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Elevated Plasma Levels of Interleukin-2 and Soluble IL-2 Receptor in Ischemic Heart Disease

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Summary

Background: T-lymphocytes are present in significant numbers in the atherosclerotic plaque, but their role in the progression and pathogenesis of coronary syndromes remains poorly understood.

Hypothesis: We sought to determine the relationship between T-lymphocyte activation and ischemic heart disease by measuring plasma levels of cytokines related to T-lymphocyte function in patients with stable and unstable angina.

Methods: Plasma levels of interleukin-2 (IL-2) and soluble IL-2 receptor (sIL-2R) were measured in 105 patients: 66 with stable angina, 24 with unstable angina, and 15 healthy controls. Patients who presented to the cardiac catheterization laboratory with unstable or stable anginal syndromes for coronary angiography or percutaneous coronary intervention enrolled in the study.

Results: Mean levels of IL-2 were significantly higher in patients with stable angina than in those with unstable angina. The differences between stable angina and control groups, or between unstable angina and control groups, were not statistically significant. Mean levels of sIL-2R were significantly higher in patients with stable angina than in either patients with unstable angina or control patients.

Conclusions: Levels of IL-2 and sIL-2 receptor are significantly elevated in patients with stable angina, but not in patients with unstable angina. The contribution of T-lymphocytes to the development of both stable and unstable angina requires further investigation.

Key words: interleukins, unstable angina, stable angina

Introduction

Rupture of an atherosclerotic plaque within the coronary circulation is the central event leading to the clinical syndrome of unstable angina.[1,2] Inflammation has been postulated to be the means by which the atherosclerotic plaque first weakens and then ruptures, causing an acute ischemic syndrome.[2] Epidemiologic studies,[3] pathologic[4] and functional[5] examination of atheroma, and analysis of activation states of circulating immune cells[6] all support the role of inflammation in the pathogenesis and progression of coronary atherosclerosis and acute coronary syndromes. Circulating levels of acute-phase reactants, such as C-reactive protein and serum amyloid A,[7] and other inflammatory markers have been demonstrated to have a close relationship to clinical outcomes in unstable coronary syndromes[8] and in outcomes after coronary intervention.[9]

T-lymphocytes are found in every developmental stage of the atherosclerotic plaque[10] and constitute between 10 and 22% of total cells in the atheroma.[11] T-cell lymphokines modulate the induction of vascular smooth muscle cell (SMC) tissue plasminogen activator production and SMC migration by both platelet-derived growth factor[12] and basic fibroblast growth factor.[13] Most T-lymphocytes found in human atherosclerotic plaques are memory T-cells in a state of late or chronic activation as evidenced by their expression of surface antigens such as HLA-DR,[14] CD45RO, and the VLA-1 integrin.[15]

To test the hypothesis that different degrees of T-cell activation exist in patients with stable versus unstable angina, we examined circulating plasma levels of interleukin-2 (IL-2) and soluble interleukin-2 receptor (sIL-2R) in patients with stable and unstable angina. We selected IL-2 and sIL-2R as the focus of this study because of a large body of evidence that the actions of IL-2 on its cell surface receptor are critical for T-cell activation.[16] Levels of sIL-2R are also a sensitive marker of immune activation, both systemically and in other tissue and fluid compartments.[17,18] In this study, we demonstrate that elevated circulating levels of IL-2 and sIL-2R are present in patients with stable angina compared with patients with unstable angina.

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Methods

Patient Population

The enrollment of patients in this study was approved by the Institutional Review Board of the Columbia-Presbyterian Medical Center, and was in accordance with the Declaration of Helsinki. We studied a total of 105 patients (79 men, 26 women) aged 59 ± 12 years. Of this group, 24 were patients with unstable angina scheduled for cardiac catheterization. Inclusion criteria were angina at rest with ST changes diagnostic of ischemia during anginal episodes, a normal creatine phosphokinase (CPK), or if the creatine kinase (CK)-MB fraction was positive, a non-Q-wave myocardial infarction (MI). Patients with no electrocardiographic (ECG) documentation during anginal attacks were enrolled if they had a history of coronary artery disease and a clinical syndrome consistent with unstable angina. To reduce the effect of a recent ischemic or thrombotic episode on the markers to be studied, at least 12 h between the last anginal episode and enrollment was required. Exclusion criteria included left ventricular ejection fraction (LVEF) < 40%, acute Q-wave MI, acute or chronic infectious or immunologic conditions, neoplastic disease, renal failure, glucocorticoid or myelosuppressive therapy, or recent coronary artery bypass graft (CABG), percutaneous transluminal coronary angioplasty (PTCA), surgery, or gastrointestinal bleeding.

During the same period, 66 patients with chronic stable angina scheduled for elective angioplasty were enrolled. At least 24 h between the last anginal episode and enrollment was required, and the same exclusion criteria applied. Fifteen control patients were recruited from healthy individuals with no history of coronary artery disease and from patients undergoing catheterization that demonstrated no angiographic disease.

Study Design

Prior to angiography, blood samples were drawn from the central venous catheter placed at the beginning of the procedure. The specimens were centrifuged at 2000 rpm and 3°C for 20 min. The plasma was then separated and frozen at −70°C. Enzyme-linked immunosorbent assays (ELISA) utilizing monoclonal antibodies to IL-2 and sIL-2R (Biosource International, Inc. Camarillo, Calif., USA) were performed in a blinded fashion. The upper and lower detection limits of the IL-2 assay were 1000 and 8.7 pg/ml, respectively. The corresponding values for the sIL-2R assay were 8000 and 32 pg/ml, respectively.

Statistical Analysis

Nonparametric testing was used for statistical tests of significance. A Kruskal-Wallis test was used to compare the means between the three groups, and a Mann-Whitney U test was used to compare differences between subgroups. Results are expressed as mean and standard error of the mean (SEM). A p value of < 0.05 was considered to be significant.

Results

Demographic, clinical, and angiographic characteristics for the patients studied are described in Table I. The stable and unstable angina groups were well matched, except for the more frequent use of heparin, beta blockers, and nitrates in patients with unstable angina. Figure 1 shows the IL-2 assay results. Levels of IL-2 were highest in the 66 patients with stable angina: mean 249 ± 47 pg/ml. The 24 patients with unstable angina had a mean IL-2 level of 66 ± 42 pg/ml, and the 15 control patients had a mean IL-2 level of 77 ± 33 pg/ml. These differences were significant at the $p = 0.01$ level by the Kruskal-Wallis test. The difference between the stable and unstable angina groups was significant at the $p = 0.003$ level. The differences between stable angina and control groups, or between unstable angina and control groups were not statistically significant ($p = 0.49$ and $p = 0.08$, respectively).

Figure 2 describes the sIL-2R assay results. Patients with stable angina had the highest sIL-2R levels: mean 1531 ± 96 pg/ml. Patients with unstable angina had a mean level of 1213 ± 88 pg/ml, and control patients a mean level of 1090 ± 127 pg/ml. These differences were significant at the $p = 0.01$ level

TABLE I Patient characteristics

	Control n = 15	Stable n = 66	Unstable n = 24	p Value (stable vs. unstable)
Age, years (mean ± SD)	45 ± 16	62 ± 10	58 ± 9	0.04
Sex, M/F	9/6	55/10	15/9	NS (>0.05)
Risk factors, n (%)				
Smoker	5 (33)	44 (69)	18 (75)	NS
Hypertension	6 (40)	45 (70)	17 (71)	NS
Diabetes	2 (13)	20 (31)	5 (21)	NS
Hyperlipidemia	7 (46)	36 (56)	14 (58)	NS
Family history of CAD	7 (46)	43 (67)	11 (46)	NS
Prior MI	0	19 (30)	12 (50)	NS
Prior CABG	0	11 (17)	1 (4)	NS
Medications, n (%)				
Aspirin	7 (46)	61 (95)	24 (100)	NS
Heparin	5 (33)	21 (31)	21 (88)	< 0.0001
Beta blockers	7 (46)	41 (64)	23 (96)	0.003
Nitroglycerin	4 (27)	26 (41)	23 (96)	< 0.0001
Ca blockers	1 (7)	33 (55)	10 (42)	NS
ACE inhibitor	3 (20)	13 (20)	4 (17)	NS
Lipid lowering	3 (20)	22 (34)	7 (29)	NS
LVEF (%)	62	55	58	NS
Diseased vessels		2.1	2.3	NS
Stenoses > 70%		2.2	2.7	NS

Abbreviations: SD = standard deviation, M = male, F = female, CAD = coronary artery disease, MI = myocardial infarction, CABG = coronary artery bypass graft, Ca = calcium, ACE = angiotensin-converting enzyme, LVEF = left ventricular ejection fraction, NS = not significant.

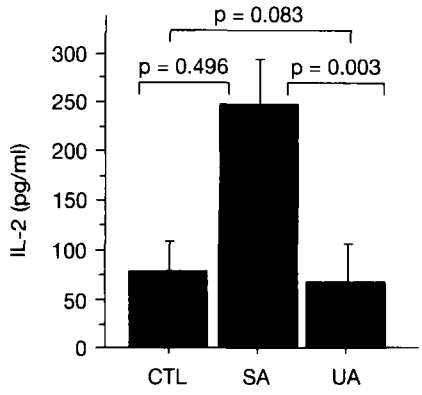

FIG. 1 Levels of interleukin-2 (IL-2) in picograms per milliliter in control patients (CTL), patients with stable angina (SA), and patients with unstable angina (UA).

by the Kruskal-Wallis test. Subgroup analysis showed that the difference between the stable and unstable angina groups was significant at the $p = 0.03$ level, and between the stable angina and control groups at the $p = 0.02$ level. The difference between unstable angina versus control groups was not statistically significant ($p = 0.37$).

Discussion

In this study, we report that T-cell activation, as reflected by levels of IL-2 and soluble IL-2 receptor, differs in patients with stable versus unstable angina. Our study is consistent with the previous finding that soluble IL-2 receptor levels are elevated in patients with stable angina presenting for elective coronary angioplasty.[9] Our results confirm these findings and extend them by the additional finding that these elevations are not present in patients with unstable angina. Indirect support for our results can also be found in pathologic studies describing plaque inflammation, particularly in those that included samples from a variety of patients and anatomic sites.[14, 19, 20]

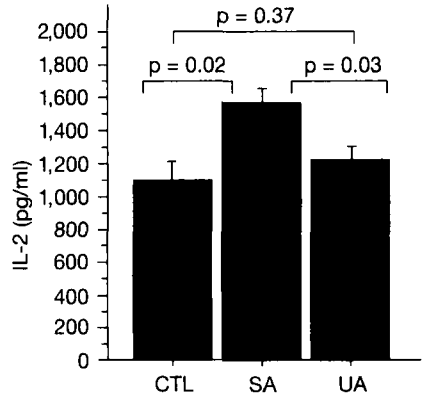

FIG. 2 Levels of soluble IL-2 receptor (sIL-2R) in picograms per milliliter in control patients (CTL), patients with stable angina (SA), and patients with unstable angina (UA).

One prior study which relates T-lymphocyte activity to unstable coronary syndromes[6] was an ex vivo analysis using measurements of thrombosis and coagulation as the indicators of immune activation. Thus, it is not surprising that their particular study design would find positive results in unstable angina, a syndrome in which the activity of the coagulation system is well documented. Moreover, the latter study examined T-lymphocyte activation in general, without focusing on heterogeneous T-cell subsets. Pathologic studies of coronary atheroma have demonstrated the T-lymphocytes in situ to be of polyclonal origin[21] and of multiple subtypes.[22] It is conceivable that different T-cell subsets have disparate effects on plaque stability, and thus disparate effects on clinical outcomes. A recent study of atherectomy specimens reveals an increase in the percentage of IL-2R positive T-lymphocytes in culprit lesions of patients with acute ischemic syndromes.[23] However, Stemme *et al.* demonstrated that IL-2 receptor expression is low in atherosclerotic plaque T-cells, and that the frequency of IL-2R(+) cells in the plaque does not differ significantly from that in peripheral blood.[15]

Furthermore, the fact that these cytokines are elevated systemically does not necessarily prove a significant cytokine paracrine activity within the microenvironment of the atheroma in the vessel wall at the site of plaque rupture. It is conceivable that elevated levels of IL-2 and sIL-2R in the plasma may be associated with either increased or decreased levels within the vessel wall. Maseri has noted that systemic markers of T-lymphocyte activation may not correlate directly with the atherosclerotic plaque and may reflect another concomitant inflammatory or infectious condition.[24] Further histologic studies are needed to determine the degree of T-cell activation in the vessel wall, as well as the presence, localization, and activity of various T-cell subsets in stable versus unstable angina.

T-lymphocytes possess the ability to produce factors that can be expected to favor plaque stability as well as those that favor plaque rupture. They produce lymphokines that modulate vascular SMC proliferation and migration[12] as well as macrophage activity,[25, 26] and the T-cell lymphokine gamma interferon inhibits scavenger receptor expression and macrophage foam cell formation.[25, 26] Furthermore, T-cell induction of vascular SMC proliferation results in greater extracellular matrix production and plaque fibrosis, an important determinant of plaque stability.[2, 27] Smooth muscle cell proliferation and plaque fibrosis may also be favored by the production of the SMC mitogens heparin-binding epidermal growth factor and basic fibroblast growth factor, both of which are produced by T-lymphocytes found in atherosclerotic plaques.[28] In this regard, it should be noted that the region of the advanced atherosclerotic plaque that possesses the highest percentage of T-cells is the fibrous cap.[29]

Limitations

The use of coronary angiography alone to define control patients, given the relative insensitivity of this technique to detect early atherosIcerosis, may have resulted in some patients mis-

takenly defined as controls who in fact had subclinical atherosclerosis. This may explain why a significant difference was found in IL-2 levels between stable versus unstable angina, but not in patients with stable angina versus "normal" patients.

The use of only two serum markers is a limited characterization of a complex immunologic interaction and communication between multiple cell lines and subtypes. This study cannot answer whether a particular T-cell subtype is responsible for the differences demonstrated here.

As the patients studied have different clinical syndromes, there are significant differences in the medical therapies in use at the time of study entry. In particular, our patients with unstable angina were treated more intensively, with more frequent use of heparin, beta blockers, and nitroglycerin at the time of enrollment. Heparin has been demonstrated to interfere with migration and effector functions of lymphocytes in animal models.[30] Therefore, the more frequent use of heparin in our patients with unstable angina may have resulted in a heparin-mediated decrease in plasma IL-2 and IL-2R levels. Variation in the use of various anti-ischemic therapies, as well as low sample sizes, may also account for the results of a recent study that found no differences in acute-phase IL-2 levels in patients with unstable versus stable angina.[31]

Conclusions

This study demonstrates that levels of interleukin-2 and the soluble IL-2 receptor are significantly elevated in patients with stable angina, but not in patients with unstable angina. The process by which T-lymphocytes may exert influence on the evolution of the atherosclerotic plaque, and the contribution of T-lymphocytes to the pathogenesis of the acute ischemic syndromes, remain topics for future research.

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