Increased Expression of Neutrophil and Monocyte Adhesion Molecules in Unstable Coronary Artery Disease

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**Background.** A rapid increase in leukocyte adhesion to endothelial cells is one of the first events in the acute inflammatory response and in the pathogenesis of vascular diseases. A subgroup of cell surface glycoproteins (the CD11/CD18 complex) play a major role in the leukocyte adhesion process; in particular, the CD11b/CD18 receptor can be up-regulated severalfold in response to chemotactic factors. The purpose of this study was to assess whether up-regulation of granulocyte and monocyte CD11b/CD18 receptors takes place during the passage of blood through the coronary tree of patients with clinical manifestations of ischemic heart disease.

**Methods and Results.** Thirty-nine patients who underwent diagnostic coronary arteriography were studied. Group 1 (15 patients) had a clinical diagnosis of unstable angina, group 2 (14 patients) had stable exertional angina, and group 3 (10 patients) had atypical chest pain. Simultaneous sampling from the coronary sinus and aorta was obtained before coronary arteriography. Cell surface receptors were detected by direct immunofluorescence evaluated by flow cytometry using monoclonal antibodies tagged with fluorescent markers. Leukocytes were stained in unseparated blood to avoid in vitro manipulation that could activate phagocytes. Group 1 and 2 patients had significant coronary artery disease (>50% coronary narrowing in at least one major coronary vessel), whereas group 3 patients had normal coronary arteries. In group 1, granulocytes and monocytes showed a significantly higher expression of the CD11b/CD18 adhesion receptor in the coronary sinus than in the aorta (both *P* < .01), whereas no difference in CD11b/CD18 expression was seen in groups 2 and 3.

**Conclusions.** Patients with unstable angina have an increased expression of granulocyte and monocyte CD11b/CD18 adhesion receptors, indicating that an inflammatory reaction takes place within their coronary tree. Activation of these leukocytes may induce coronary vasoconstriction, favor thrombotic processes, and further activate platelets, thus having potential implications on the pathogenesis of unstable coronary artery disease. (Circulation 1993;88:358-363)

**Key Words** • phagocytes • integrins • angina

Adhesion of neutrophils and monocytes to endothelial cells is the initial event in the acute inflammatory response and in the pathogenesis of vascular disease. Recent experimental and clinical observations have begun to define the molecular determinants on the surface of leukocytes that contribute to the adhesive process. These studies have established the in vivo critical role of CD11/CD18 leukocyte adhesion molecules, a family of cell-surface glycoproteins consisting of three heterodimers sharing a common β-subunit with a distinct α-subunit (CD11a, CD11b, CD11c). Regarding adhesion to endothelial cells, CD11b/CD18 and CD11a/CD18 receptors are particularly important. Whereas CD11a/CD18 is constitutively expressed in the plasma membrane and is not upregulated, CD11b/CD18 can be upregulated severalfold from intracellular granules by chemotactic factors such as C5a, interleukin-8, and platelet activating factor. Adhesion of neutrophils and monocytes to endothelial cells of coronary arteries and subsequent leukocyte activation may be relevant in the progression and evolution of atherosclerotic coronary disease. Recent data also suggest a role for inflammation in the pathophysiology of unstable angina. This study was undertaken to assess whether up-regulation of CD11b/CD18 adhesion receptors of neutrophils and monocytes occurs during the passage of blood through the coronary tree of patients with coronary heart disease.

**Methods**

**Study Patients**

Thirty-nine patients who underwent diagnostic coronary arteriography were studied. All patients gave writ-
ten informed consent, and the protocol was approved by the hospital ethics committee. Fifteen patients (group 1) had a clinical diagnosis of unstable angina defined as class IIIB or IIIIB of the Braunwald classification. All these patients had chest pain at rest associated with transient ST-segment changes on ECG unaccompanied by serum creatine kinase elevation or a new Q wave in the ECG.\textsuperscript{14} Fourteen patients (group 2) suffered from stable exertional angina and had a positive exercise test defined as the development of ST-segment depression of >1 mm. The remaining 10 patients (group 3) complained of chest pain but had a negative exercise test. Cardioactive drugs, which included nitrates, $\beta$-blockers, and calcium antagonists, were not discontinued in most of the patients at the time of coronary arteriography. Group 1 patients were also taking antiaggregating agents (12 patients, aspirin; 3 patients, ticlodipine). At the time of the study, no patient had congestive heart failure or an acute infective disease. No attempt was made to alter the patients' medications, and those taking nonsteroidal anti-inflammatory drugs or steroids were excluded. Significant coronary artery disease was defined as >50% narrowing in the luminal diameter of any individual coronary vessel.

**Study Protocol**

Patients were studied in the fasting state after premedication with diazepam (10 mg). At the beginning of the procedure, an 8F pigtail catheter was advanced from the femoral artery and placed in the ascending aorta, and a 7F Gorlin catheter was inserted in an antecubital vein and placed in the coronary sinus. Simultaneous sampling (5 mL of heparinized blood) was obtained from the aorta and coronary sinus before the injection of contrast medium. Aortic and coronary sinus blood was collected into heparinized plastic syringes and immediately placed in polystyrene tubes at 4°C.

**Monoclonal Antibodies and Direct Immunofluorescence**

Cell surface receptors were detected by direct immunofluorescence evaluated by flow cytometry. The leukocytes were stained in whole (unseparated) blood to avoid any in vitro manipulation that might activate phagocytes.\textsuperscript{15} The following monoclonal antibodies were tested: OKM1 (Ortho Diagnostics, Milan, Italy), used as the anti-CD11b/CD18 complex; LFA-1 (Sorin Biomedica Saluggia, Italy), used against the CD11a/CD18 complex; T11-FITC (Coulter Immunology, San Giuliano Milanese, Italy), the MoAb against the T-lymphocyte CD2 receptor; and Mo2-FITC (Coulter Immunology, San Giuliano Milanese, Italy), used against the monocyte CD14 receptor. Anti-mouse control FITC was obtained from AMD Alma Export, Firenze, Italy. All these MoAbs were incubated at a final concentration of 10 $\mu$L per 100 $\mu$L of whole blood for 30 minutes on ice, washed twice before and after lysis of erythrocytes by adding 2 mL of ice-cold erythrocyte-lysing solution (NH$_4$Cl 2.08 g; Na$_2$EDTA 0.0108 g; NaHCO$_3$ 0.21 g in 250 mL H$_2$O), and analyzed. Nonspecific immunofluorescence was determined by using the control MoAbs, and the cell purity for granulocytes and monocytes was evaluated on the bitmap gating of the flow cytometer. Analysis was performed by flow cytometry using a fluorescence-activated cell sorter (Epics Profile II, Coulter Immunology). The laser 488-nm band was run at 500 mW of power. Gating for granulocytes and monocytes was determined by the dot blot generated by forward angle versus right angle scatter. Fluorescence and forward angle scatter of microscopy DNA check (Coulter Immunology) were used for instrument calibration. Fluorescence intensity of each cell was recorded as a mean channel number over the logarithmic range of 1 to 1024. The quantity of mean per cell expression of membrane glycoproteins was reported as mean channel of fluorescence intensity (Log/FL). Statistical analysis was performed with the Student’s $t$ test for paired data calculated with the Apple IIsi STATVIEW program.

**Results**

**Clinical and Angiographic Data**

In group 1, there were 11 men and 4 women, with a mean age of 54 years (range, 41 to 69). Ten patients had single-vessel disease and five had multivessel disease; mean ejection fraction was .59 (range, .46 to .80). In group 2, there were 13 men and 1 woman, with a mean age of 58 years (range, 48 to 70). Six patients had single-vessel disease and eight had multivessel disease, with mean ejection fraction of .52 (range, .24 to .76). Group 3 included seven men and three women, with a mean age of 56 years (range, 35 to 70). All patients had normal coronary arteries: Three had mild to moderate mitral insufficiency caused by mitral valve prolapse and three had mild to moderate aortic regurgitation. Mean ejection fraction was .60 (range, .54 to .74).

**Expression of Neutrophil and Monocyte Adhesion Receptors**

Mean neutrophil and monocyte counts are shown in Table 1: No difference was found within each group between coronary sinus and aortic blood. Percentage of positive cells is shown in Table 2. More than 90% of monocytes and granulocytes bound LFA-1 and OKM1 (the MoAbs against the CD11a/CD18 and the CD11b/CD18 complex). The T-lymphocyte-specific anti-CD2 was bound by only 1% or less of cells, whereas the monocyte-specific anti-CD14 was bound by 94% to 99% of monocytes and by less than 1% of granulocytes, indicating the purity of the bitmap gating for granulocytes and monocytes analyzed by the flow cytometer.

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**Table 1. Monocyte and Granulocyte Counts in Aorta and Coronary Sinus in the Three Groups of Patients**

<table>
<thead>
<tr>
<th>Group</th>
<th>Monocytes Aorta</th>
<th>Monocytes Coronary sinus</th>
<th>Granulocytes Aorta</th>
<th>Granulocytes Coronary sinus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>240±0.06</td>
<td>280±0.04</td>
<td>3600±0.40</td>
<td>3800±0.80</td>
</tr>
<tr>
<td>Group 2</td>
<td>180±0.07</td>
<td>260±0.05</td>
<td>3400±0.60</td>
<td>3600±0.50</td>
</tr>
<tr>
<td>Group 3</td>
<td>240±0.05</td>
<td>200±0.04</td>
<td>3900±0.80</td>
<td>3800±0.70</td>
</tr>
</tbody>
</table>

Values are mean±SEM (mm$^3$).
Table 2. Percentage of Positive Cells on Bitmap Gating of Monocytes and Granulocytes in Aorta and Coronary Sinus in the Three Groups of Patients

<table>
<thead>
<tr>
<th>MoAbs</th>
<th>Monocyte bitmap (% of positive cells)</th>
<th>Granulocyte bitmap (% of positive cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aorta</td>
<td>G1</td>
</tr>
<tr>
<td>Negative control</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td>Anti-CD2</td>
<td>0.8</td>
<td>0.7</td>
</tr>
<tr>
<td>Anti-CD14</td>
<td>96.0</td>
<td>94.3</td>
</tr>
<tr>
<td>Anti-CD11a/CD18</td>
<td>94.5</td>
<td>99.0</td>
</tr>
<tr>
<td>Anti-CD11b/CD18</td>
<td>97.4</td>
<td>98.4</td>
</tr>
</tbody>
</table>

G1, group 1 (unstable angina); G2, group 2 (stable angina); G3, group 3 (normal coronary arteries).

Table 3 shows the individual values of the mean channel of the fluorescence intensity for the anti-CD11b/CD18 complex of granulocytes and monocytes. In patients with unstable angina, neutrophils and monocytes showed a significantly higher expression of the CD11b/CD18 complex in the coronary sinus than in aortic blood ($P<0.01$ and $P<0.01$, respectively; Figs 1 and 2). Such difference was not observed in patients with stable angina or in those with normal coronary arteries. No difference was found in CD11a/CD18 expression from monocytes and granulocytes between coronary sinus and aortic mean channel values in each of the three patient groups (Fig 3).

Discussion

In this study, neutrophils and monocytes taken from the coronary sinus of patients with unstable angina were found to have increased expression of CD11b/CD18 adhesion receptors, thus demonstrating that an inflammatory reaction takes place in the coronary tree of patients with unstable angina. In vitro and in vivo studies show that activation of neutrophils results in upregulation of these surface glycoproteins. The chemoattractant peptide FMLP is able to induce a threefold increase in CD11b expression on purified neutrophils pretreated with cytochalasin-B and with platelet activating factor. The importance of the CD11/CD18 integrin for tissue injury in vivo has been demonstrated in a number of animal models. Addition of anti-CD18 MoAbs can reduce tissue injury and mortality in ischemia reperfusion-induced shock in rabbits and myocardial infarct size in dogs. Moreover, neutrophils accumulating at sites of acute inflammation in vivo have been shown to increase their surface density of CD11b/CD18 receptors compared with intravascular neutrophils simultaneously isolated from the same animals. The physiological significance of enhanced neutrophil CD11b/CD18 expression is also underlined by its detection in clinically active systemic lupus erythematosus as well as in the synovial fluid of patients with rheumatoid arthritis.

In our study, upregulation of the CD11b/CD18 complex was not accompanied by any difference between aortic and coronary sinus leukocyte count. This finding seems to exclude entrapment of these cells in the coronary vasculature. Adhesion of leukocytes to endothelial cells is a complex phenomemon involving receptors and ligands on both surfaces, and it is possible that stimulation of CD11b/CD18 receptor expression was insufficient to promote trapping of neutrophils in microvessels. In this study we did not correlate our findings with other biochemical indices of neutrophil activation such as elastase release. However, Dinerman et al recently found that plasma levels of a neutrophil elastase-derived fibrinopeptide (B-β 30-43) were 13-fold higher in patients with unstable angina compared with control subjects. Previous data had suggested an inflammatory component in “active” angina: Berk et al observed high levels of C-reactive protein, an interleukin-1 acute-phase reactant, in the peripheral blood of patients with unstable angina. Furthermore, adventitial infiltration of inflammatory cells involving (in most cases) autonomic nerve fibers was observed at autopsy in 12 patients with crescendo angina at rest. In nine of such cases, a coronary thrombus superimposed on atherosclerotic coronary plaques was also found. Moreover, Forman et al reported the case of a patient with variant angina complicated by sudden death in whom mast cell infiltration was found at the site of angiographic documentation of coronary spasm. These pathological observations provide a link between an active atherosclerotic process and an inflammatory response in patients with unstable angina culminating in sudden cardiac death.

Chemotactic factors able to induce activation of neutrophils and monocytes are potentially released into the coronary circulation of patients with unstable angina. The presence of an acute thrombotic process leads to increased plasmin activity, which can cause the activation of the complement system. Yasuda et al found increased plasma levels of iC3b and C3d in 17 patients with unstable angina, suggesting complement activation in this clinical setting. Moreover, the terminal C5b-9 has been localized in atherosclerotic plaques and could come in contact with the circulating blood after plaque rupture. Likewise, break or ulceration of coronary lesions may provoke the release of tumor necrosis factor (TNF)-α, which is a constituent of coronary atherosclerotic plaques. This cytokine can induce neutrophil-mediated endothelial damage, and recent studies suggest that a specific CD11b/CD18-mediated signal triggers toxicity of TNF-activated neutrophils.

Activation of neutrophils and monocytes may have important consequences in unstable angina because these leukocytes, once stimulated, may release a variety of potentially toxic and vasoactive substances, in particu-
TABLE 3. Percentage Positivity of Granulocytes and Mean Channel of Fluorescence Intensity for the Anti-CD11b/CD18 Complex

<table>
<thead>
<tr>
<th>Patients</th>
<th>Monocytes</th>
<th>Granulocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aorta</td>
<td>Coronary sinus</td>
</tr>
<tr>
<td>Group 1</td>
<td>%</td>
<td>MC</td>
</tr>
<tr>
<td>1</td>
<td>98.7</td>
<td>33.8</td>
</tr>
<tr>
<td>2</td>
<td>94.6</td>
<td>26.6</td>
</tr>
<tr>
<td>3</td>
<td>98.7</td>
<td>8.9</td>
</tr>
<tr>
<td>4</td>
<td>95.4</td>
<td>5.9</td>
</tr>
<tr>
<td>5</td>
<td>98.7</td>
<td>11.3</td>
</tr>
<tr>
<td>6</td>
<td>94.6</td>
<td>5.7</td>
</tr>
<tr>
<td>7</td>
<td>99.0</td>
<td>7.6</td>
</tr>
<tr>
<td>8</td>
<td>98.9</td>
<td>9.0</td>
</tr>
<tr>
<td>9</td>
<td>96.5</td>
<td>12.3</td>
</tr>
<tr>
<td>10</td>
<td>99.0</td>
<td>11.4</td>
</tr>
<tr>
<td>11</td>
<td>100.0</td>
<td>20.8</td>
</tr>
<tr>
<td>12</td>
<td>98.8</td>
<td>12.6</td>
</tr>
<tr>
<td>13</td>
<td>94.8</td>
<td>14.5</td>
</tr>
<tr>
<td>14</td>
<td>98.7</td>
<td>9.5</td>
</tr>
<tr>
<td>15</td>
<td>94.8</td>
<td>9.5</td>
</tr>
<tr>
<td>Group 2</td>
<td>97.4±0.5</td>
<td>13±2.0</td>
</tr>
<tr>
<td>Group 3</td>
<td>98.4±0.3</td>
<td>11.8±2.6</td>
</tr>
</tbody>
</table>

%: mean channel values.

ular, the lipoxynase-derived metabolites of arachidonic acid, leukotrienes C4, D4, and E4.9-31 These substances have been shown to induce coronary vasoconstriction and decrease coronary flow in a variety of preparations. In addition, the respiratory burst results in the formation of oxygen-derived free radicals, which are able to alter microvascular permeability and influence vascular smooth muscle tone. Moreover, activated monocytes release interleukin-1, which induces biosynthesis and cell surface expression of procoagulant activity in cultured endothelial cells.32 Recently, Neri Sernieri et al33 found that monocytes from patients with
unstable angina showed high tissue factor–like procoagulant activity, whereas preparations from patients with stable angina and normal control subjects developed only small amounts of procoagulant activity. These authors speculated that activation of monocytes may be responsible for the increased thrombin formation in unstable angina. Finally, leukocyte-derived products enhance platelet aggregation, whereas products of platelet activation may aid neutrophil accumulation at inflammatory sites. \textsuperscript{34-36} Therefore, in unstable angina, activated leukocytes and platelets potentiate each other’s effects, favoring the occurrence of coronary vasoconstriction and thrombosis.

**References**


Increased expression of neutrophil and monocyte adhesion molecules in unstable coronary artery disease.
A Mazzone, S De Servi, G Ricevuti, I Mazzucchelli, G Fossati, D Pasotti, E Bramucci, L Angoli, F Marsico and G Specchia

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