Increasing Levels of Interleukin (IL)-1Ra and IL-6 During the First 2 Days of Hospitalization in Unstable Angina Are Associated With Increased Risk of In-Hospital Coronary Events

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Background—A growing body of evidence suggests a role for inflammation in acute coronary syndromes. The aim of this study was to assess the role of proinflammatory cytokines, their time course, and their association with prognosis in unstable angina.

Methods and Results—We studied 43 patients aged 62±8 years admitted to our coronary care unit for Braunwald class IIIB unstable angina. In each patient, serum levels of interleukin-1 receptor antagonist (IL-1Ra), interleukin-6 (IL-6) (which represent sensitive markers of biologically active IL-1β and tumor necrosis factor-α levels, respectively), and troponin T were measured at entry and 48 hours after admission. Troponin T–positive patients were excluded. Patients were divided a posteriori into 2 groups according to their in-hospital outcome: group 1 comprised 17 patients with an uneventful course, and group 2 comprised 26 patients with a complicated in-hospital course. In group 1, mean IL-1Ra decreased at 48 hours by 12%, and IL-6 diminished at 48 hours by 13%. In group 2, IL-1Ra and IL-6 entry levels were higher than in group 1 and increased respectively by 37% and 57% at 48 hours ($P<0.01$).

Conclusions—These findings indicate that although they receive the same medical therapy as patients who do not experience an in-hospital event, patients with unstable angina and with complicated in-hospital courses have higher cytokine levels on admission. A fall in IL-1Ra and IL-6 48 hours after admission was associated with an uneventful course and their increase with a complicated hospital course. These findings may suggest novel therapeutic approaches to patients with unstable angina. **(*Circulation*. 1999;99:2079-2084.)**

Key Words: interleukins ■ inflammation ■ angina ■ prognosis

Recent findings suggest that inflammation may play an important role in the pathogenesis of acute coronary syndromes. Unstable angina is characterized by increased levels of the acute-phase reactants fibrinogen, C-reactive protein (CRP), and serum amyloid A protein and of the cytokine interleukin (IL)-6, the major inducer of CRP production in the liver, and their elevation is associated with a worse short- and long-term prognosis.[1–3] In addition, IL-1 is likely to be involved in the inflammatory process associated with the acute coronary syndromes because it is known to induce IL-6, to increase gene expression for clotting factors and inhibitors of fibrinolysis, and to contribute to transendothelial passage of neutrophils by increasing the surface expression of endothelial adhesion molecules and the production of IL-8.[4–6] IL-1α is expressed on the surface of activated macrophages[7,8] as a biologically active molecule, and IL-1β is released from activated platelets.[9–12]

The IL-1 receptor antagonist (IL-1Ra), another member of the IL-1 family, is often measured as an indicator of disease severity because IL-1 levels in the circulation are usually very low, even in septic shock patients.[13] IL-1Ra correlates better with disease severity than IL-1 itself in experimental endotoxemia, burns, and various infectious diseases[14–17]; in fact, there is evidence that IL-1Ra, like CRP, is an acute-phase protein.[18] Because IL-1Ra is a specific antagonist of IL-1, elevated levels of IL-1Ra could indicate a desirable clinical scenario for reducing the inflammation caused by IL-1.[12] However, exogenous administration of IL-1Ra in patients with rheumatoid arthritis has demonstrated that endogenous levels are insufficient to affect disease progression.[19] Therefore, we measured IL-1Ra and IL-6 blood levels in patients with unstable angina on admission and after 48 hours of full medical therapy.

Methods

Patient Population

We studied 43 patients (38 men, 5 women) aged 62±8 years (range, 45 to 73 years) admitted to our coronary care unit (CCU) due to the

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severity of their unstable angina. The inclusion criteria were angina at rest with ≥2 ischemic episodes or 1 episode lasting >20 minutes during the preceding 24 hours (Braunwald class IIIB), with diagnostic ST-segment shift. These patients required full medical therapy, including intravenous nitrates and heparin with optimal anticoagulation (activated partial thromboplastin time between 1.5 and 2.5 times the basal value). At admission, there was no evidence of myocardial infarction as detected by enzymatic techniques. The exclusion criteria were left bundle-branch block, dilated cardiomyopathy, valvular heart disease, previous myocardial infarction within 4 weeks, atrial fibrillation, the presence of a pacemaker or an ECG abnormality that would invalidate ST-segment analysis, or positive (>0.1 μg/L) troponin T. Patients with known or suspected thrombotic disorders other than unstable angina, malignancy, infection, or inflammatory disease or recent (<1 month) surgery or trauma were also excluded. Between March 1995 and October 1996, 210 patients were admitted to our CCU with a diagnosis of unstable angina; 51 patients were excluded because of the absence of an ischemic episode during the prior 24 hours, 24 exhibited an increase in total creatine kinase or troponin T within the 48 hours of the study, and 43 had suffered a recent MI. In addition, 19 patients were excluded because of neoplastic disease,[5] inflammatory diseases,[11] and left bundle-branch block.[7] In 30 additional patients, the attending physician of the CCU at the time of their admission did not consider full medical therapy to be indicated. The onset of symptoms of unstable angina ranged from 2 to 40 days (12.6±10 days). Twenty-seven of the 43 patients were secondary referrals to our center. The study was approved by the Ethics Committee of the Catholic University, and each patient gave informed consent.

Study Design

Blood was drawn from patients with unstable angina on admission to the CCU for assessment of serum levels of IL-1Ra, IL-6, and troponin T. The latter was measured to assess a possible role of myocardial cell damage in inducing the inflammatory response. IL-1Ra, IL-6, and troponin T were also measured 48 hours after admission in each patient, and those patients with raised levels of troponin T were excluded as part of the study design. Patients were monitored in the CCU, and each underwent coronary angiography. In 26 patients, an angiogram was performed within 4 days (in 5, within 48 hours) of hospitalization because of the presence of refractory angina. In the remaining 17, angiography was performed later as part of the study.

The patients were divided into 2 groups according to their in-hospital outcome: group 1 comprised 17 patients with an uneventful course, and group 2 comprised 26 patients with death (3), myocardial infarction (1), or refractory angina (22) despite full medical therapy.

Laboratory Assays

IL-1Ra levels were measured by a specific radioimmunoassay as previously described.[20] The lower level of detection for the assay is 125 pg/mL. In healthy subjects, levels of IL-1Ra vary from 125 to 230 pg/mL.[14]

IL-6 was measured by a commercial kit (Quantikine human IL-6, R&D Systems) with a range from 3 to 300 pg/mL. Troponin T was measured by a commercially available enzyme immunoassay (Boehringer Mannheim).

Statistical Analysis

Because the data were not normally distributed, nonparametric tests were used; results are expressed as median and range. ANOVA with Newman-Keuls correction was used for comparison among groups. The Spearman test was used for correlations, and discontinuous variables were tested by a contingency χ^2 test. We considered as relevant an increase or decrease in IL-1Ra or IL-6 in the 48-hour sample that was above or below the upper limit of normal (respectively, 0.2 ng/mL and 3 pg/mL) or any change >25% when both samples (entry and discharge) were elevated. Percent changes were calculated as (levels at 48 hours−levels at entry)/levels at entry. The odds ratio (OR) was calculated for any increase at 48 hours in IL-1Ra and IL-6 by logistic regression analysis, with age, smoking habit, cholesterol >200 mg/dL, diabetes, and family history of ischemic heart disease as possible confounding variables. All tests were 2-tailed.

Results

Clinical characteristics of the patients and risk factors are presented in the Table and were similar between the 2 groups. During hospitalization, 17 (40%) of 43 patients had waning of symptoms in response to full medical therapy (group 1); 26 (60%) of 43 patients had events (group 2). Of these, 3 patients died (1 suddenly, 2 preceded by a myocardial infarction), 1 had a nonfatal acute myocardial infarction, and 22 required urgent revascularization because they failed to respond to full medical therapy. In group 1, mean IL-1Ra was 0.184 ng/mL (0.012 to 1.37) at entry and decreased to 0.176 ng/mL (0.006 to 0.8) at 48 hours (P=NS); in addition, IL-6 diminished from 4.7 pg/mL (0.1 to 12.6) at entry to 0.94 pg/mL (0.05 to 10.4) at 48 hours (P=NS). In group 2, IL-1Ra was higher at entry than in group 1 ($P<0.05$) and increased from 0.347 ng/mL (0.06 to 0.85) to 0.426 ng/mL (0.09 to 1.23; $P<0.01$) at 48 hours. In addition, IL-6 was higher than in group 1, although not significantly, and increased from the entry value of 7.3 pg/mL (0.1 to 22.9) to 9.5 pg/mL (0.1 to 47.5; $P<0.01$) at 48 hours (Figure 1). Had we considered only the entry levels of IL-6, we would have found a significant difference between groups 1 and 2 by Mann-Whitney U test ($P=0.018$).

In addition to these changes in mean serum levels, we examined the percent change in IL-1Ra and IL-6 in each patient after 48 hours (Figure 2). IL-1Ra in group 1 decreased by 12% (range, −74% to +75%) but in group 2 increased by 37% (range, −44% to +203%); a reduction in IL-1Ra was observed in 10 (59%) of 17 group 1 patients but only in 2 (8%) of 26 group 2 patients ($P<0.001$). A significant increase of IL-1Ra was observed in 3 group 1 patients (18%) and in 18 group 2 patients (69%) ($P=0.003$). Moreover, IL-1Ra did not change in 4 patients in group 1 and 6 in group 2 (Figure 2A). IL-6 decreased by 13% (range, −99% to +82%) in group 1 and increased by 57% (range, −81% to +406%) in group 2. A decrease in IL-6 was observed in 12 group 1 patients (71%) but only in 3 group 2 patients (12%) ($P<0.001$) (Figure 2B). An increase in IL-6 was observed in 4 group 1 patients and in 17 group 2 patients ($P=0.011$). No differences in IL-1Ra or IL-6 levels were observed between patients with hard end points (death or myocardial infarction) and patients with a soft end point (refractory angina) (Figure 3).

In the pooled population, an increase in IL-1Ra carried an OR (adjusted for age, cholesterol, smoking status, diabetes, and hypertension) for in-hospital events of 11 (95% CI, 1.4 to 81; $P=0.023$), and an increase in IL-6 levels carried an OR of 7 (95% CI, 1.3 to 38; $P=0.025$). IL-1Ra was significantly correlated with IL-6 ($r=0.39$, $P=0.01$).

Group 2 patients had more ischemic episodes than group 1 patients, but the difference was not significant (2.4±1.6 versus 1.6±1.3; $P=0.09$). In addition, group 2 patients exhibited a longer ischemic burden during the 48 hours of the study (24±13 minutes versus 15±10 minutes; $P=0.023$); however, no correlation was found between number and

Baseline Characteristics of Patients

	Group 1	Group 2	*P*
Clinical characteristics			
Number of patients	17	26	
IL-1Ra levels at entry, ng/mL median (range)	0.184 (0.012–1.37)	0.347 (0.06–0.85)	0.009
IL-6 levels at entry, pg/mL median (range)	4.7 (0.1–12.6)	7.3 (0.1–22.9)	0.018
IL-1Ra at 48 hours, ng/mL median (range)	0.176 (0.006–0.8)	0.426 (0.09–1.23)	<0.001
IL-6 at 48 hours, pg/mL median (range)	0.94 (0.005–10.4)	9.5 (0.1–56.7)	<0.001
Age, y (SD)	59 (9)	64 (7)	0.062
Sex, M/F	17/0	21/5	0.15
Risk factors, n (%)			
Family history of IHD	8 (47)	11 (42)	0.39
Family history of IHD under age 50 y	4 (24)	2 (7)	0.15
Total cholesterol >200 mg/dL	8 (47)	9 (35)	0.62
HDL cholesterol <35 mg/dL	13 (69)	22 (82)	0.38
LDL cholesterol >130 mg/dL	10 (59)	9 (35)	0.08
Diabetes	1 (6)	4 (15)	0.64
Hypertension	5 (29)	14 (54)	0.21
Smoking history	11 (65)	12 (46)	0.38
Therapy, n (%)			
Calcium antagonists	11 (65)	18 (71)	0.81
β-Blockers	5 (29)	4 (15)	0.47
Aspirin	17 (100)	26 (100)	...
Intravenous nitrates	17 (100)	26 (100)	...
Intravenous heparin	17 (100)	26 (100)	...

IHD indicates ischemic heart disease.

duration of ischemic episodes during the first 48 hours and an increase in IL-1Ra or IL-6.

Discussion

Our findings indicate that patients with unstable angina and with worsening disease exhibit a higher cytokine level than patients who do not experience an in-hospital event despite the same medical therapy. In addition, a fall in IL-1Ra and IL-6 after 48 hours was associated with a good outcome, and conversely, an additional increase was associated with a complicated in-hospital course. Others have reported a fall in IL-1Ra and IL-6 in patients with rheumatoid arthritis within 24 hours after initiation of anti–tumor necrosis factor (TNF)-α therapy.[21,22] In patients with septic shock, a fall in IL-6 levels is associated with survival.[23]

Previous Studies

Elevated levels of IL-1 have been described in acute myocardial infarction as soon as 2 hours after symptoms onset and 6 to 9 hours before an increase in IL-6. In addition, elevated levels of IL-1 have been found to be associated in this disease with myocardial dysfunction. Elevated levels of IL-1 have also been described in patients with angina compared with normal subjects. We and others have described elevated levels of IL-6 in unstable angina.[3]

Pathophysiological Implications

IL-1 and IL-6 are related cytokines; IL-1, in particular, is a prototypic proinflammatory cytokine and elicits the production of IL-6, whereas IL-6 does not induce IL-1 production. IL-1 also induces production of nitric oxide, prostaglandins, leukotrienes, and platelet activating factor, each of which is an inflammatory mediator. IL-1 upregulates adhesion molecules on cultured endothelial cells and acts synergistically with bradykinin and other cytokines, particularly IL-6 and TNF-α, producing, among other effects, insulin resistance, IL-8 synthesis by endothelial cells, antigen-induced T-cell generation of IL-2, and hepatic synthesis of acute-phase proteins. At the cardiovascular level, IL-1 induces gene expression for clotting factors and inhibitors of fibrinolysis, transendothelial passage of neutrophils,[4] induction of endothelial adhesion molecules, and induction of granulocyte-macrophage colony stimulating factor and macrophage colony stimulating factor.[24] IL-1 has also been found on macrophage surfaces and in platelet granules[25,26] and may contribute to their role in disease. Taken together, the activities of IL-1 and IL-6 suggest that this cytokine may contribute to the pathogenesis of acute coronary syndromes.

However, it should be emphasized that neither IL-1Ra nor IL-6 per se possesses proinflammatory properties. IL-1Ra is a pure receptor antagonist of IL-1 activity. It is a member of the IL-1 gene family (together with IL-1α and IL-1β) and has no biological activity other than to block IL-1 cell-surface receptors. However, as a member of the IL-1 gene family, its production increases under the same inflammatory conditions that stimulate IL-1α and IL-1β. Because IL-1α and IL-1β lack a signal peptide, these potent proinflammatory cytokines

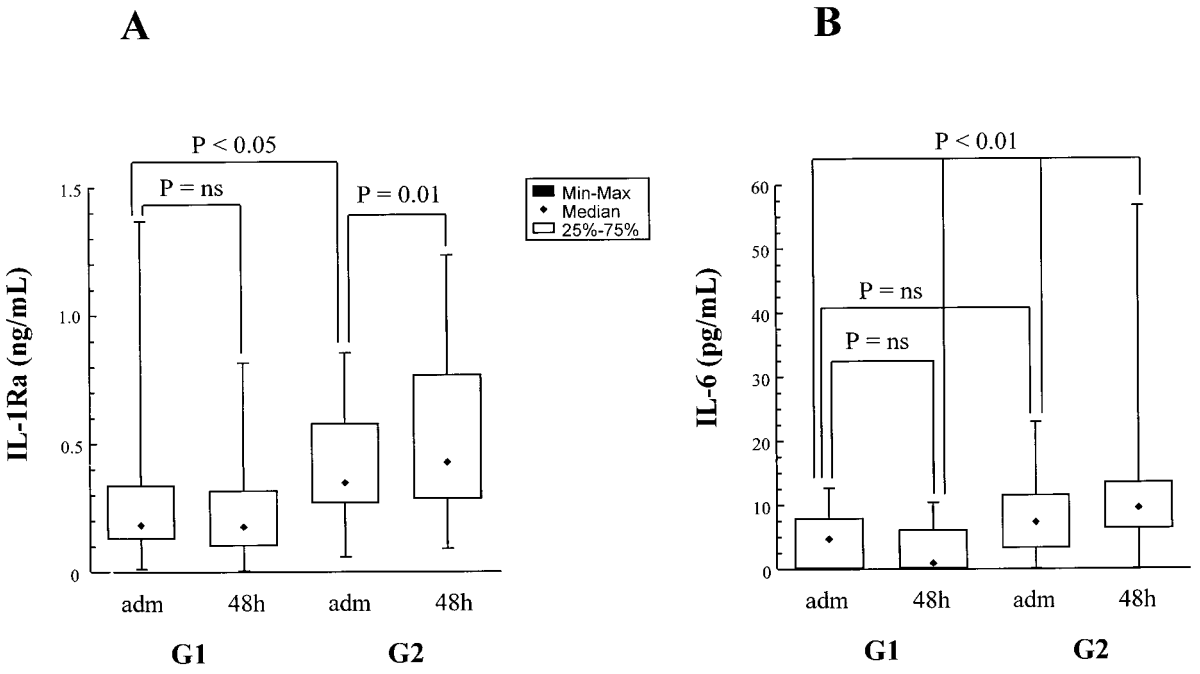

Figure 1. Levels of IL-1Ra (A) and IL-6 (B) at admission (adm) and after 48 hours. Levels are displayed as box and whiskers with median levels (♦), 25% to 75% percentiles, and range. In group 1 (G1), levels of IL-1Ra were higher, but not significantly, at admission than after 48 hours and were significantly lower at any time compared with group 2 (G2). In G2, levels of IL-1Ra at admission, conversely, were significantly lower than at 48 hours. In G1, levels of IL-6 were higher at admission than after 48 hours, but not significantly, and were significantly lower at any time compared with G2 at 48 hours. In G2, conversely, levels of IL-6 at admission were significantly lower than at 48 hours. Min indicates minimum; Max, maximum.

are not readily secreted from the cells into the systemic circulation.[12] Hence, levels of IL-1α and IL-1β in the circulation in patients with infectious or inflammatory disease are often marginal.[27] On the other hand, IL-1Ra has a signal peptide and is readily secreted from cells into the circulation. During experimental endotoxemia in humans, IL-1β increases in the circulation by a factor of 2 to 2.5, whereas IL-1Ra increases by a factor of 10 to 20.[14,15,28] Therefore, measurement of IL-1Ra rather than IL-1α or IL-1β is a more reliable assessment of an increase in production of IL-1 family members in inflammatory diseases.

Although IL-6 is associated with infection and inflammation, and although IL-6 in the circulation often correlates with severity of disease, the injection of large amounts of IL-6 into humans is not associated with hypotension or the systemic symptoms that are observed after the injection of 1000-fold–less IL-1α, IL-1β, or TNF-α. Instead, IL-6 levels indicate the presence of inflammation, and high levels of IL-6 are a poor prognostic sign in many diseases, including unstable angina,[3] but particularly in patients with septic shock and death.[28,29] IL-6 induces acute-phase responses in the liver, produces fever, and stimulates the bone marrow. In the present study, the prognostic values of measuring IL-1Ra and IL-6 appeared to be similar.

In animal studies, IL-6 production is in part under the control of IL-1β.[30–32] In humans, IL-1Ra is regulated by TNF-α.[33] Hence, measurement of IL-1Ra and IL-6 in these patients may

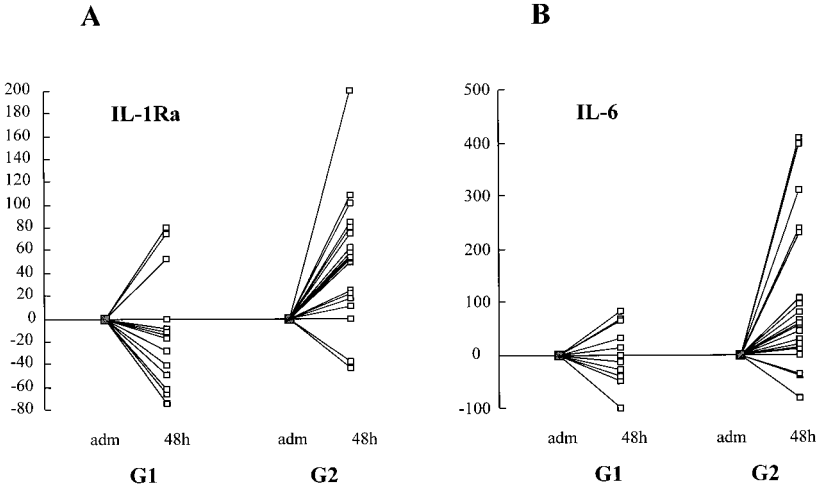

Figure 2. Percent changes in IL-1Ra (A) and IL-6 (B) at 48 hours from baseline levels in group 1 (G1) and group 2 (G2). Percent changes were calculated as (levels at 48 hours−levels at entry)/levels at entry. adm indicates admission.

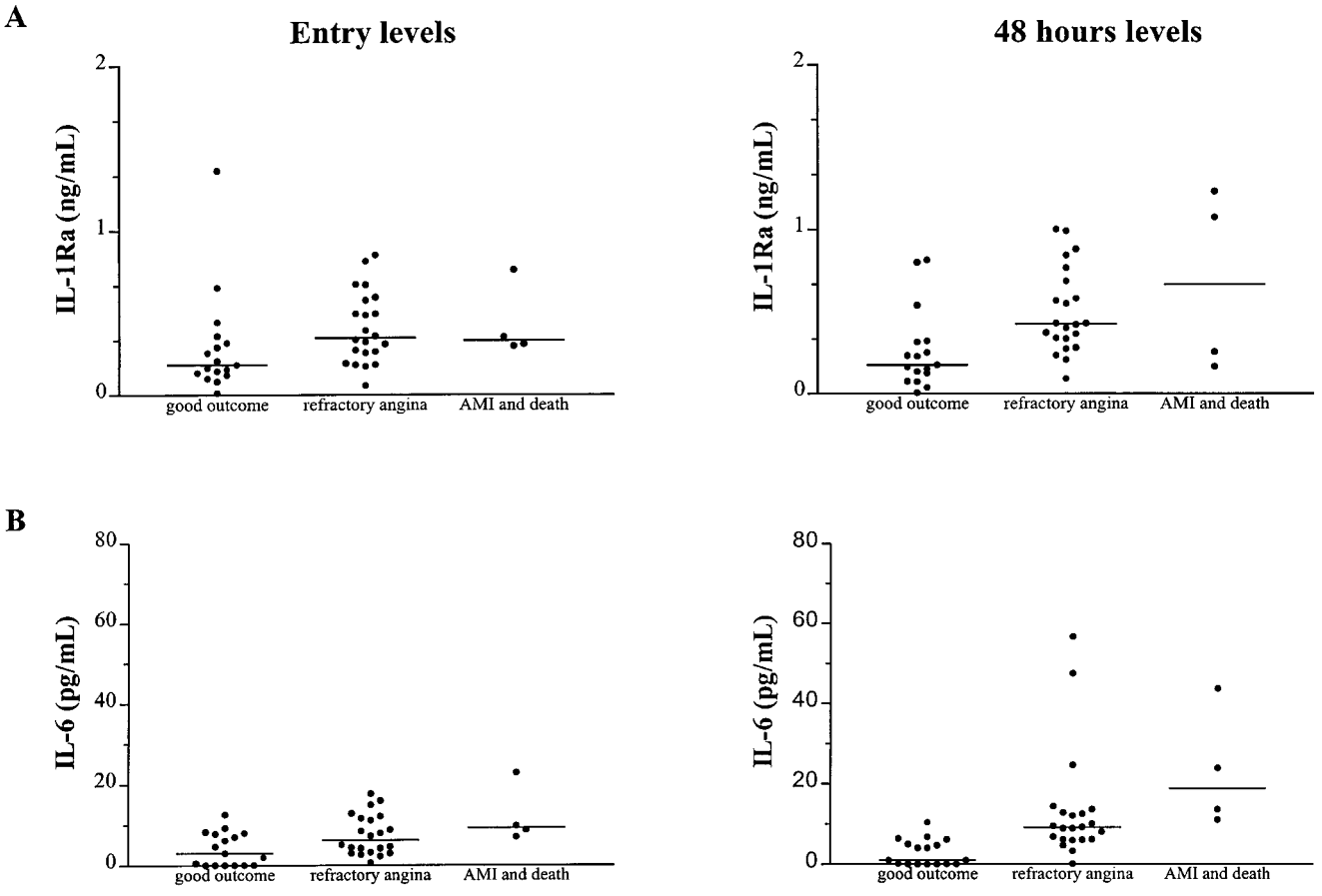

Figure 3. Individual levels of IL-1Ra (A) and IL-6 (B) according to different outcomes: good, refractory angina, and acute myocardial infarction (AMI) or death. Left, Individual levels at entry; right, levels after 48 hours.

be considered a method for determining biologically active IL-1 and TNF in the inflammatory process in the coronary vasculature. The inflammatory process in any tissue is the result of proinflammatory and anti-inflammatory cytokines. In vascular tissue, the levels of soluble cytokine receptors and cytokines such as IL-10 can easily reduce the biological consequence of IL-1 or TNF. Hence, measurement of the biological activity of these cytokines is more relevant than their immunoreactivity. A similar conclusion has been reached in patients with septic shock, in whom it has been proposed that elevated IL-6 levels indicate the presence of biologically active TNF.

What remains unclear is the source of these cytokines, and why with the same treatment, some patients have a dramatic fall in IL-6 levels that is associated with an uncomplicated in-hospital course, whereas others have an increase in IL-1Ra and IL-6 that is associated with a complicated in-hospital course. IL-1 and IL-1Ra can be induced by a variety of stimuli, including viruses, bacteria, and soluble microbial products; this observation is intriguing in light of recent studies demonstrating an association among different infective agents and ischemic heart disease.[34–36] IL-1 may represent a link between viral and microbial infection and ischemic heart disease; in fact, chlamydia infection of human blood monocytes induces the production of IL-1 and IL-8 (C.A. Dinarello, MD, et al, unpublished observations, 1998). The present study, however, was not designed to assess the nature of this link, and hence, the cause for the increase in IL-1 and IL-6 remains unexplained. On the other hand, the increase in IL-1Ra and IL-6 levels after 48 hours in the group of patients with more aggressive disease might be interpreted as a consequence of the disease activity; however, the lack of significant correlations in the present study between ischemic episodes and IL-1Ra or IL-6 levels, our previous observations that ischemic and thrombotic episodes are not sufficient to elicit an acute-phase response,[37,38] and particularly the fact that we studied only patients with negative troponin T during the first 48 hours and thus without sign of detectable myocardial cells damage argue against this possibility.

Clinical Implications

Our findings may open the avenue to novel diagnostic and therapeutic applications. In particular, our findings suggest that blocking of IL-1 and possibly of IL-6 or TNF-α activity might result in a decrease in disease severity and in a more favorable patient outcome. Our findings can also be viewed in light of recent reports of a reduction in cardiovascular events (recurrence of angina, myocardial infarction, and death) in unstable angina and in survivors of myocardial infarction after macrolide antibiotic treatment for *Chlamydia pneumoniae*,[36,39] which also possess strong anti-inflammatory effects.

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Increasing Levels of Interleukin (IL)-1Ra and IL-6 During the First 2 Days of Hospitalization in Unstable Angina Are Associated With Increased Risk of In-Hospital Coronary Events

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