Objective - To compare circulating plasma levels of immunoinflammatory markers in patients with known de novo coronary artery disease and patients with postangioplasty restenosis.

Methods - Using enzymatic immunoabsorbent assay, we measured plasma levels of soluble interleukin-2 receptors, tumor necrosis factor alpha, and soluble tumor necrosis alpha receptors I and II in 11 patients with restenosis postcoronary angioplasty (restenosis group), in 10 patients with primary atherosclerosis (de novo group) who were referred for coronary angiography because of stable or unstable angina, and in 9 healthy volunteers (control group). Levels of soluble interleukin-2 receptors were significantly higher in the de novo group compared with that in the restenosis and control groups. Levels were also higher in the restenosis group compared with that in the control group. Plasma levels of tumor necrosis alpha and receptor levels were significantly higher in the de novo group compared with that in the restenosis and control groups, but levels in the restenosis group were not different from that in the controls.

Conclusion – Coronary artery disease, either primary or secondary to restenosis, is associated with significant immunoinflammatory activity, which can be assessed by examining the extent of circulating plasma levels of inflammatory markers. Moreover, patients with de novo lesions appear to have increased inflammatory activity compared with patients with restenosis.

Keywords: coronary artery disease, inflammation, tumor necrosis factor alpha, interleukin-2
sentation was classified as stable or unstable angina class IB, IIB, or III B according to Braunwald’s criteria. Use of cardiac drugs as prescribed by the attending cardiologists was recorded. Exclusion criteria were: systemic inflammatory disease, heart failure, ejection fraction less than 40%, decompensated diabetes mellitus, current use of antiinflammatory (except for aspirin) or immunosuppressive drugs, acute myocardial infarction or surgery in the last three months, systemic infections, or neoplasia. To minimize chances of asymptomatic coronary artery disease, the control group was formed by subjects under 35 years of age, with no symptoms or signs of cardiac disease, absence of risk factors for coronary artery disease, and no significant medical history. They did not undergo coronary angiography. The study protocol was previously approved by the Ethics and Research Committee of Hospital de Clínicas de Porto Alegre, and we obtained written informed consent from all subjects prior to enrollment.

Clinicians used Judkin’s technique to perform coronary arteriography on the patients. The presence and severity of coronary angiographic lesions were evaluated from at least three projections by one investigator who was blinded to the clinical characteristics of the patients. Coronary restenosis was defined as the recurrence of a 50% stenosis in the site of a previously dilated vessel. Patients in the group of de novo coronary atherosclerosis had a 70% stenosis in at least one coronary artery. The number of affected vessels was also recorded.

Patients had 20 mL of blood drawn from a venous site to an EDTA-containing tube immediately prior to coronary angiography. Control subjects had blood drawn in a quiet laboratory room. Samples were immediately centrifuged at 2000 rpm for 10 minutes, and the plasma was stored at -20°C for up to 6 months for future combined analysis. Enzyme-linked immunoabsorbent assays were then run using duplicate samples, to minimize interassay variability, to measure plasma circulating levels of soluble interleukin-2 receptors (sensitivity <6 pg/mL; range 78.1 – 5000 pg/mL), tumor necrosis alpha (sensitivity <4.4 pg/mL; range 15.6 – 1000 pg/mL), soluble receptor I of tumor necrosis alpha (sensitivity <3 pg/mL; range 7.8 – 500 pg/mL), soluble receptor II of tumor necrosis alpha (sensitivity <1 pg/mL; range 7.8 – 500 pg/mL) (R&D Systems, Minneapolis). Lipid profile, creatine phosphokinase (CPK), and MB fraction were also measured in all subjects.

Continuous variables are expressed as means ± SD. Tumor necrosis alpha was not normally distributed and is presented as median [range]. Differences among the three groups were analyzed by ANOVA with Scheffe’s procedure, except for tumor necrosis alpha, for which the Kruskall-Wallis test was used. Associations among variables were evaluated using Pearson’s correlation coefficient or Spearman’s rank-order correlation coefficient, and differences between groups of patients were analyzed by the Students t test or Fisher’s exact test for categoric variables. A p value of <0.05 was considered significant for all tests. Linear regression models were used to analyze variables adjusting for baseline clinical differences among groups.

Results

Characteristics of patients and control subjects are shown in the Table I. According to inclusion criteria, normal individuals were significantly younger than both groups of patients. Unstable angina accounted for the totality of cases in the de novo lesions group, but only 7/11 patients in the restenosis group had unstable angina; however, this was not statistically significant. The time from the last episode of angina in the group of patients with restenosis was significantly shorter than that of patients with de novo lesions (77±214 versus 124±144 hours; p<0.05). No statistically significant differences occurred regarding the use of cardiovascular drugs, which included beta-blockers, aspirin, nitrates, calcium channel blockers, ticlopidin, or intravenous heparin. The lipid profile was also similar between the two groups of patients, and no change in cardiac enzyme profiles were observed. The mean elapsed time from coronary angioplasty in the group of patients with restenosis was 165±35 days.

Significantly more patients in the de novo group presented with one vessel disease compared with the restenosis group (Table I). Other angiographic characteristics, such as proximal left anterior descending artery involvement and severity of stenosis, were not significantly different between the groups.

Individual data as well as mean values for immunoinflammatory markers are presented in the Figure 1. Levels of soluble interleukin-2 receptors were significantly higher in the group of patients with de novo coronary lesions compared with that in normal controls and patients with restenosis (2283±542 pg/mL, 1640±576 pg/mL and 796±470 pg/mL, respectively, p<0.05). In addition, soluble interleukin-2 receptor levels were also higher in the restenosis group compared with levels in the normal controls. Plasma levels of tumor necrosis alpha were significantly higher in patients with de novo lesions [0.65 pg/mL (0-3.0 pg/mL)] compared with that in the restenosis group [0 pg/mL (0-2.8 pg/mL); p<0.05] and compared with that in the normal controls [0 pg/mL (0-0.3 pg/mL); p<0.05]. Circulating levels of soluble tumor necrosis alpha receptor I were also significantly higher in the group of patients with de novo lesions compared with that in patients with restenosis (1223±194 pg/mL versus 1021±108 pg/mL; p<0.05) and compared with that in normal controls (888±162 pg/mL; p<0.05). As for circulating levels of soluble tumor necrosis alpha receptor II, values were significantly higher in the group of patients with de novo lesions compared with that in patients with restenosis (2958±716 pg/mL versus 2267±447 pg/mL; p<0.05) and compared with that in normal controls (2123±345 pg/mL; p<0.05). Taken together, these results demonstrate increased levels of these inflammatory markers in the group of patients with de novo lesions compared with that in both other groups. In addition, levels of these markers observed in patients with restenosis were not statistically different compared with levels in the normal controls, except for soluble interleukin-2 receptor levels. Significant correlations between tumor necrosis alpha and soluble tumor necrosis alpha receptor I (r = 0.6; p<0.05) and

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between tumor necrosis alpha and soluble tumor necrosis alpha receptor II (r = 0.6; p < 0.05) were observed. Similarly, soluble receptor levels correlated with each other (r = 0.85; p < 0.05). After adjustment for differences in age among groups, results remained unchanged.

**Discussion**

Atherosclerosis can be considered an immunoinflammatory process evolving over a number of years according to the presence or absence of risk factors and genetic background. Restenosis can be viewed as an accelerated form of this process with associated wound-healing characteristics. In this study, we demonstrated an enhanced state of inflammatory activity detected in the plasma of patients presenting with coronary artery disease syndromes secondary to both de novo or restenotic lesions compared with that in the normal controls.

Patients enrolled in this study constituted a typical group of individuals referred by their attending cardiologists for coronary angiography for known or suspected coronary artery disease. No major clinical or laboratory differences were observed between the two groups of patients, except for an increased number of patients presenting with unstable angina in the group with de novo lesions. Although this difference did not reach statistical significance, this is in keeping with the notion that, overall, restenotic lesions are less likely to cause unstable syndromes. Use of medications was also similar in both groups at the time of blood sampling, except for two patients receiving heparin intravenously in the de novo atherosclerosis group.

Atherogenesis can be viewed as an inflammatory process in which vascular cells can play important roles mediating several immunoinflammatory mechanisms secondary to endothelial injury. Endothelial cells express adhesion molecules in their surface that will induce T cells and monocytes/macrophages to adhere to the vascular endothelial surface and subsequently migrate to the subendothelial space. Increased plasma levels of different inflammatory media-

| Table I - Clinical and angiographic characteristics of the study population |
|-----------------------------|-----------------------------|-----------------------------|
|                            | De Novo (n=10)              | Restenosis (n=11)           | Controls (n=9)             |
| N                           | 10                          | 11                          | 9                          |
| Age (years)                 | 64 ± 6                      | 58 ± 13                     | 28 ± 4 **                  |
| Gender (male)               | 6                           | 7                            | 10                         |
| Unstable angina (n)         | 10                          | 7                            | 7                          |
| Previous acute myocardial   |                            |                              |                            |
| infarction (n)              | 1                           | 5                            |                            |
| LDL-Cholesterol (mg/dl)     | 123 ± 28                    | 141 ± 47                    | 121 ± 39                   |
| HDL-Cholesterol (mg/dl)     | 41 ± 9                      | 38 ± 9                      | 42 ± 9                     |
| Severity of stenosis (%)    | 97 ± 4                      | 88 ± 14                     |                            |
| One vessel disease (n)      | 9                           | 2                            |                            |
| Proximal LAD involvement (n)| 5                           | 3                            |                            |

LAD: left anterior descending artery; * p < 0.05 between both group of patients; ** p < 0.05 between controls and both group of patients. Values are expressed as absolute numbers or mean ± SD.
reaction in this group of patients, offsetting a potential increased inflammatory activity expected to be present in patients with restenotic lesions.

Our data should be interpreted in the light of specific study limitations. First, our limited sample size may not support definitive answers, but regardless of the number of study individuals, our data collectively show a consistent pattern of heightened inflammatory activity in the de novo group as opposed to the restenosis group. Second, we chose a control group that was significantly younger than both other groups. While this may have influenced our analysis, interleukin 2 soluble receptor levels may correlate with age only up to 4 years when the immune system is still being developed. Tumor necrosis factor alpha was not influenced by age in similar studies. Third, as mentioned above, our data may not reflect intraplaque events, because only peripheral blood was studied. It can be speculated that blood sampling from the coronary sinus could more appropriately identify differences occurring inside the atherosclerotic plaque. Finally, because coronary syndromes can be complex, it would be interesting to investigate patients presenting with clinical syndromes as comparable as possible (all stable angina or all unstable angina patients with primary and restenotic lesions).

In conclusion, our study reinforces the notion that coronary artery disease is associated with a pattern of immunoinflammatory activity and suggests that this can be assessed by measuring plasma levels of tumor necrosis alpha and its receptors and soluble interleukin-2 receptors. Our data also indicate significant increases in circulatory levels of these markers in patients with primary atherosclerosis when compared with that in patients with restenotic lesions or in normal controls. Finally, a moderate degree of immunoinflammatory activity was shown in patients with restenosis as increased levels of soluble interleukin-2 receptors only were observed in this group compared with controls.

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