Myocardial Ischemia Induces Interleukin-6 and Tissue Factor Production in Patients With Coronary Artery Disease
A Dobutamine Stress Echocardiography Study

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Background—Interleukin-6 (IL-6) and macrophage colony stimulating factor plasma levels are elevated in acute coronary syndromes. IL-6 has an inherent negative inotropic action and, with tissue factor (TF), mediates the ischemia-reperfusion myocardial injury. We hypothesized that inducible ischemia leads to cytokine production, TF expression, and consequently persistent left ventricular dysfunction after dobutamine stress echocardiography (DSE) in coronary artery disease patients.

Methods and Results—DSE was performed in 103 patients with angiographically documented coronary artery disease. Blood samples were obtained at rest, at peak stress, and 30 minutes after cessation of dobutamine infusion for measurement of macrophage colony stimulating factor, IL-6, and TF. New or worsening wall motion abnormalities at peak stress and their duration into recovery were noted. Median IL-6 and TF levels were increased at peak stress and at 30 minutes into recovery compared with rest (2.7 and 2.4 versus 2.1 pg/mL for IL-6, 310 and 385 versus 266 pg/mL for TF \( P < 0.01 \) in patients with an ischemic response; \( n = 55 \)). Compared with rest, a greater release of IL-6 at peak stress and recovery was observed in patients with increasing number of ischemic segments at peak DSE (2 versus 3 to 4 versus 5 to 6 versus 7 to 8 segments; \( P = 0.03 \)). The time to recovery of wall motion abnormalities was also associated with IL-6 levels at peak stress and recovery (\( r = 0.51 \) and \( r = 0.39 \), \( P < 0.05 \)). Macrophage colony stimulating factor levels remained unchanged throughout DSE.

Conclusions—Reversible ischemia induced during DSE increases IL-6 and TF plasma levels. IL-6 is related to the extent of left ventricular dysfunction at peak stress and to persistent LV dysfunction during recovery. (Circulation. 2005;112:3272-3279.)

Key Words: echocardiography ▪ hypoxia ▪ interleukins ▪ ischemia ▪ tissue factor

Interleukin 6 (IL-6) and macrophage colony stimulating factor (MCSF) are elevated after acute myocardial infarction and in patients with stable and unstable coronary artery disease (CAD).\(^1\)\(^-\)\(^5\) Additionally, MCSF has been linked to daily life ischemia\(^6\)\(^-\)\(^8\) in patients with chronic CAD. MCSF induces the production of procoagulant cytokines such as IL-1b and IL-6\(^-\)\(^1\)\(^8\) at atherosclerotic plaques, whereas IL-6 promotes tissue factor (TF) expression from endothelial cells and circulating monocytes,\(^10\)\(^-\)\(^12\) which is responsible for the thrombogenicity of the atherosclerotic plaque.\(^13\) High circulating TF plasma levels have been reported in patients with CAD.\(^14\)\(^,\)\(^15\) Furthermore, both IL-6\(^1\)\(^6\) and TF\(^1\)\(^7\) mediate cardiac ischemia-reperfusion injury after coronary occlusion in experimental models. However, the relation between demand-driven myocardial ischemia and proinflammatory cytokines or TF plasma levels has not been well established in patients with CAD.

Production of proinflammatory cytokines is stimulated by muscular effort during exercise.\(^18\) It would therefore be difficult to provide a causal link between myocardial ischemia after exercise stress testing and cytokine production. Dobutamine stress echocardiography (DSE) is a well-established diagnostic technique for the detection of myocardial ischemia by inducing reversible regional wall motion abnormalities (WMAs).\(^19\)\(^,\)\(^20\) Additionally, WMAs may persist during recovery, and the time to recovery has been linked to the severity of myocardial ischemia at peak stress, the presence of multivessel disease, and the absence of collateral circulation at angiography.\(^21\) IL-6 has a direct negative inotropic action on human myocardium,\(^22\) has been associated with elevated intracardiac pressures in patients with ischemic...
cardiomyopathy, and is induced during experimental ischemic models. However, the relation between IL-6 plasma levels and the extent of new or worsening WMAs after DSE has not been investigated.

We hypothesized that inducible ischemia after DSE increases plasma levels of MCSF, IL-6, and TF, leading to persistent left ventricular (LV) dysfunction during recovery, and that there is an association between extent of ischemia and IL-6 release during DSE.

Methods

Study Sample
We recruited 103 consecutive patients with angiographically documented CAD who underwent DSE to assess the location and severity of ischemia either after angiography to define the target lesion for future revascularization or after a previous revascularization procedure as part of the routine clinical workup. Patients with recent (<6 months) myocardial infarction or acute coronary syndrome were excluded from the study to ensure that the increased cytokine levels observed after the acute event would not interfere with measurements. Other exclusion criteria were coronary angioplasty or surgery within the previous 6 months, cerebral vascular disease, peripheral vascular disease, impaired renal or liver function, and evidence of active infection. All antianginal medication (β-blockers or calcium channel blockers) was withdrawn at least 48 hours before DSE. All patients gave informed consent before inclusion in the study.

Cardiac Catheterization
The presence of CAD was established during routine diagnostic coronary angiography within 3 months before enrollment. Stenosis >50% was considered clinically significant.

DSE Study
Each patient underwent 2D echocardiography with a Philips HDI 5000 system. Standard tomographic views of the left ventricle (LV) were obtained at rest and continuously during dobutamine infusion. Imaging was continuously recorded on VHS videotapes and was digitized online with the equipment’s software in a quad screen format every 3 minutes at the end of each dose of dobutamine (5 to 40 μg·kg⁻¹·min⁻¹) and during recovery. Recording was completed only after all ischemic regions had returned to baseline. Digital imaging was also obtained at fixed 5-minute intervals during recovery to facilitate simultaneous and synchronized analysis of each myocardial segment throughout and to monitor the time to recovery of WMAs as previously described. Failure to achieve a heart rate of 90% maximal predicted levels for age was followed by a bolus administration of up to 1 mg atropine at the end of dobutamine infusion. Dobutamine stress echocardiography was considered positive for myocardial ischemia when new or worsening WMAs were detected in at least 2 contiguous segments. The dobutamine stress test was continued regardless of the occurrence of new or worsening WMAs until symptoms occurred or maximal heart rate was achieved. For the analysis of regional WMAs, a 16-segment protocol of the 4 tomographic views of the LV was used. Each segment was scored as follows: 1 = normal, 2 = hypokinetic, 3 = akinetic, and 4 = dyskinetic. A total wall motion score (WMS) was used to quantify the magnitude of ischemia as the sum of all 16 segments. The increases in WMS and the heart rate–systolic blood pressure product between rest and peak stress and the percent increase in WMS% and systolic blood pressure were calculated.

The number of myocardial segments with new or worsening WMAs was noted in each patient and used as a measure of the extent of ischemia. The ratio (%) between the number of ischemic segments and the total 16 LV segments was also used to describe the percent area of LV with an ischemic response at peak DSE. Patients with ischemic response were categorized into 4 groups—those with 2 ischemic segments (12.5% of LV), those with 3 or 4 segments (<25% of LV), those with 5 or 6 segments (<37.5% of LV), and those with 7 or 8 segments (<50% of LV) —to investigate the presence of a “dose-response” relation between extent of ischemia and levels of biochemical markers. Finally, the ejection fraction was calculated with the machine’s software using the methods of disks.

Echocardiograms were analyzed by 2 experienced observers on 2 separate days with a time interval of 1 month. For the definition of WMAs, the 2 observers had a concordance of 89% (contingency coefficient, 0.76; k = 0.80). Interobserver variability and intraobserver variability were defined as the mean ± SD difference in the measurement of total WMS by the 2 observers and between the online and offline recordings for observer 1 (G.A.) and were 0.85 ± 0.8 (95% CI, 0 to 2.5) and 0.67 ± 0.7 (95% CI, 0 to 1.9), respectively.

Blood Sampling
Blood samples were drawn at rest, at peak stress, and 30 minutes after cessation of dobutamine infusion from the right antecubital vein. The samples were drawn into plastic tubes containing 1:9 volumes of 0.103 mol/L trisodium citrate and centrifuged at 3000 g for 15 minutes at 40°C. Aliquots of plasma were stored at −70°C until subsequent analysis.

Laboratory Assays
The laboratory measurements were performed by personnel unaware of the clinical data. Plasma MCSF concentrations were measured with a commercial ELISA (human MCSF Quantikine R&D system). The sensitivity of the assay is 20 pg/mL. IL-6 and TF were measured by high-sensitivity immunoassays (human IL-6 Quantikine [high sensitivity] R&D systems and human TF IMUBIND 845, American Diagnostica Inc) that detect values as low as 0.094 and 15.3 pg/mL, respectively. The intra-assay coefficient of variation was <5% for all assays.

Statistical Analysis
In a pilot study of 20 patients (10 with and 10 without ischemia at DSE), we found that the SD of the change in IL-6, TF, and MCSF between rest and peak DSE was 1.03, 140, and 164 pg/mL, respectively. From previous studies, we assumed that a 25% increase in the levels of the inflammatory indexes at peak DSE compared with rest in ischemic compared with nonischemic patients is clinically significant; thus, with an α = 0.05 (2 tailed) and a power of 80%, the sample size was calculated to 44 patients per group.

Biochemical data are expressed as medians and quartiles. SPSS version 11.5 (SPSS Inc) software was used. All nonnormal variables were transformed into ranked data for further analysis. Biochemical data were analyzed by ANOVA for repeated measurements (general linear model, SPSS version 11.5) with time of measurement (rest, peak DSE, and recovery) as a within-subject factor and presence of ischemia (yes versus no) in all patients or extent of ischemia (4 subgroups with increasing number of ischemic segments) as ischemic patients used as a between-subject factor. The Greenhouse-Geisser correction was used when the sphericity assumption, as assessed by Mauchly’s test, was not met. The F and corresponding probability values of the interaction between time of measurement of the biochemical markers and presence or extent of ischemia were calculated. Post hoc comparisons were performed with Bonferroni’s correction. Differences between 2 groups were analyzed with the Mann-Whitney U test. Spearman’s rank-correlation test was used to assess relations between variables. Multiple relations were tested by stepwise regression analysis after logarithmic transformation of the nonnormal variables. Categorical variables were compared by contingency test. A value of P < 0.05 (2 tailed) was considered statistically significant.

Results

Study Sample
Patients’ characteristics are listed in Table 1. Of the 103 patients, 55 had a positive DSE. The resting mean ejection
fraction was 46±12% in all patients (48±11% in patients with ischemia at DSE).

DSE Results

The results of DSE are summarized in Table 2. In brief, heart rate increased from 69±14 to 106±30 bpm (P<0.01) and systolic and diastolic blood pressures from 136±25 to 154±24 mm Hg (P<0.01) and 84±17 to 88±12 mm Hg (P<0.01) at peak stress in all patients. Of the 55 patients with a positive response at peak DSE, 12 patients had 2 ischemic segments, 15 patients had 3 or 4 ischemic segments, 19 patients had 5 or 6 segments, and 9 patients had 7 or 8 segments. There was no difference in age, gender, atherosclerotic risk factors, number of diseased vessels, and medication between patients with and without ischemia (Table 1). However, patients with no ischemia had lower resting ejection fraction and higher WMS, as well as a higher incidence of previous revascularization procedures (percutaneous coronary intervention or CABG) compared with patients with ischemia after DSE (P<0.05; Tables 1 and 2).

Biochemical Indexes During DSE

ANOVA showed that IL-6 and TF plasma levels increased at peak DSE and remained elevated at 30 minutes of recovery compared with rest in patients with ischemia (Figure 1A and 1C) but not in patients without evidence of ischemia at peak stress (F for interaction=18.09, P<0.001; F=57.18, P<0.001, respectively; Figure 1B and 1D). Compared with patients without evidence of ischemia, those with ischemia had higher IL-6 levels at peak stress and during recovery (2.10 versus 2.78 and 2.21 versus 2.48 pg/mL, respectively; P>0.01). Conversely, MCSF levels remained unchanged throughout the stress and into recovery in both ischemic and nonischemic patients (rest, range 462 pg/mL [25th, 75th percentile 239 to 590 pg/mL] versus peak stress, 380 pg/mL [222 to 547 pg/mL] versus recovery, 400 pg/mL [253 to 566 pg/mL]; F for interaction=0.64; P>0.5).

Compared with baseline, there was greater release of IL-6 at peak stress and during recovery in patients with a greater extent of ischemia at peak DSE as measured by the number of ischemic segments (<2 versus 3 to 4 versus 5 to 6 versus 7 to 8 segments.

TABLE 1. Clinical Characteristics of the Study Sample

<table>
<thead>
<tr>
<th></th>
<th>DSE(+) (n=55)</th>
<th>DSE(-) (n=48)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>62±9 (38–75)</td>
<td>61±9 (32–72)</td>
<td>0.7</td>
</tr>
<tr>
<td>Gender, M/F, n (%)</td>
<td>45/10 (82)</td>
<td>43/5 (89)</td>
<td>0.5</td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td>5.9±0.99 (4.8–8.1)</td>
<td>5.0±0.5 (2.0–6.0)</td>
<td>0.1</td>
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<tr>
<td>Triglycerides, mmol/L</td>
<td>1.8±1.2 (0.8–5.7)</td>
<td>1.2±0.5 (0.8–2.0)</td>
<td>0.3</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>28±4 (22–36)</td>
<td>27±3 (20–35)</td>
<td>0.8</td>
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<tr>
<td>Smoking, n (%)</td>
<td>39 (70)</td>
<td>29 (60)</td>
<td>0.6</td>
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<tr>
<td>Hyperlipidemia, n (%)</td>
<td>43 (78)</td>
<td>31 (64)</td>
<td>0.1</td>
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<tr>
<td>Hypertension, n (%)</td>
<td>21 (38)</td>
<td>17 (36)</td>
<td>0.8</td>
</tr>
<tr>
<td>Parental CAD, n (%)</td>
<td>22 (40)</td>
<td>18 (37)</td>
<td>0.7</td>
</tr>
<tr>
<td>Diabetes, n (%)</td>
<td>10 (18)</td>
<td>9 (18)</td>
<td>0.9</td>
</tr>
<tr>
<td>CCS anginal class, n (%)</td>
<td>22 (40)</td>
<td>19 (40)</td>
<td>0.7</td>
</tr>
<tr>
<td>Diseased coronary arteries, n (%)</td>
<td>18 (33)</td>
<td>21 (44)</td>
<td>0.08</td>
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<td>Previous revascularization, n (%)</td>
<td>8 (14)</td>
<td>35 (73)</td>
<td>&lt;0.01</td>
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<tr>
<td>Previous MI, n (%)</td>
<td>29 (53)</td>
<td>28 (58)</td>
<td>0.6</td>
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<td>Drugs, n (%)*</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>β-Blockers</td>
<td>37 (68)</td>
<td>26 (54)</td>
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<td>Ca²⁺ blockers</td>
<td>22 (40)</td>
<td>14 (30)</td>
<td>0.4</td>
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<tr>
<td>Long-acting nitrates</td>
<td>19 (34)</td>
<td>24 (50)</td>
<td>0.1</td>
</tr>
<tr>
<td>Lipid lowering</td>
<td>23 (42)</td>
<td>19 (40)</td>
<td>0.8</td>
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<tr>
<td>Antiplatelets</td>
<td>48 (88)</td>
<td>41 (85)</td>
<td>0.7</td>
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<tr>
<td>Diuretics</td>
<td>8 (15)</td>
<td>7 (15)</td>
<td>0.9</td>
</tr>
<tr>
<td>ACE inhibitors</td>
<td>18 (32)</td>
<td>18 (38)</td>
<td>0.5</td>
</tr>
</tbody>
</table>

*Medical treatment at inclusion.

+ Indicates positive for ischemia; (−), negative for ischemia; CCS, Canadian Cardiovascular Society; and MI, myocardial infarction. Revascularization refers to previous percutaneous coronary intervention or CABG. Values are expressed as mean±SD.
of the 16 LV segments; F for interaction = 2.44; P = 0.03; Figure 2). This was also observed when the percent ischemic area of the LV was used to assess the extent of ischemia (12.5% versus <25% versus <37.5% versus <50%, respectively; P = 0.03; Figure 2), suggesting a dose-response relation between extent of ischemia and IL-6 release after DSE. Increasing numbers of ischemic myocardial segments or percent ischemic area at peak DSE was associated with increasing IL-6 levels at baseline (1.68 versus 2.18 versus 2.56 versus 2.93 pg/mL; P < 0.05), peak stress (1.8 versus 2.42 versus 3.05 versus 3.5 pg/mL; P < 0.01), and recovery period (1.8 versus 2.38 versus 2.82 versus 3.25 pg/mL; P < 0.01; Figure 2).

In all patients with >4 ischemic segments (>25% of LV), the time to recovery of WMAs exceeded 5 minutes, whereas in 23 (85%) patients with <4 ischemic segments, the time to recovery of WMAs was <5 minutes (P < 0.01). There were no differences in age, gender, atherosclerotic risk factors, number of diseased vessel, medication, resting WMS, and peak double product between the 4 subgroups of patients with increasing number of ischemic segments.

**Correlations Among Biochemical Markers**

Resting MCSF values were related to resting IL-6 in all patients (r = 0.41, P < 0.01). However, this relation was not evident at peak stress and recovery, possibly because of the greater increase in IL-6 levels by ischemia compared with MCSF.

Furthermore, among patients with myocardial ischemia, those with high resting MCSF levels demonstrated a greater absolute and percent increase in TF at peak stress (r = 0.31, P = 0.021; r = 0.30 P = 0.026) and into recovery (r = 0.44, P = 0.01; r = 0.36, P = 0.02) compared with baseline, suggesting that the presence of high resting MCSF levels may promote TF production during or after a reversible ischemic episode. There was a significant relation between MCSF and TF plasma levels at peak stress and recovery (r = 0.40, P = 0.012; r = 0.51, P = 0.01, respectively). This relation was not evident in patients without ischemia throughout DSE. There was no relation between biochemical markers and WMS at rest, peak stress, and recovery or between changes in biochemical markers and increase in WMS and double product between rest and peak stress.

**Cytokine Levels and Time to Recovery of WMAs**

The time to recovery of WMAs was associated with IL-6 plasma levels at peak stress and recovery (r = 0.51 and r = 0.39, respectively; P < 0.05). Patients with WMAs lasting >5 minutes into recovery (n = 32) had higher IL-6 at peak stress and recovery than those with WMAs lasting <5 minutes (P < 0.01; Figure 3). There was no relation between MCSF or TF levels and the time to recovery of WMAs.

**Ischemia-Induced Changes of Cytokines**

**IL-6 Levels**

In this study, myocardial ischemia increased the production of IL-6 at peak stress and during recovery period. This increase was not observed in patients without evidence of ischemia at DSE. Furthermore, an increasing number of ischemic segments or percent area of ischemic LV at peak stress was associated with a greater increase in IL-6 levels at peak stress and recovery compared with baseline. Our findings suggest that the extent of ischemia may be associated with a greater release of IL-6. Although this early rise in IL-6 at peak stress may appear somewhat surprising, other investigators have also demonstrated that IL-6 mRNA and protein were rapidly induced in ischemic myocardial segments in a canine model and that the highest levels of IL-6 mRNA and protein were observed in the most ischemic segments.16 The same investigators have shown that the induction of IL-6 mRNA demonstrated peak levels after 30 minutes of stimulation, which is similar to the elevation of IL-6 levels at 30 minutes after DSE that we have observed. Other investigators have demonstrated that cardiac release of IL-6 was increased immediately and 5 minutes after recanalization of the left anterior descending coronary artery by means of primary balloon angioplasty in patients with acute myocardial infarction24 and that glandular epithelial cells release preformed IL-1β and IL-6 from their granules by 10 minutes after α- and β-adrenergic stimulation.26
Mast cells,\textsuperscript{27–29} basophils,\textsuperscript{29} eosinophils,\textsuperscript{30} monocytes,\textsuperscript{8,31} pulmonary,\textsuperscript{32} and vascular endothelial cells\textsuperscript{33} synthesize and store large amounts of IL-6. Mast cell degranulation also leads to release of preformed cytokines\textsuperscript{34} (including IL-6\textsuperscript{27}), induces IL-6 production by endothelial cells,\textsuperscript{33} and thus initiates the inflammatory process in experimental models of ischemia-reperfusion.\textsuperscript{34,35} Ischemia may induce localized cytokine production that facilitates influx of leukocytes in the ischemic area and their entrapment in microcirculation, resulting in production of larger amounts of IL-6.\textsuperscript{16,35}

Thus, we can speculate that the increased release of IL-6 after DSE observed in this study may suggest that reversible myocardial ischemia triggers an initial rapid release of preformed IL-6 from circulating monocytes or cardiac mast cells,\textsuperscript{31,34} followed by enhanced production of newly synthesized IL-6 by hypoxic myocytes,\textsuperscript{36} vascular endothelial cells,\textsuperscript{33} or adherent leukocytes\textsuperscript{8,16,26} after cessation of the ischemic insult. Alternatively, we may assume that the elevation of IL-6 during DSE may be the consequence of the acute hemodynamic impairment resulting from the development of extensive WMAs at DSE, and acute deterioration of systolic LV function has been shown to occur in patients with congestive heart failure.\textsuperscript{23} However, the relation between increasing IL-6 levels at rest and increasing number of ischemic myocardial segments at peak DSE suggests

\textbf{Figure 1.} Individual values of IL-6 and TF plasma levels at baseline (rest), peak DSE (peak stress), and 30 minutes after cessation of dobutamine (recovery) in patients with ischemic (A, C) or nonischemic (B, D) response at peak stress. Biochemical factors increased at peak stress and recovery vs rest in ischemic (A, C) but not in nonischemic patients (B, D) (F for interaction=18.09, \(P<0.001\) for IL-6; \(F=57.18, P<0.001\) for TF by ANOVA).
that the relation between IL-6 and WMAs may not be merely a
marker of the acute impairment of LV systolic function at peak
stress. Conversely, IL-6 levels may mediate or at least promote
the manifestation of WMAs during DSE because they exert a
direct reversible negative inotropic action on myocardium.22

Sympathetic activation may also be another important trig-
nering factor for IL-6 release during stress.26,37 However, pa-
tients with no ischemia during DSE did not show any increase in
IL-6 throughout the test, even though they achieved a similar
heart rate–blood pressure product and presumably a similar level
of sympathetic activation as patients with ischemia. Our findings
are in agreement with those of Kukielka et al,16 who have shown
that coronary occlusions not associated with ischemia because of
collateral blood flow did not elicit measurable levels of IL-6
mRNA despite identical occlusion and reperfusion interval.
However, an enhanced catecholamine response to ischemia itself
may be an additional pathophysiological mechanism contribut-
ing to increased IL-6 release from cardiac or peripheral sources
after DSE.

IL-6 has been reported to be elevated after treadmill exercise
and has been shown to be of skeletal muscle origin.18 In our
study, the lack of skeletal muscle contraction during DSE
suggests that the source of elevated IL-6 with DSE may be the
ischemic myocardium. This is further supported by studies
demonstrating an increased release of IL-6 in the coronary sinus
of patients with CAD but not in patients with congestive heart
failure, suggesting that elevated IL-6 levels in the peripheral
blood of patients with CAD derive from a cardiac source.38

**Relation of IL-6 Levels to Delayed Recovery of
Regional WMAs**

Experimental studies22 have shown that IL-6 exerts a direct
concentration-dependent and, more importantly, reversible
negative inotropic action on human pectinate and hamster
papillary muscle preparations. In our study, IL-6 levels at
peak DSE and at 30 minutes of recovery were associated with
an increased duration of WMAs after cessation of dobuta-
mime infusion. Finkel et al22 also have demonstrated that a
negative inotropic effect of IL-6 on myocardial cells was
observed within 2 minutes, was maximal after 5 minutes,
remained constant for 20 minutes, and was completely
reversed within 40 minutes after IL-6 removal.

In our study, we showed that increased production of IL-6 at
peak DSE, caused by either ischemia16 or impaired hemodynam-
ics of LV,22 extends into the first 30 minutes of recovery and
thus may mediate the delayed recovery of WMAs after DSE.

We have previously shown20 a close relation between severity
of WMAs at peak DSE and the duration of WMAs into
recovery. In the present study, we extend our previous findings
by demonstrating that increased production of IL-6 may be the
biochemical link between severity of WMAs at peak DSE and
delayed recovery of WMAs after DSE.

**MCSF Levels**

In the present study, demand-driven ischemia induced by
DSE was not associated with an increase in MCSF plasma
levels 30 minutes into recovery after DSE. This finding may
be explained by the long transcription time of MCSF gene
after stimulation (3 to 24 hours),39 which may not permit
detection of a significant rise of MCSF protein into the
circulation within 30 minutes after the onset of ischemia.
Using 24-hour Holter monitoring, we have previously shown
that elevated resting MCSF levels by enhanced thromboxane
A2 production may facilitate ischemic episodes that are attributable to transient reduction of coronary flow (reduced supply) and not to increases of oxygen demand. Aspirin treatment reduced MCSF levels in parallel to the number and duration of ischemic episodes during Holter monitoring. Thus, we proposed that elevated MCSF levels may be one of the causes and not the result of reduced coronary blood flow. The findings of the present study provide additional evidence that demand-driven myocardial ischemia may not raise MCSF levels shortly after a reversible ischemic event. However, this may not preclude a late increase in MCSF (>30 minutes). Furthermore, in the present study, the concurrent use of aspirin, statins, and ACE inhibitors by a large percentage of patients with an ischemic response at DSE (88%, 42%, and 32%, respectively) may have blunted an early (<30 minutes) rise in MCSF after ischemia because these medications have been shown to reduce MCSF production.

**Tissue Factor**

In this study, we have shown for the first time that ischemia elicited by DSE induces increased production of TF levels at peak stress that extends at least for 30 minutes into recovery. Conversely, in patients without ischemia, TF levels remained unchanged. Increased production of oxygen radicals or cytokines during ischemia may induce increased expression of TF in circulating monocytes, vascular endothelial cells, resident macrophages, and smooth muscle cells. Experimental studies have confirmed an increased activity of the TF-thrombin pathway during ischemia-reperfusion, resulting in subsequent myocardial injury. In our study, however, unlike IL-6 levels, TF levels were not associated with the extent of RWMA at peak stress or their delayed recovery after DSE, suggesting that the TF pathway may not mediate the occurrence of reversible WMAs after an ischemic insult. Moreover, the lack of a direct relation between IL-6 and TF levels throughout DSE suggests that this cytokine may not contribute to activation of the TF pathway during or after an episode of reversible ischemia. Conversely, among patients with an ischemic response, those with increased resting MCSF levels demonstrated a greater increase in TF levels at peak stress and recovery period after DSE compared with baseline. Furthermore, there was a significant relation between MCSF and TF levels throughout the DSE. Our findings suggest that MCSF may play an important role in activating the TF pathway and, in addition to the elevated IL-6 levels, may contribute to the development of a procoagulant state after reversible ischemia.

MCSF is an atherogenic growth factor that causes monocyte activation and promotes TF expression at atherosclerotic lesions. Furthermore, MCSF is a major triggering factor for increased monocyte chemotactic protein-1 production, which is closely linked to increased circulating levels of TF in patients with acute coronary syndromes. Thus, endothelial dysfunction induced by ischemia at DSE may lead to monocyte chemotactic protein-1 production that is further enhanced by elevated MCSF levels, causing increased TF plasma levels. These pathophysiological mechanisms may explain the relation between circulating levels of MCSF and TF observed in our study during and after reversible ischemia elicited during DSE.

**Study Limitations**

The following limitations should be acknowledged. Inflammatory indexes were measured in peripheral blood. This does not allow firm conclusions on the release of these factors within the coronary circulation.

Medication may affect plasma levels of the measured inflammatory indexes. The effect of dobutamine per se on inflammatory markers is not known. However, both patient groups with and without ischemia received similar antianginal, antiplatelet, and lipid-lowering treatment, and both received the same amount of dobutamine during stress. Thus, any possible influence of the medication or dobutamine per se on cytokine plasma levels was distributed equally within the study groups.

Patients with no ischemia during DSE had slightly higher baseline levels of IL-6 and TF compared with patients with ischemia at DSE. This difference may be attributed to the lower baseline ejection fraction and higher WMS compared with patients with ischemia during DSE.

Finally, increased vascular shear stress in patients with more extensive vascular disease might have influenced cytokine plasma levels. Although study subgroups did not differ in age, sex, atherosclerotic risk factors, or anatomic extent of CAD, suggesting a similar extent of vascular disease, further controlled trials are needed to investigate this possibility.

**Conclusions**

In this study of patients with chronic CAD, we demonstrated that myocardial ischemia elicited during DSE caused a significant increase in IL-6 and TF plasma levels at peak stress that were maintained through 30 minutes into recovery. Thus, demand-driven ischemia may contribute to a prothrombotic state in patients with chronic CAD by inducing procoagulant cytokines such as IL-6 and TF. Patients with an ischemic response who had high resting MCSF levels demonstrated a greater increase in TF levels at peak DSE and recovery, suggesting an important role of MCSF in activation of the TF pathway under conditions of hypoxia. Patients with extensive myocardial ischemia during DSE had a greater release of IL-6 during stress and recovery. Furthermore, IL-6 levels at peak stress and recovery period were associated with the time to recovery of newly induced WMAs by DSE, probably by exerting a negative inotropic action on myocardium.

**Acknowledgment**

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Myocardial Ischemia Induces Interleukin-6 and Tissue Factor Production in Patients With Coronary Artery Disease: A Dobutamine Stress Echocardiography Study
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