Systemic Inflammatory Parameters in Patients With Atherosclerosis of the Coronary and Peripheral Arteries

Michael Erren, Holger Reinecke, Ralf Junker, Manfred Fobker, Helmut Schulte, Josef O. Schurek, Jürgen Kropf, Sebastian Kerber, Günter Breithardt, Gerd Assmann, Paul Cullen

Abstract—Plasma concentration of markers of inflammation are increased in patients with atherosclerosis. However, it is unclear whether the pattern and magnitude of this increase vary with the site and extent of disease. In 147 patients undergoing semiquantitative coronary angiography, we measured the acute-phase reactants C-reactive protein (CRP) or serum amyloid A (SAA); the proinflammatory cytokine interleukin 6 (IL-6); the active and total fractions of the anti-inflammatory cytokine transforming growth factor-β (TGF-β); the macrophage activation marker neopterin; and the infection marker procalcitonin. Compared with 62 patients without either coronary artery disease (CAD) or peripheral artery disease (PAD), 57 patients with CAD but no PAD showed greater median CRP (0.4 versus 0.2 mg/dL, \(P=0.004\)) and IL-6 (3.8 versus 1.6 pg/mL, \(P=0.007\)) levels and a lower level of active-TGF-β (57 versus 100 ng/mL, \(P=0.038\)). Moreover, CRP, IL-6, and neopterin levels showed a positive and the active TGF-β level a negative correlation with the extent of coronary atherosclerosis. Compared with these 57 patients with CAD alone, 15 patients with PAD and CAD had higher median levels of SAA (17 versus 7 mg/mL, \(P=0.008\)), IL-6 (12 versus 4 pg/mL, \(P=0.002\)), neopterin (14 versus 11 mg/dL, \(P=0.006\)), and total TGF-β (11834 versus 6417 ng/L, \(P=0.001\)). However, these strong univariate associations of markers of inflammation and atherosclerosis were lost in multivariate analysis once age, sex, and high density lipoprotein cholesterol or fibrinogen were taken into account. Increased plasma levels of CRP, SAA, IL-6, TGF-β, neopterin, and procalcitonin constitute an inflammatory signature of advanced atherosclerosis and are correlated with the extent of disease but do not provide discriminatory diagnostic power over and above established risk factors. (Arterioscler Thromb Vasc Biol. 1999;19:2355-2363.)

Key Words: atherosclerosis ■ coronary artery disease ■ peripheral artery disease ■ markers of inflammation ■ acute-phase response

For some time, evidence has been accumulating that inflammation plays an important role in both the initiation and progression of atherosclerosis of the coronary, carotid, and peripheral arteries.1-3 For example, levels of C-reactive protein (CRP), an acute-phase reactant, >0.3 mg/dL but still within the reference range, have been found to predict unstable angina pectoris, myocardial infarction, and sudden cardiac death.4 In addition, 2 recent reports from the Physicians’ Health Study indicate that in apparently healthy men, even slight increases in CRP above this cut-point increase the risk for future myocardial infarction, stroke, and peripheral artery disease (PAD).5,6 Serum amyloid A (SAA), an apolipoprotein predominantly bound to HDL, is an acute-phase reactant that indicates systemic inflammatory activity with at least the sensitivity of CRP.7 SAA levels were also reported to be increased in coronary atherosclerosis.8 A third acute-phase reactant of interest is fibrinogen.9,10 Numerous workers have reported an increase in fibrinogen in atherosclerosis,11,12 but it is unclear whether this elevation is mainly a cause or a result of the disease, although both may be true. The acute-phase response is under tight control of interleukin 6 (IL-6),13 which is produced to a large extent by macrophages.14 Expression of IL-6 has been demonstrated in atherosclerotic plaques,14-17 but little information exists on circulating levels of IL-6 in patients with atherosclerosis of the coronary or peripheral arteries.

In recent years, numerous experimental studies have demonstrated an important pathophysiological role of the cytokine transforming growth factor-β (TGF-β) in the pathogenesis of atherosclerosis.18-21 Clinical studies have concentrated on both the active and total fractions of this cytokine. One study found that serum levels of active TGF-β were much lower in patients with atherosclerosis of all 3 coronary arteries than in persons without coronary atherosclerosis, with no overlap between these groups.22 In another study, increased plasma concentrations of active TGF-β were associated with the presence of coronary artery disease (CAD).23

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The role of latent bacterial and viral infections in the pathogenesis of atherosclerosis has been the subject of much recent debate.\textsuperscript{24} Neopterin, a specific marker of macrophage activation,\textsuperscript{25} is commonly used to screen for subclinical viral infections 26 and has been found to be elevated in the circulation of patients with atherosclerosis of the carotid,\textsuperscript{27} coronary,\textsuperscript{28} and peripheral\textsuperscript{29} arteries. Another newer marker of atherosclerosis is the peptide hormone procalcitonin (PCT). An elevated level of PCT is thought to indicate systemic exposure of the body to bacterial endotoxins and/or exotoxins.\textsuperscript{30} At present, it is unknown whether microbially induced atherosclerosis is associated with elevated levels of PCT.

Despite the studies performed to date, it is not known whether the pattern of inflammation parameters differs between atherosclerosis of the coronary and peripheral arteries. In addition, the mechanisms relating the increased levels of markers of inflammation to atherosclerosis are, in general, unknown. We therefore undertook the present study in 147 consecutive patients undergoing semiquantitative coronary angiography to address the following questions: (1) Is there a consistent pattern of markers of inflammation in human atherosclerosis? (2) If so, does the pattern of markers in CAD differ from that in PAD? (3) Are the plasma concentrations of markers of inflammation correlated with the extent of atherosclerotic disease? (4) To what extent are markers of inflammation correlated with each other and/or with metabolic risk markers of atherosclerosis?

**Methods**

Patients

All patients admitted to our university hospital for elective coronary angiography in the months of May and June 1997 were screened for inclusion in the study. Patients were excluded if they were critically ill or hemodynamically unstable, if they had undergone surgery or suffered a myocardial infarction within the previous 3 months, if they were receiving drugs (except heparin) parenterally, or if they were critically ill or hemodynamically unstable, if they had undergone surgery or suffered a myocardial infarction within the previous 3 months, if they were receiving drugs (except heparin) parenterally, or if they had a stenosis of ≥50% of the left main stem coronary artery.

Unstable angina pectoris was defined as pain at rest with at least 2 episodes during the previous 48 hours, at least 1 of which lasted >20 minutes, or ST-segment deviations that were diagnostic of myocardial ischemia during anginal attacks, with no elevation of the MB fraction of creatine kinase or lactate dehydrogenase on admission to hospital.

**TABLE 1.** Demographic and Clinical Characteristics of Patients

<table>
<thead>
<tr>
<th></th>
<th>All Patients</th>
<th>No CAD, No PAD</th>
<th>CAD, No PAD</th>
<th>CAD, PAD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>147</td>
<td>62</td>
<td>57</td>
<td>15</td>
</tr>
<tr>
<td>Male sex, %</td>
<td>73</td>
<td>56</td>
<td>88‡</td>
<td>73</td>
</tr>
<tr>
<td>Age, y</td>
<td>59±12</td>
<td>56±13</td>
<td>61±10⁺</td>
<td>63±9§#</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>26±4</td>
<td>26±5</td>
<td>26±4</td>
<td>27±4</td>
</tr>
<tr>
<td>Risk factors for CAD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive family history for cardiovascular disease, %</td>
<td>33</td>
<td>39</td>
<td>32</td>
<td>20</td>
</tr>
<tr>
<td>Blood pressure &gt;140 mm Hg systolic and/or 90 mm Hg diastolic, %</td>
<td>51</td>
<td>34</td>
<td>54*</td>
<td>80‖</td>
</tr>
<tr>
<td>Current Smokers, %</td>
<td>21</td>
<td>15</td>
<td>26</td>
<td>20</td>
</tr>
<tr>
<td>Diabetes mellitus, %</td>
<td>26</td>
<td>15</td>
<td>32</td>
<td>60#</td>
</tr>
<tr>
<td>History of CAD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Angina pectoris, %</td>
<td>63</td>
<td>39</td>
<td>77†</td>
<td>100*⁺⁺⁺</td>
</tr>
<tr>
<td>Unstable angina pectoris, %</td>
<td>4.8</td>
<td>0</td>
<td>8.7⁺</td>
<td>13**</td>
</tr>
<tr>
<td>Previous myocardial infarction, %</td>
<td>34</td>
<td>11</td>
<td>54‡</td>
<td>27‖</td>
</tr>
<tr>
<td>Previous coronary angioplasty, %</td>
<td>31</td>
<td>15</td>
<td>42†</td>
<td>27</td>
</tr>
<tr>
<td>Previous coronary artery bypass grafting, %</td>
<td>14</td>
<td>0</td>
<td>18†</td>
<td>47⁺⁺⁺⁺</td>
</tr>
<tr>
<td>Receiving statins, %</td>
<td>32</td>
<td>15</td>
<td>40†</td>
<td>67⁺⁺⁺⁺</td>
</tr>
<tr>
<td>Receiving acetylsalicylic acid, %</td>
<td>54</td>
<td>27</td>
<td>81‡</td>
<td>47†</td>
</tr>
<tr>
<td>Receiving phenprocoumon, %</td>
<td>6.8</td>
<td>6</td>
<td>5</td>
<td>13</td>
</tr>
<tr>
<td>Findings on coronary angiography</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal coronary arteries, %</td>
<td>47</td>
<td>100</td>
<td>0††</td>
<td>0+++</td>
</tr>
<tr>
<td>1-Vessel disease, %</td>
<td>23</td>
<td>0</td>
<td>49‡</td>
<td>20⁺⁺⁺⁺</td>
</tr>
<tr>
<td>3-Vessel disease, %</td>
<td>16</td>
<td>0</td>
<td>26‡</td>
<td>40⁺⁺⁺⁺</td>
</tr>
<tr>
<td>Left ventricular ejection fraction, %</td>
<td>65±19</td>
<td>69±15</td>
<td>61±18*</td>
<td>61±27</td>
</tr>
</tbody>
</table>

Values are mean±SD, when shown. Eleven patients with unconfirmed PAD and 2 patients with confirmed PAD without CAD were excluded from the subgroup analysis.

For calculation of statistical significance, the following comparisons were made: (1) CAD, No PAD vs No CAD, No PAD: *P<0.05, †P<0.01, ‡P<0.001. (2) CAD, PAD vs CAD, No PAD: §P<0.05, ¶P<0.01, ††P<0.001. (3) CAD, PAD vs No CAD, No PAD: #P<0.05, **P<0.01, †††P<0.001.
TABLE 2. Compositions of Subgroups With CAD and PAD

<table>
<thead>
<tr>
<th></th>
<th>PAD Absent</th>
<th>PAD Present</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAD absent</td>
<td>62</td>
<td>2</td>
<td>64</td>
</tr>
<tr>
<td>CAD present</td>
<td>57</td>
<td>15</td>
<td>72</td>
</tr>
<tr>
<td>Total</td>
<td>119</td>
<td>17</td>
<td>136</td>
</tr>
</tbody>
</table>

Numbers of patients with CAD and angiographically confirmed PAD are shown. Eleven patients with unconfirmed PAD were excluded from this subgroup analysis.

branches. Ninety-three patients (63%) had a history of angina pectoris. One-, 2-, and 3-vessel disease was present at about the same frequency. Owing to exclusion of hemodynamically unstable or critically ill patients, only 7 participants (5%) had symptoms of unstable angina pectoris. Twenty-eight patients (19%) had symptoms and/or clinical signs of PAD. In 17 of these 28 patients (11 men, 6 women), atherosclerosis of the lower-limb arteries was confirmed angiographically. Twenty patients had carotid bruits or a history of intermittent claudication, of whom 12 had evidence of carotid atherosclerosis on B-mode ultrasound examination. All 12 patients with sonographically proven carotid stenosis also had angiographic evidence of lower-limb atherosclerosis. Eleven patients with unconfirmed PAD were excluded from the analysis. Of the remaining 136 patients, 62 were angiographically free of atherothrombotic disease, 57 had CAD alone, 2 had PAD alone, and 15 patients had both CAD and PAD (Table 2). A third of all patients were receiving statins for treatment of hypercholesterolemia, half were receiving acetylsalicylic acid, and 7% were on phenprocoumon.

Coronary Angiography

In the 93 patients with angina pectoris, coronary angiography was performed to define the extent of CAD; in the remaining 54 patients, the investigation was performed as part of a work-up of cardiac arrhythmia. Angiography was performed predominantly by the technique of Judkins and, rarely, the technique of Sones. A Judkins catheter was introduced via the femoral artery, and Ultravist 370 (Schering AG) was injected selectively in the left ventricle and the right coronary artery, and main posterior descending branch. The angiographic movie was first divided into 8 segments: left main stem, left anterior descending artery, diagonal branch, first septal perforator, left circumflex artery, marginal or posterolateral branch, right coronary artery, and main posterior descending branch. The most severe stenosis in each of these segments was then scored, with 0 points for no stenosis, 1 point for 1% to 49% reduction in vessel diameter, 2 points for 50% to 74% reduction, 3 points for 75% to 99% reduction, and 4 points for total occlusion of the segment. Scores for each segment were added and therefore the total score could range from 0 to 32 points.11

Assessment of PAD

PAD was suspected if the patient reported intermittent claudication, if pedal or popliteal pulses were undetectable, or if arterial bruits were heard over the iliac or popliteal arteries. Most of these patients were examined angiographically; definite PAD was defined as the presence of ≥1 stenosis of ≥50% in the iliac, femoral, popliteal, or crural arteries.35 Patients with suspected PAD in whom angiography could not be performed for technical reasons or in whom angiography was inconclusive were classified as "unconfirmed PAD." All other patients were classified as having no PAD.

Blood Sampling

Between 8 and 10 AM of the day of coronary angiography but before it was performed and after an overnight fast, blood was drawn by venipuncture without use of a tourniquet with a 21-gauge needle directly into chilled 10-mL plain tubes and into 10-mL tubes containing EDTA (final concentration 0.1%) or 3.13% trisodium citrate (% of volume). Samples were immediately placed on ice and transported to the laboratory. Within 30 minutes after venipuncture, platelet-poor plasma was isolated by centrifugation at 1200g for 30 minutes at 4°C and immediately stored in aliquots of 1 mL at −86°C. After being allowed to clot for 2 hours at room temperature, serum was isolated by centrifugation at 800g for 20 minutes at room temperature and stored in aliquots of 1 mL at −86°C until analysis. Storage time of samples ranged from 2 to 4 months. Measurements of all parameters were performed in a single batch at the end of the collection period from material that was thawed only once.

Immunologic and Clotting Parameters

CRP was measured by means of a particle-enhanced immunonephelometric assay with a lower detection limit of 0.0175 mg/dL (manufacturer’s data; N Latex CRP mono, Dade Behring Diagnostics) on a nephelometric analyzer (BN II, Dade Behring Diagnostics). SAA was measured by solid-phase sandwich ELISA (Cytoscreen, BioSource). IL-6 was also measured by solid-phase sandwich ELISA (IL6-ELISA-CB, BioSource) on a Cobas Core II analyzer (Roche Diagnostics). Active and total TGF-β were measured both in platelet-poor plasma (2000 to 5000 platelets/μL) and in serum. No correlation was found between the TGF-β concentration in plasma and the platelet count. Total TGF-β was measured using a sandwich ELISA assay developed at the University of Marburg, Germany34; active TGF-β was measured using a commercial test kit (Biotrak, Amersham) according to the manufacturer’s instructions. An automated pipetting robot (Tecan Genesis RSP 150) was used for all pipetting steps, and an automated washer was used for washing between incubation. Neopterin was measured by competitive enzyme immunoassay (Elitest, Brahms). PCT was measured using an immunoluminometric assay (Lumitest PCT, Brahms) on a Stratec SL300 autoanalyzer (Stratec Elektronik). Clottable fibrinogen was determined according to the method of Clauss35 on a Behring BCS coagulation analyzer using Multifibrin U (Dade Behring Diagnostics). Plasminogen activator inhibitor 1 (PAI-1) activity was measured with the Chromolize PAI-1 assay kit from Biopool.

Other Laboratory Parameters

Total serum cholesterol and triglycerides were measured using enzymatic assays.36 HDL cholesterol (HDL-C) was determined by a precipitation method (based on Reference 37; Boehringer Mannheim) on a Hitachi 917 autoanalyzer. LDL-C was calculated using the Friedewald formula. Lp(a) was measured by nephelometry using a latex-enhanced immunoassay (Immuno) on a Hitachi 917 autoanalyzer. Serum glucose was measured using the glucose oxidase method38 and uric acid by the UA Plus method.39 Homocysteine was measured by reverse-phase high-performance liquid chromatography with a commercial assay kit (Chromsystems) on an isocratic liquid chromatograph (Kontron) interfered with a fluorescence detector (model RF-535, Shimadzu).

Statistics

Because many variables were not normally distributed, nonparametric statistical tests were used throughout. Groups were compared using a 2-tailed Mann-Whitney U test. The correlations between inflammatory markers or metabolic parameters and the coronary stenosis score were calculated using Spearman rank correlations. Probabilities <0.05 were regarded as significant. In preliminary analyses, several immunologic and metabolic parameters were shown to vary with sex, smoking status, and age. The numbers of women and smokers in this study, however, were too small to allow meaningful examination of these subgroups separately (Table 1). To avoid confounding, appropriate corrections were therefore made whenever the sex distribution and number of smokers varied significantly between the subgroups. No age correction was performed because of the similar age distribution in all subgroups. These corrections are likely to have increased the chance of overlooking true associations, rather than increasing the chances of identifying associations where none were present. Thus, it is unlikely that our...
TABLE 3. Levels of Immune and Metabolic Parameters in Patients With and Without CAD and/or PAD

<table>
<thead>
<tr>
<th>Parameter</th>
<th>No CAD, No PAD</th>
<th>CAD, No PAD</th>
<th>CAD, PAD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Markers of immune activation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRP, mg/dL</td>
<td>0.20 (0.02, 4.5)</td>
<td>0.40 (0.03, 14.00)†</td>
<td>0.55 (0.14, 1.90)**</td>
</tr>
<tr>
<td>SAA, mg/mL</td>
<td>8 (1, 360)</td>
<td>7 (2, 414)</td>
<td>17 (6, 66)†</td>
</tr>
<tr>
<td>Fibrinogen, mg/dL</td>
<td>328 (233, 713)</td>
<td>394 (232, 942)†</td>
<td>485 (356, 754)**</td>
</tr>
<tr>
<td>IL-6, pg/mL</td>
<td>1.6 (0, 73.0)</td>
<td>3.8 (0, 147.0)†</td>
<td>12 (2, 32)**</td>
</tr>
<tr>
<td>Active TGF-β, ng/L</td>
<td>100 (56, 145)</td>
<td>57 (38, 108)*</td>
<td>72 (32, 719)</td>
</tr>
<tr>
<td>Total TGF-β, ng/L</td>
<td>6325 (2844, 34 243)</td>
<td>6417 (3804, 15 135)</td>
<td>11 834 (3745, 26 442)§</td>
</tr>
<tr>
<td>Neopterin, mg/dL</td>
<td>10 (8, 13)</td>
<td>11 (9, 13)</td>
<td>14 (12, 21)§#</td>
</tr>
<tr>
<td>PCT, ng/mL</td>
<td>0.08 (0.07, 0.11)</td>
<td>0.08 (0.07, 0.09)</td>
<td>0.09 (0.08, 0.10)†¶</td>
</tr>
<tr>
<td>Metabolic parameters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDL-C, mg/dL</td>
<td>142±36</td>
<td>149±48</td>
<td>147±38</td>
</tr>
<tr>
<td>HDL-C, mg/dL</td>
<td>51±18</td>
<td>40±13†</td>
<td>35±15**</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>143 (108, 212)</td>
<td>138 (103, 215)</td>
<td>214 (158, 280)§ ¶</td>
</tr>
<tr>
<td>Lp(a), mg/dL</td>
<td>8 (0, 213)</td>
<td>30 (1, 146)</td>
<td>13 (0, 180)</td>
</tr>
<tr>
<td>PAI-I, U/L</td>
<td>17 (0, 50)</td>
<td>11 (0, 50)</td>
<td>24 (0, 50)</td>
</tr>
<tr>
<td>Homocysteine, μmol/L</td>
<td>17±5</td>
<td>20±7</td>
<td>23±16</td>
</tr>
<tr>
<td>Glucose, mg/dL</td>
<td>117±27</td>
<td>138±63*</td>
<td>128±27</td>
</tr>
<tr>
<td>Uric acid, mg/dL</td>
<td>7.2±2.0</td>
<td>7.3±1.5</td>
<td>9.4±3.1§ ¶</td>
</tr>
</tbody>
</table>

Data were corrected for sex and smoking status. Normally distributed data are given as mean±SD; skewed data are given as median plus (25th, 75th) percentiles.

For calculation of statistical significances, the following comparisons were made: (1) CAD, No PAD vs No CAD, No PAD: *P<0.05, †P<0.01, ‡P<0.001. (2) CAD, PAD vs CAD, No PAD: §P<0.05, ¶P<0.01. (3) CAD, PAD vs No CAD, No PAD: ¶P<0.05, #P<0.01, **P<0.001.

Comparison of Patients With CAD and PAD With Patients Without Atherosclerosis

With the exception of active TGF-β, all parameters of inflammation that were significantly different between patients with and without CAD also differed significantly between patients with CAD and PAD compared with patients without atherosclerosis. Although total TGF-β was significantly greater in patients with CAD and PAD compared with those with CAD alone, the difference between patients with and without atherosclerosis was not significant. Triglyceride and uric acid were significantly greater in patients with CAD and PAD than in those without atherosclerosis.

Correlation of Metabolic and Immunologic Parameters With Coronary Stenosis Score

Levels of CRP, fibrinogen, and IL-6 showed a significant positive correlation and active TGF-β a significant negative correlation with the extent of coronary stenosis (Table 4). Among the metabolic risk factors, HDL-C showed a negative correlation and glucose a positive correlation with the extent of coronary atherosclerosis as measured by the coronary stenosis score.
TABLE 4. Correlation of Immunologic and Metabolic Disease/Risk Markers With the Extent of CAD

<table>
<thead>
<tr>
<th>Markers of immune activation</th>
<th>Correlation Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP</td>
<td>0.29†</td>
</tr>
<tr>
<td>SAA</td>
<td>NS</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>0.31†</td>
</tr>
<tr>
<td>IL-6</td>
<td>0.21*</td>
</tr>
<tr>
<td>Active TGF-β</td>
<td>-0.24*</td>
</tr>
<tr>
<td>Total TGF-β</td>
<td>NS</td>
</tr>
<tr>
<td>Neopterin</td>
<td>NS</td>
</tr>
<tr>
<td>PCT</td>
<td>NS</td>
</tr>
<tr>
<td>Metabolic risk markers</td>
<td></td>
</tr>
<tr>
<td>LDL-C</td>
<td>NS</td>
</tr>
<tr>
<td>HDL-C</td>
<td>-0.40‡</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>NS</td>
</tr>
<tr>
<td>Lp(a)</td>
<td>NS</td>
</tr>
<tr>
<td>PAI-I</td>
<td>NS</td>
</tr>
<tr>
<td>Homocysteine</td>
<td>NS</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.20*</td>
</tr>
<tr>
<td>Uric acid</td>
<td>NS</td>
</tr>
</tbody>
</table>

Spearman rank correlations between markers of immune activation, atherosclerosis-associated metabolic markers, and the coronary stenosis score are shown.

| P<0.05, †P<0.01, ‡P<0.001. |

Correlation Between Immunologic and Metabolic Laboratory Parameters

To explore the relationships among the markers of inflammation, among the atherosclerosis-associated metabolic markers, and between these 2 sets of parameters, nonparametric correlations were calculated between and among these groups (Table 5). As expected, many correlations were found among the atherosclerosis-associated metabolic factors. However, a large number of correlations also existed between metabolic and immunologic markers. In particular, neopterin, a specific marker of monocyte-macrophage activation, was closely correlated with homocysteine (0.68, P<0.01) and uric acid (0.32, P<0.01) levels. Also of note was the close correlation between fibrinogen, a sensitive marker of the acute-phase response, and not only CRP (0.57, P<0.001), SAA (0.50, P<0.001), and IL-6 (0.50, P<0.001) but also neopterin (0.36, P<0.01) and PCT (0.34, P<0.01). By contrast, of the metabolic parameters, only HDL-C showed a negative correlation with fibrinogen (−0.25, P<0.01). Several close correlations also existed among other immunologic markers, the closest being between the proinflammatory cytokine IL-6 and the acute phase reactants CRP (0.64, P<0.001) and SAA (0.47, P<0.001), followed by IL-6 and the marker of macrophage activation, neopterin (0.44, P<0.001) as well as between IL-6 and the marker of infection, PCT (0.40, P<0.01). Correlations of lower significance also existed between neopterin and SAA as well as active TGF-β and between PCT and CRP as well as SAA. The number and strength of correlations among the immunologic markers were in general similar to those among the atherosclerosis-associated metabolic parameters.

Logistic Regression Analysis

In logistic regression analyses taking account of age and sex, levels of HDL-C, fibrinogen, and triglycerides were significantly associated with the presence of CAD. Taking age, sex, and triglycerides into account, of the immunologic parameters only CRP was found to be significantly associated with the presence of CAD. However, CRP was no longer significantly associated with CAD when fibrinogen or HDL-C was added to age, sex, and triglycerides in the logistic regression analysis. Thus, in the presence of fibrinogen or HDL-C, markers of inflammation or of the acute-phase response did not additionally discriminate between patients with and without CAD.

Discussion

The main result of this study was the finding of increased levels of parameters of inflammation in CAD and PAD, whereby more parameters were elevated and the elevation was more clear-cut in patients with both CAD and PAD compared with patients with CAD alone. This result would seem to indicate that the levels of immunologic markers may indicate the extent of atherosclerotic disease, a conclusion that is supported by the positive correlation between CRP, fibrinogen, and IL-6 and the negative correlation between active TGF-β and the extent of coronary sclerosis. It is of note that these elevations of immunologic parameters occurred despite the near absence of significant differences in the levels of atherosclerosis-associated metabolic parameters between patients with and those without atherosclerosis. Previous workers have shown an increase in the level of circulating immunologic parameters in atherosclerosis in both cross-sectional and prospective studies. An elevated level of CRP was found to be indicative of “active” CAD manifesting as unstable angina pectoris, plaque rupture, or acute myocardial infarction, whereas in prospective studies, even slight differences in the median CRP levels were found to be predictive of myocardial infarction, stroke, and PAD in men. In the present report, CRP levels were slightly higher than those reported in these studies. This may reflect differences in the methods used and may also be due to differences in patient selection. CRP levels in our patients with CAD were significantly higher than in those without CAD. Levels of CRP in patients with CAD and PAD did not differ significantly from those in patients with CAD alone but were greater than those in patients without atherosclerosis. This lack of a further significant increase of CRP in PAD plus CAD compared with CAD alone may reflect the play of chance, in view of the small number of patients with PAD analyzed. Median levels of SAA, an acute-phase protein that behaves in a very similar fashion to CRP, were significantly
elevated in PAD. Thus, our data indicate that significant elevations of CRP may be present in stable coronary atherosclerosis in the absence of “activation.” Moreover, in contrast to a report by Liuzzo and colleagues but in agreement with a previous report from our group, the level of CRP was positively correlated with both the grade and extent of coronary disease, indicating that this parameter is not only a marker of disease presence but also a quantitative indicator of disease extent.

In our patients, fibrinogen was also elevated in both CAD and PAD and was significantly correlated with the extent of CAD, indicating that this parameter is not only a marker of disease presence but also a quantitative indicator of disease extent.

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What is the cause of this increase in acute-phase reactants in atherosclerosis? During immunologic reactions, CRP, SAA, and fibrinogen synthesis in the liver increase in response to stimulation by proinflammatory cytokines. In our patients, IL-6 was elevated in both CAD and PAD, and the level of IL-6 was correlated with the extent of CAD. A main source of IL-6 is activated macrophages, and IL-6 has been shown to strongly influence levels of CRP, SAA, and fibrinogen in vivo. Moreover, IL-6 is present within the arterial wall. In the participants in our study, the level of neopterin, a specific marker of activated macrophages, was higher in patients with PAD than in patients without PAD. This increase in neopterin levels may be an indicator of macrophage activation. Several studies have confirmed that activated macrophages are present within the atherosclerotic plaque. Taken together with our results, these findings suggest that the source of the proinflammatory cytokines in atherosclerosis may, at least partially, be the macrophages within the arterial wall.

It is also possible that at least some of the increased CRP levels in our patients with atherosclerosis may have been synthesized by activated macrophages within the arterial wall. Such activation of plaque macrophages may reflect chronic infection of the arterial wall or it may be the result of their interaction with oxidized or otherwise-modified lipoproteins.

To further explore markers of infection in atherosclerosis, we also measured the level of PCT, which was increased in patients with PAD. PCT is thought to derive mainly from cells of neuroendocrine origin throughout the body and is produced almost exclusively as part of a systemic response to bacterial endotoxins or exotoxins. In sepsis, however, the
level of PCT is generally 1 to 3 orders of magnitude greater than that seen in our patients. Thus, the pathophysiological significance of the slight increase in PCT seen in our patients with PAD is uncertain but may reflect a low-grade inflammatory response, perhaps in response to primary or secondary bacterial infection.57

It is also possible that the increase in markers of inflammation in atherosclerosis is not a response to infection or modified lipoproteins within the arterial wall but reflects a low-grade primary activation of the immune system.62 For example, studies by Wick and others have found evidence of inflammatory activity with cellular infiltrates in the arteries of children and young adults before the development of atherosclerosis.63–65 Conversely, the increased level of parameters of inflammation may contribute directly to atherogenesis. CRP has been shown to increase the opsonization and uptake of lipid complexes by macrophages,66 and SAA modifies HDL particles such that their affinity for monocytes/macrophages is increased.67 Moreover, inflammatory parameters directly influence metabolic parameters; IL-6, for example, sharply lowers HDL-C and alters lipoprotein composition.68

In our patients, active TGF-β was reduced in coronary atherosclerosis, while total TGF-β was increased in PAD. Moreover, the levels of active TGF-β were inversely correlated with the extent of coronary atherosclerosis. This finding of a decrease in active TGF-β in patients with CAD is in agreement with the report of Grainger et al.,22 even though the association in our patients was less marked and these levels showed some degree of overlap in patients with 3-vessel disease and those with normal coronary arteries. Results from different studies on TGF-β in atherosclerosis are inconsistent. This may be due to differences in preanalytic design, including measurement in different matrices (serum22 versus plasma23) and the use of different methods for measurement of TGF-β. The phylogenetically highly conserved TGF-β possesses low immunogenicity. Available antibodies therefore have low specificity and affinity and detect different epitopes. To circumvent these problems, some groups have used recombinant truncated forms of the TGF-β receptor as capture ligand.69 However, the lack of international reference material prevents efforts to standardize these tests. To address these issues, we measured active TGF-β in plasma and serum in all our patients by using a number of different antibody-based assay systems (Amersham, BioSource, Promega, Genzym, and the Marburg-assay) and a TGF-β receptor–based system (R&D Inc; data not shown). The results of these measurements showed a low correlation between the tests as well as differences in concentrations of active TGF-β of 1 to 2 orders of magnitude. The best correlation of active TGF-β with CAD was found with the Amersham assay (r = −0.24, P < 0.05). The details of TGF-β measurements in our patients will be presented in detail elsewhere.

Multiple highly significant correlations were seen in the present study between the levels of CRP, SAA, IL-6, neopterin, PCT, and fibrinogen. Correlations with PAI-1 and TGF-β were less marked. This may indicate that the former 6 parameters form a core inflammatory response in atherosclerosis, which may be driven by activation of macrophages within the arterial wall. It is notable that the level of neopterin, a specific marker of macrophage activation,25 was closely correlated not only with immunologic parameters (including fibrinogen) but also with the metabolic parameters urate, triglycerides, and homocysteine and inversely with HDL-C. Correlation coefficients can provide no information on cause and effect. However, these correlations are consistent with the hypothesis that at least part of these metabolic changes may be secondary to activation of inflammation.

Compared with the clearly increased levels of many inflammatory parameters in atherosclerosis in the present study, differences in the metabolic parameters between patients with and without CAD or PAD were fewer and of lesser extent, although HDL-C was negatively correlated and glucose was positively correlated with the extent of coronary atherosclerosis. The relative lack of differences in metabolic parameters between patients with and without atherosclerosis may have been partly a result of drug therapy or of modifications of life style or dietary habits undertaken by the patients in response to the diagnosis of atherosclerosis. Nevertheless, even with the relatively small number of patients in this present study, marked changes in immunologic parameters were seen, which may indicate that these markers are superior indicators of existent disease in the individual patient.

Despite the differences seen in univariate analysis, multivariate logistic regression analysis revealed that only CRP provided additional discriminatory power in the identification of CAD, once age and sex were taken into account. However, when fibrinogen, a positive acute-phase reactant, or HDL-C, a negative acute-phase reactant, was taken into account, none of the parameters of inflammation analyzed in the present study provided additional information. It would therefore appear that determination of parameters of inflammation does not provide information over and above that provided by fibrinogen or HDL-C. Many of the studies that have shown an increase in immunologic parameters in atherosclerosis either did not adjust for age (eg, recent analyses of CRP levels in the Physicians’ Health Study by Ridker and colleagues5–6) or failed to perform multivariate regression analysis (eg, studies of TGF-β in CAD by Grainger et al.22). In studies in which such analyses were performed, differences between patients with and without atherosclerosis were much attenuated when age was included in the regression equation.27 It is of note that on multivariate analysis, the most effective discrimination between patients with and without CAD was achieved with the well-known “classic” risk factors determined in such prospective studies as the Framingham Heart Study70 or the Münster Heart Study,71 despite the sometimes striking differences in the levels of parameters of inflammation seen on univariate analysis. The discriminatory power of the markers of inflammation in the present study was explained to a great degree by fibrinogen and HDL-C. This may reflect the fact that HDL-C and fibrinogen are not only indicators of atherosclerotic risk in their own right, as components of lipid metabolism or the clotting system, but also positive and negative acute-phase reactants, respectively.

In summary, the present study suggests that increased circulating levels of CRP, SAA, IL-6, neopterin, PCT, and fibrinogen represent a core inflammatory signature of advanced atherosclerotic disease and that the level of these markers may reflect the whole-body burden of atherosclerotic tissue. However, on multivariate logistic regression analysis, the discriminatory power of these markers of inflammation...
was explained by fibrinogen or HDL-C. Thus, performance of these expensive and technically demanding assays cannot, at present, be recommended for the assessment of atherosclerotic disease in clinical practice.

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