Serum Levels of the Antiinflammatory Cytokine Interleukin-10 Are Decreased in Patients With Unstable Angina

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Background—Proinflammatory cytokines play a role in acute coronary events. However, the potential role of antiinflammatory cytokines in the modulation of the atherosclerotic process remains unknown. Interleukin (IL)-10, which is expressed in human atherosclerotic plaques, has potent deactivating properties in macrophages and T cells. The aim of this study was to assess whether serum concentrations of IL-10 differed between patients with unstable and stable angina pectoris.

Methods and Results—A total of 95 patients with angina pectoris and angiographically documented coronary artery disease were studied. Of these, 50 patients had chronic stable angina (with stable symptoms over 3 months), and 45 patients had Braunwald class IIIB unstable angina with ST-segment changes. Serum IL-10 and IL-6 concentrations were measured on admission using commercially available immunoassays. Serum IL-10 concentrations were lower in unstable angina patients compared with those who had chronic stable angina (28.4 versus 14.0 pg/mL; 95% CI, 9.8 to 19.0; \( P < 0.0001 \)), even after adjustment for variables that were significantly different on univariate analysis. IL-6 concentrations were higher in the unstable angina group (20.9 versus 11.4 pg/mL; 95% CI, 1.0 to 12.6; \( P = 0.04 \)).

Conclusions—Patients with unstable angina had significantly lower serum IL-10 concentrations than did patients with chronic stable angina. This important finding is in keeping with previous data from animal model studies that suggest that IL-10 has a protective role in atherosclerosis. (Circulation. 2001;104:746-749.)

Key Words: interleukins | coronary disease | angina

Dynamic instability of a coronary atherosclerotic plaque is now seen as the foundation for the development of the clinical syndromes we recognize as unstable angina and myocardial infarction. A complex intravascular inflammatory response is an integral component of this dynamic instability.\(^1\)\(^-\)\(^3\) Compared with patients who have chronic stable angina, patients with acute coronary syndromes have coronary plaques with more extensive macrophage-rich areas.\(^4\) Activated macrophages within plaques produce a range of proteases (metalloproteinases) that lead to proteolytic destruction of connective tissue matrix.\(^5\)\(^-\)\(^8\) Vulnerable plaques also have activated T cells that express proinflammatory cytokines such as interferon-\(\gamma\), tumor necrosis factor-\(\alpha\), and interleukin (IL)-1 and IL-6, which affect extracellular matrix collagen production and activate macrophages.\(^3\)

Although proinflammatory cytokines have been shown to play a role in atherogenesis and the development of acute coronary syndromes,\(^9\)\(^-\)\(^12\) little is known of the role of antiinflammatory cytokines in this setting. IL-10, which is produced by various inflammatory cells, especially macrophages,\(^13\) is a major inhibitor of cytokine synthesis, suppresses macrophage function, and inhibits the production of proinflammatory cytokines.\(^13\)\(^-\)\(^14\) IL-10 expression has been identified within human atherosclerotic plaques,\(^15\)\(^-\)\(^16\) with high levels of expression being associated with significantly decreased cell death and iNOS expression.\(^16\) Recent in vitro and in vivo studies in animals have shown a protective role for IL-10 in both atherosclerotic lesion formation and stability.\(^17\)\(^-\)\(^18\) We hypothesized that IL-10 levels may be decreased in patients with plaque instability and acute coronary syndromes compared with chronic stable angina patients, and therefore, we compared serum concentrations of IL-10 in patients with unstable and stable angina.

Methods

Patients
We studied 95 patients (73 of whom were men) who were consecutively admitted to our institution for the assessment of angina chest pain. Of the 95 patients, 50 had chronic stable angina and 45 unstable angina. Stable angina was defined as typical exertional chest pain relieved by rest, glyceryl trinitrate administration, or both, with positive responses to exercise ECG stress testing and \(> 50\%\) diameter stenosis in \(\geq 1\) coronary artery at catheterization. Unstable
angina was defined according to Braunwald, and only patients with class IIIB unstable angina were included in the study. All unstable angina patients had diagnostic ST-segment changes, T-wave inversion, or both, as well as negative cardiac enzymes. No patient included in the study had evidence of ongoing systemic or cardiac inflammatory processes. Blood samples for cytokine and biochemical analysis were taken at the time of admission into hospital. The patients’ baseline clinical characteristics at initial presentation are summarized in the Table.

### Angiographic Analysis
Coronary angiography was carried out according to the Judkins technique, and images of the coronary tree were obtained in routine, standardized projections with the digital Philips Integris 3000 system (Philips Medical Systems International) in all patients. Two experienced cardiologists who had no knowledge of the patients’ clinical characteristics and biochemical results visually reviewed all angiographic images to assess the extent of coronary artery disease. Sullivan’s scoring system was used to assess the extent of atherosclerotic disease in the coronary artery tree, which has been fully described in previous studies from our group. Briefly, this system assesses vessel score, stenosis score, and extension score. Vessel score is based on the number of coronary arteries showing $≥75\%$ stenosis. Stenosis score is aimed at reflecting the most severe stenosis observed in each of the main coronary vessels assessed and is graded from 1 to 4, with grade 4 representing total coronary occlusion. Finally, extension score refers to the proportion of the coronary luminal surface area involved by atheroma.

### Laboratory Analysis
Serum IL-10 concentrations were measured using a high-sensitivity, quantitative sandwich enzyme immunoassay (Quantikine HS, R&D Systems).
IL-10 concentrations in unstable and chronic stable angina groups. Box plot represents the median, quartiles, and extreme values of IL-10 in the stable and unstable angina groups.

Systems Europe Ltd. The lower limit of detection was 0.7 pg/mL. No significant cross-reactivity or interference was observed with recombinant human or recombinant mouse IL-10. Serum IL-6 concentrations were measured using an ELISA immunoassay (OptEIA, PharMingen). The lower limit of detection was 3.4 pg/mL. All other biochemistry measurements were carried out by the analytical unit of the biochemistry department of our institution using standard methods.

Statistical Analysis

Results are presented as mean±1 SD for continuous, normally distributed variables and as percentages for categorical data. Normality was tested using the Kolmogorov-Smirnov test. Continuous variables were analyzed using 2-tailed t test for unpaired observations. Categorical data and proportions were analyzed using χ2 or Fisher’s exact test when required. Multiple logistic regression analysis was used to assess the independent adjusted relationship between IL-10 and type of angina (unstable or stable) with independent variables being those with P<0.05 in univariate analysis. A P value <0.05 was considered statistically significant. The SPSS 8.0 statistical software package (SPSS Inc) was used for all calculations.

Results

Baseline Characteristics

Baseline characteristics of patients with stable and unstable angina are presented in the Table. A similar proportion of patients in the 2 groups had a history of myocardial infarction or previous coronary interventions (coronary artery bypass graft or percutaneous transluminal coronary angioplasty). Established risk factors for coronary artery disease were similar in the 2 groups. Total cholesterol levels did not differ significantly between the 2 groups. The medications taken by both groups at study entry were similar, except for β-blocking drugs (P=0.09) and lipid-lowering agents (P=0.04), both of which were significantly more common in the stable angina group. Angiographic findings were similar in the 2 groups. The chronic stable angina group had slightly higher stenosis scores, and the unstable angina group had slightly higher extension scores, but this did not reach statistical significance.

IL-10 Concentrations

Serum IL-10 concentrations were significantly lower in the unstable angina group compared with the patients who had stable angina (mean difference, 14.4 pg/mL; 95% CI, 9.8 to 19.0; P<0.0001; see the Figure). Multiple logistic regression analysis showed that the difference in IL-10 concentrations between these groups remained statistically significant (P<0.0001) after inclusion of all variables that were statistically significant in univariate analysis (IL-6 concentrations and lipid-lowering medication). IL-6 concentrations were significantly higher in the unstable angina group (mean difference, 8.6 pg/mL; 95% CI, 1.0 to 12.6 pg/mL; P=0.04).

Discussion

Our study showed for the first time that serum IL-10 concentrations are significantly lower in patients with unstable angina compared with patients with stable angina. This suggests that decreased serum IL-10 concentrations are associated with clinical instability. We have also found that serum IL-6 concentrations are significantly higher in patients with unstable angina, as has been reported previously. IL-10 is a potent antiinflammatory cytokine, secreted by lymphocytes of the Th2 subtype and also in large amounts by macrophages. It inhibits many cellular processes that could play an important role in plaque progression, rupture, or thrombosis, including nuclear factor-κB activation, metalloproteinase production, tissue factor and cyclo-oxygenase-2 expression, and cell death. Several studies have shown the release of IL-10 into plasma during human myocardial ischemia/reperfusion injury and during cardiopulmonary bypass. Additionally, IL-10 expression has been demonstrated in both early and advanced human atherosclerotic plaques, with high levels of expression being associated with significantly decreased cell death and iNOS expression.

The results of the present study are in keeping with recent in vitro and in vivo studies in animals, which have suggested a protective role of IL-10 in both atherosclerotic lesion formation and stability. Mallat et al17 reported a significantly increased susceptibility to atherosclerosis in IL-10–deficient C57BL/6J mice. Furthermore, in vivo transfer of murine IL-10 reduced lesion size. These findings were corroborated by Finderski Oslund et al,18 who found that IL-10 transgenic mice, which overexpress IL-10 2- to 4-fold, displayed significantly less atherosclerotic lesion formation than either wild-type or IL-10–deficient mice. Additionally, in vitro experiments from this group showed IL-10 to inhibit the stimulatory effects of oxidized lipids on human aortic endothelial cells, as assessed by monocyte binding. Yang et al24 showed that endogenous IL-10 regulated infarct size and mortality in a mouse myocardial ischemia/reperfusion model. They also demonstrated that IL-10 regulated neutrophil infiltration, intercellular adhesion molecule-1 expression, and production of tumor necrosis factor-α and iNOS after coronary occlusion and reperfusion.

As expected, more patients with stable angina were taking lipid-lowering medication (P=0.04), and there was also a trend toward increased use of aspirin in this group (P=0.10). Both of these medications have antiinflammatory effects and may have influenced the results. However, multiple logistic regression analysis showed that the difference in IL-10 concentrations between the 2 groups remained unchanged (P<0.0001) after inclusion of all variables that were statistically significant in univariate analysis, including IL-6 concentrations and lipid-lowering medication. Cardiac troponin levels were not systematically measured in our patients, and
this limitation precludes an interesting analysis of the relationship between IL-10 and cardiac troponins in this study.

Our investigation represents a small observational study, and it was beyond the scope of the study to assess the relationship between IL-10 and patient outcome. However, our results suggest that reduced levels of IL-10 may favor plaque instability and the development of acute coronary syndromes. In view of the highly significant relationship between IL-10 concentrations and clinical presentation found in this study, further studies in humans are warranted to elucidate the role of IL-10, both as a marker of plaque instability and as a therapeutic agent in unstable angina. Indeed, because of its potent antiinflammatory properties, the therapeutic potential of IL-10 is currently under investigation for a variety of chronic diseases, including rheumatoid arthritis, inflammatory bowel disease, psoriasis, multiple sclerosis, allergic eosinophillic inflammation, Wegener’s granulomatosis, and cardiac allograft rejection.35

Undoubtedly, the association between inflammation, coronary plaque instability, and clinical presentation is extremely complex, and thus the demonstration of reduced concentrations of IL-10 in unstable angina, as shown in our study, does not necessarily indicate that exogenous antiinflammatory cytokine administration might protect from the development of acute coronary syndromes. However, our findings highlight an intriguing relationship between clinical presentation with unstable angina and reduced IL-10 levels that deserve further investigation.

Conclusions
For the first time we have demonstrated a significant association between lower serum concentrations of the antiinflammatory cytokine IL-10 and unstable angina. This association supports previous data from animal studies and suggests that decreased IL-10 concentrations can contribute to atheroma- to ple plaque instability in humans.

References
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