal to the disrupted plaque. Thus, the precise site of, and stimulus for, the inflammatory response that accompanies UA remains unclear.

The aim of this study was to clarify these issues by examining whether the systemic inflammatory response in UA was due to ischemic myocardial injury and/or plaque inflammation. We examined the relationship between stable coronary atherosclerosis and circulating levels of inflammatory markers. Secondly, in patients with UA we determined whether a temporal relationship existed between systemic markers of inflammation and the sensitive marker of myocardial necrosis, cardiac troponin T (TnT). Finally, we determined whether the focus of systemic inflammation in patients with UA exists across the culprit atherosclerotic plaque and/or the myocardium.

METHODS
Three groups of patients were prospectively studied. All had given informed consent, and the protocol was approved by the St. Thomas’ Hospital Local Institutional Review Board.

Group A consisted of 94 patients undergoing diagnostic cardiac catheterization at our institution for the investigation of stable angina. Before arteriography, an arterial blood sample was taken. Patients in this group were segregated according to the absence or presence (defined as a diameter...
stenosis >70% on quantitative coronary angiography) of coronary artery disease. Those with coronary artery disease were subdivided further by the number of vessels involved.

Group B consisted of 91 patients admitted with Braunwald IIIb UA (7) to our own institution or to two local general hospitals. Patients had experienced chest pain at rest associated with electrocardiogram changes within the preceding 48 h. Exclusion criteria included myocardial infarction (MI) within six weeks, evidence of significant myocardial necrosis on admission (total creatine kinase >2 times upper limit of normal) or coexistent conditions likely to be associated with an acute phase response. All patients received standard medical therapy, consisting of aspirin, subcutaneous low molecular weight heparin, intravenous nitrates and beta-blockers, where not contraindicated. No patients received a glycoprotein IIb/IIIa antagonist. Peripheral blood was sampled on admission, at 6 h, 24 h, 48 h and 7 days.

In group C there were a further 36 patients with Braunwald IIIb UA undergoing cardiac catheterization on clinical grounds. Before angiography the coronary sinus was cannulated and simultaneous blood samples taken from the coronary sinus and aortic root. In patients proceeding to percutaneous coronary intervention, where a culprit lesion had been identified, a Multifunctional Probing catheter (Boston Scientific Corp., Maple Grove, Minnesota) was passed into the distal coronary artery and blood sampled from this site before balloon dilation.

**Laboratory assays.** Serum was immediately separated from all blood samples and stored at −80°C. Levels of C-reactive protein (CRP), interleukin (IL)-6 and tumor necrosis factor (TNF)-alpha were determined by a solid-phase, two-site chemiluminescent immunometric assay (Diagnostic Products Corp., Los Angeles, California). Cardiac troponin T was measured using an enzyme-linked immunoassay kit (Boehringer Mannheim, Berkshire, United Kingdom). The results of these assays were not available to those personnel involved in the clinical care of these patients.

Patients were classified according to the presence or absence of a major adverse cardiac event (MACE), defined as the 30-day need for urgent revascularization, progression to MI or death.

**Statistical analysis.** The results are presented as the mean ± standard error for variables that were normally distributed and as the median (range) where this was not the case. Nonparametric data was normalized before statistical analysis. The levels of CRP, IL-6 and TNF-alpha among the patient subgroups in group A were compared with a Kruskal-Wallis one-way analysis of variance on ranks. Where differences were present between subgroups, this was assessed using Dunn’s method. The same method was used to compare these stable patients with those in the unstable group B. The temporal relationships of CRP, IL-6, TNF-alpha and TnT between those who subsequently experienced MACE and those who did not were determined by one-way repeated measures analysis of variance. Where a difference was found between those who experienced MACE and those who did not, this was tested with a Bonferroni t test. The mean level of each marker was determined for each patient. Linear regression analysis was then employed in this group to determine those variables that related to outcome and multiple linear regression to identify those that were independently related. The relationship of aortic to coronary sinus cytokine levels was determined with a Student t test for paired data.

**RESULTS**

**Stable patients undergoing angiography.** In patients undergoing routine angiography (age 60.1 ± 10.5, 72% men), hypercholesterolemia was prevalent (46% of patients), though diabetes was an uncommon finding (5%). A total of 15% of these patients had had previous coronary revascularization. There was no consistent relationship found between the presence or extent of coronary disease and the level of inflammatory markers (Fig. 1). However, the levels of CRP and IL-6 were both significantly lower in these stable patients than in the patients with UA (Fig. 2).

**Patients with UA.** Demographics were similar between patients who did or did not have a MACE (Table 1). The levels of IL-6, CRP and TnT were elevated after hospital admission among patients who experienced MACE compared with those who did not (Fig. 3). The systemic rise in the level of IL-6 observed among the MACE group occurred in parallel to that of TnT and preceded the rise in CRP by approximately 6 h. However, a direct correlation between either CRP or IL-6 levels with TnT was not found at any time point. By seven days after admission, there was no longer any difference in the level of IL-6 between these two groups (Fig. 3), whereas both CRP and TnT remained elevated at this time in those experiencing MACE (Fig. 3). No difference was found in the level of TNF-alpha between these two groups at any time point.

Linear regression analysis showed the normalized mean levels of CRP, IL-6 and TnT over the first 48 h after hospital admission to be predictive of 30-day MACE. Multiple regression analysis over this time period showed both IL-6 (p = 0.002) and TnT (p = 0.002), but not CRP (p = 0.06), to be independently predictive of 30-day MACE. Best subsets regression analysis suggested a com-
Combination of both IL-6 and TnT to be most predictive of subsequent MACE (Mallows $C_p$ of 4.8, where $n = 5$).

**Coronary sinus and aortic root sampling.** Among the 36 patients (age $64.8 \pm 8.1$ years, 68% men) with Braunwald IIIb UA undergoing cardiac catheterization, 35 had disease of at least one epicardial coronary artery, and 22 (63%) underwent urgent revascularization. These patients were similar in their demographics to those in group B. No difference in cytokine levels was observed between those who did and those who did not undergo revascularization.

The levels of both TNF-alpha and IL-6 were significantly greater in the coronary sinus compared with the aortic root suggesting intracardiac production of these substances (Fig. 4). These patients were subdivided according to the presence or absence of an elevated TnT level at the time of catheterization. In those with TnT elevation ($n = 17$), the coronary sinus levels of TNF-alpha and IL-6 were both significantly greater than aortic levels (Fig. 4). However, a quantitative relationship between the TnT level and the absolute transmyocardial gradient of TNF-alpha or IL-6

**Figure 1.** The levels of tumor necrosis factor (TNF)-alpha, interleukin (IL)-6 and C-reactive protein (CRP) are shown for the stable patients undergoing angiography. No consistent relation was found between the level of these inflammatory markers and the extent of angiographic coronary disease. A = normal coronary arteries; B = single vessel disease; C = double vessel disease; D = triple vessel disease.

**Figure 2.** The levels of tumor necrosis factor (TNF)-alpha, interleukin (IL)-6 and C-reactive protein (CRP) are shown for the stable patients and the unstable angina patients divided according to the subsequent occurrence of major adverse cardiac events (MACE). The levels of IL-6 and CRP are substantially higher in those with unstable angina compared with those with stable symptoms ($p < 0.05$). A = stable patients; B = unstable patients without MACE; C = unstable patients with MACE.
was not found. In those without evidence of myocardial necrosis (TnT negative), this intracardiac production of TNF-alpha and IL-6 was no longer evident (Fig. 4). In those proceeding directly to coronary intervention, blood was successfully sampled distal to the culprit coronary lesion in 11 patients. In this group, no differences were found in the levels of TNF-alpha or IL-6 between the proximal and distal coronary artery despite the presence of a transcatheter cytokine gradient between the aortic root and coronary sinus (Fig. 5).

**DISCUSSION**

This study supports the premise that the myocardium is the principal site for the systemic elevation in inflammatory markers found in UA. Furthermore, early activation of the inflammatory response in these unstable patients was associated with an adverse outcome.

**Relationship between inflammatory markers and coronary disease.** Among patients with stable angina, no relationship existed between the levels of TNF-alpha, IL-6 or CRP and the extent of coronary artery disease. It has previously been observed that clinically silent plaque rupture is common (8). Furthermore, fractured fibrous caps with intense inflammation are a common finding in the abdominal aorta at necropsy, and asymptomatic carotid plaque rupture may be found in almost one-fifth of elderly persons at autopsy (9). Based on these observations, it might be expected that those with a heavy burden of coronary disease would have demonstrably higher inflammatory markers than those without. The absence of a relationship between the number of diseased vessels (as a surrogate measure of stable plaque burden) and systemic inflammatory response implies that there is a process in addition to plaque rupture ongoing among those with UA.

**Inflammatory markers in unstable patients.** In this unstable group, plaque disruption was the presumed underlying etiology in all patients. A systemic inflammatory response was present in 40% of these patients, and this was associated with subsequent MACE. Among these patients, both IL-6 and TnT were elevated at the time of hospital admission with further elevations in the levels of these two proteins occurring in parallel over the subsequent 48 h. This suggests that myocardial necrosis and systemic inflammatory activity are closely related in this group. If plaque disruption was the sole mediator of the inflammatory response, then it would have been difficult to explain the dichotomy that exists between these two groups of patients. **Transcatheter cytokine gradient.** The rise in the level of both IL-6 and TNF-alpha between the aortic root and coronary sinus in patients with UA suggests intracardiac synthesis of these substances. Importantly, when the patients are divided according to their troponin T status, this intracardiac inflammatory response appears to be present principally in those with evidence of myocardial injury. Microscopic multifocal MI associated with embolized platelet microthrombi has been well described in UA (10) and is believed to be the mechanism for the elevation in tropon in T found in these patients (11). As no gradient in cytokine concentrations could be demonstrated between the aorta and the vessel distal to the culprit lesion in patients with UA, the inflammatory response we measured appears to lie within the “downstream” myocardium. The relationship between intracardiac cytokine synthesis and troponin T elevation further suggests that the inflammatory response is related to necrosis within the myocardium.

Interestingly, the systemic levels of TNF-alpha were similar in all groups. Though TNF-alpha is one of the determinates of IL-6 synthesis, it is also modulated by IL-1. Interleukin-1 receptor antagonist has been found previously to be elevated in UA (12) and may account for the systemic increases in IL-6 that have been observed here. **IL-6 and prognosis.** These data suggest a prognostic role for IL-6 in the early risk stratification of patients with UA. In those who had a subsequent MACE, both IL-6 and TnT were elevated at hospital admission with CRP becoming elevated some hours later. Elevation of TnT in UA is increasingly seen as having important prognostic implications (13). These results suggest that elevation of IL-6 may carry similar prognostic connotations. During the first 48 h after admission, the levels of IL-6, CRP and TnT remained elevated in those who subsequently had a MACE and may permit discrimination of these patients over this time period. One week after admission, the prognostic value of IL-6 had been lost, though both CRP and TnT remained elevated in those who experienced a MACE. We were, however, unable to demonstrate a distinct correlation between the levels of these inflammatory markers and TnT at these time points. Though the systemic half-life of CRP is longer than IL-6, they are both somewhat shorter than TnT, which may, in part, explain this observation.

**A role for immunosuppression?** An inflammatory response has previously been observed among patients with acute MI and found to be related to infarct size and

Table 1. The Demographics of the Unstable Patients Divided According to the Subsequent Occurrence of MACE

<table>
<thead>
<tr>
<th></th>
<th>MACE (n = 37)</th>
<th>No MACE (n = 54)</th>
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<tbody>
<tr>
<td>Age (mean ± SD), yrs</td>
<td>65.9 ± 11.2</td>
<td>67.8 ± 8.3</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>23/14</td>
<td>36/18</td>
</tr>
<tr>
<td>Hypertension</td>
<td>9 (24%)</td>
<td>15 (28%)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>3 (8%)</td>
<td>2 (6%)</td>
</tr>
<tr>
<td>Smoking</td>
<td>5 (19%)</td>
<td>6 (11%)</td>
</tr>
<tr>
<td>Family history of IHD</td>
<td>14 (38%)</td>
<td>18 (33%)</td>
</tr>
<tr>
<td>Previous stable angina</td>
<td>8 (22%)</td>
<td>19 (35%)</td>
</tr>
<tr>
<td>Previous episode of unstable angina</td>
<td>9 (24%)</td>
<td>15 (28%)</td>
</tr>
<tr>
<td>Previous history of MI</td>
<td>6 (16%)</td>
<td>8 (15%)</td>
</tr>
<tr>
<td>Peripheral vascular disease</td>
<td>2 (5%)</td>
<td>4 (2%)</td>
</tr>
<tr>
<td>Cerebrovascular disease</td>
<td>2 (5%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Previous PTCA</td>
<td>1 (3%)</td>
<td>3 (6%)</td>
</tr>
<tr>
<td>Previous CABG</td>
<td>3 (8%)</td>
<td>5 (9%)</td>
</tr>
</tbody>
</table>

CABG = coronary artery bypass graft; IHD = ischemic heart disease; MACE = major adverse cardiac event; MI = myocardial infarction; PTCA = percutaneous transluminal coronary angioplasty.
prognosis (14–16). In light of this observation, the effect of immunosuppression with methylprednisolone after MI was tested and found to be deleterious (17). A possible beneficial role for immunosuppression in UA has been suggested. However, as the systemic inflammatory response appears to reflect low level myocardial necrosis, it is interesting that immunosuppression in this setting resulted in similar adverse outcomes to those seen in the previous infarct studies, as recently shown in the Methylprednisolone in Unstable Angina (MUNA) trial (18).

**Clinical implications.** It has previously been shown that, in patients with UA undergoing coronary intervention, the IL-6 level in those with a normal baseline level does not change after percutaneous transluminal coronary angioplasty (PTCA) (19). However, in those with an already elevated IL-6, there is often a further significant rise after PTCA (19). Percutaneous transluminal coronary angioplasty among patients with UA is known to be associated with distal embolization within the coronary artery of platelet microthrombi and a significant risk of periprocedural MI (20,21). Although speculative, if elevation of IL-6 is due to myocardial microinfarction from previous platelet aggregate embolization, this may predispose to further embolization at the time of PTCA with a further rise in IL-6. Those with a normal IL-6 level before their procedure are unlikely to have had significant recent embolization, implying relative absence of thrombus within the coronary artery.

The glycoprotein IIb/IIIa inhibitors reduce the risk of periprocedural MI in patients with UA undergoing PTCA (20), particularly in those with elevated TnT (22). Furthermore, abciximab has recently been shown to reduce the inflammatory response among these patients (23). Incorporating data on inflammatory status may allow improved targeting of those patients at risk of periprocedural adverse events for early antiplatelet therapy.

**Study limitations.** Measurement of coronary blood flow would allow the amount of IL-6 and TNF-alpha produced...
within the heart to be accurately determined. However, this would require further manipulation at the time of catheterization among patients who are unstable, which may be problematic. We have postulated that the source of cytokine production lies within the myocardium and occurs in response to microinfarction. However, further sampling of blood in the distal coronary artery and selective cannulation of the cardiac veins would help to establish this further. In addition, larger studies are needed to determine the effectiveness of these markers in risk stratification and also to test their role in patients undergoing coronary intervention.

**CONCLUSIONS**

Adverse events in UA are associated with early elevation of IL-6 and troponin T. The systemic appearance of IL-6 occurs in parallel to that of troponin T among those having a subsequent MACE. Both TNF-alpha and IL-6 are actively generated within the heart among these patients, and this inflammatory response is found principally in those with evidence of myocardial necrosis. This suggests that the systemic inflammatory response may be the result of the myocardial microinfarction known to occur in this group. The admission levels of IL-6 appear to be an independent predictor of MACE in UA and, in combination with troponin T, may allow improved risk stratification of these patients. Further studies are warranted, as an improved understanding of this inflammatory process may lead to novel therapeutic approaches and better application of currently available therapies.

**REFERENCES**

3. van der Wal AC, Becker AE, van der Loos CM, Das PK. Site of intimal rupture or erosion of thrombosed coronary atherosclerotic plaques is characterized by an inflammatory process irrespective of the dominant plaque morphology. Circulation 1994;89:36–44.