Increased Expression of Neutrophil and Monocyte Adhesion Molecules LFA-1 and Mac-1 and Their Ligand ICAM-1 and VLA-4 Throughout the Acute Phase of Myocardial Infarction

Possible Implications for Leukocyte Aggregation and Microvascular Plugging

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Objectives. This study sought to evaluate expression of adhesion molecules on neutrophils and monocytes throughout the acute phase of myocardial infarction.

Background. Neutrophil and monocyte counts increase within days from onset of acute myocardial infarction. Because leukocytes are recruited to the involved myocardial region, we postulated that these activated cells would display an increased expression of adhesion molecules necessary for effective endothelial transmigration.

Methods. We measured the expression of neutrophil and monocyte lymphocyte function associated antigen-1 (LFA-1), Mac-1, very late after activation antigen-4 (VLA-4) and intercellular adhesion molecule-1 (ICAM-1) by flow cytometry throughout the acute phase of acute myocardial infarction in 25 patients and 10 age-matched control subjects.

Results. Expression of Mac-1 on neutrophils increased significantly, whereas no expression of VLA-4 and ICAM-1 was detected. The expression of LFA-1, Mac-1, VLA-4 and ICAM-1 on the monocyte cell membrane in patients with an acute myocardial infarction was increased compared with that in control subjects by 22% (on day 7), 67%, 13% and 44% (all on day 4), respectively (all p < 0.001). Elevated density of monocyte-specific CD14 in the AMI versus the control group was also shown (30%, p < 0.001).

Conclusions. Increased expression of neutrophil and monocyte adhesion molecules may contribute to their adhesion to endothelium in the ischemic territory. This adhesion could feasibly precipitate vasoconstriction or add a local thrombotic effect due to tissue factor expression secondary to Mac-1 engagement. In addition, the manifestation of increased density of LFA-1 and Mac-1 by activated leukocytes with monocytes also expressing ICAM-1 suggests that leukocytes may form microaggregates that could cause microvascular plugging. This mechanism may facilitate the occurrence of the “no-reflow” phenomenon or slow coronary filling after acute myocardial infarction.

Infiltration of necrotic myocardial tissue by neutrophils within the first day and by monocytes beyond the third day of onset of acute myocardial infarction has long been documented by histopathologic examination (1,2). Increased neutrophil count is known to occur very commonly within the first day from onset of myocardial infarction (3). We have previously documented (Meisel SR, et al., in preparation), and more recently Tashiro et al. (4) have reported, an increase in monocyte count 2 to 3 days after myocardial infarction. Because these increases occur at the time that neutrophils and monocytes begin to accumulate in the involved myocardium, we hypothesized that this phenomenon represents peripheral recruitment of leukocytes to necrotic cardiac muscle. The monocytes play a major part in the necessary debridement of necrotic tissue through their scavenger function and thus set the stage for the healing process (1,2). The role of the neutrophils, in contrast, is less clear. However, the presence of leukocytes in the myocardium requires endothelial transmigration, which is facilitated by the expression of adhesion molecules by leukocytes and endothelial cells alike (5). We surmised, therefore, that the increase in neutrophil and monocyte counts during myocardial infarction would be accompanied by a corresponding increase in their expression of cell-surface adhesion molecules to enhance endothelial transmigration required for tissue infiltration.

Upregulation of neutrophil and monocytic adhesion molecules is clinically relevant because activated leukocytes could adhere to altered coronary endothelium downstream to the culprit lesion and modify local vasomotor function (6). Furthermore, this adherence could lead to the enhancement of
monocyte tissue factor expression due to engagement of Mac-1 receptor (7), thus adding a local thrombotic procoagulant effect as well. This mechanism may explain the occasional occurrence of the “no-reflow” phenomenon in the acute stage of myocardial infarction or persistent ischemia despite rean-
alization of the occluded infarct-related artery.

The goal of the present study, therefore, was to evaluate the changes in the expression of neutrophil and monocyte membrane-associated adhesion molecules throughout the acute phase of myocardial infarction.

Methods

Subjects. The study included 25 patients (18 men, 7 wom-
en; mean [±SD] age 59 ± 14 years), 9 with an anterior myocardial infarction, and 16 with an acute myocardial infarction in other anatomic locations (inferior or inferoposterior). Nineteen patients (76%) received thrombolytic therapy, and no patient had a clinical course complicated by cardiogenic shock or significant congestive heart failure. Patients, recruited from June 1994 to March 1995, were consecutively included if they fulfilled the following criteria: 1) onset of severe symp-
toms <3 h before admission, to exclude patients with myocardial reinfarction or a “stuttering” pattern; 2) admission was between 7 AM and 3 PM to ensure prompt analysis of samples. Ten normal subjects, matched for age and gender (mean age 54 ± 9 years; seven men, three women), without any known underlying disease and taking no medication were selected as a control group. Acute myocardial infarction was defined by at least two of the three following major criteria: clinical presenta-
tion; typical and unequivocal electrocardiographic changes; and significant cardiac enzyme elevation (more than three times the upper limit of the normal range).

Adhesion molecules. The expression of the following adhe-
sion molecules on neutrophils and monocytes was measured using flow cytometry: 1) CD11a, which is the alpha-chain of the heterodimer CD11a/CD18, also termed lymphocyte function associated antigen-1 (LFA-1). 2) CD11b, which is the alpha-
chain of the heterodimer CD11b/CD18, also termed Mac-1. 3) CD29d, which is the alpha-chain of the heterodimer CD29d/CD49, also termed very late after activation antigen-4 (VLA-4). 4) CD54, termed intercellular adhesion molecule-1 (ICAM-1).

Another membrane molecule measured was CD14, which is the receptor that binds the complex lipopolysaccharide-
(SPSS for the PC) was used to evaluate statistical significance of differences between groups and day of sampling. Variables included in the analysis were LFA-1, Mac-1, VLA-4, ICAM-1 and CD14, and these were controlled by the same variables obtained in the control subjects. Pattern of changes between sampling days compared with control trend was also evaluated in the analysis. A resulting p value < 0.05 was deemed to signify statistical significance.

Results

Patients and control subjects. Patients and control subjects were matched for age and gender. Seventy-six percent of patients underwent thrombolytic therapy. Subgroup analysis of adhesion molecule expression according to the administration of thrombolytic therapy revealed no difference between patients who underwent such treatment and those who did not (data not shown); therefore, postmyocardial infarction patients were analyzed as one group for the evaluation of changes in adhesion molecule expression.

The average changes in the expression of the measured cell surface molecules on neutrophils and monocytes throughout the hospital period are displayed graphically in Figures 1 to 4. The expression of Mac-1 in neutrophils was increased in patients by 133% (p < 0.001) on day 1 compared with that in age-matched control subjects. No expression of VLA-4 and ICAM-1 was detected in neutrophils. The results demonstrate that the expression of the adhesion molecules LFA-1, Mac-1 and VLA-4 on monocytes was increased in patients compared with that in control subjects throughout the length of the hospital stay (days 1, 4 and 7) in a statistically significant manner (p < 0.001). The elevation in LFA-1 and Mac-1 was already evident at the day of presentation, demonstrating an early response. ICAM-1, in contrast, increased significantly in patients compared with control subjects from day 4 onward (p < 0.001) (Fig. 4). In monocytes, the average maximal

Figure 1. Intensity changes of LFA-1 expression in monocytes (A) and neutrophils (B) after an acute myocardial infarction. Intensity is expressed in mean channels. Statistical significance of difference: A, p<sub>group</sub> = 0.001 (p<sub>group</sub> represents statistical significance of difference between groups); p<sub>day</sub> = 0.003 (p<sub>day</sub> represents statistical significance of difference between groups); interaction = 0.474 (showing statistical significance of divergent trend between groups). B, p<sub>group</sub> and p<sub>day</sub> = NS. Solid bars = control group; open bars = infarction group.

Figure 2. Intensity changes of Mac-1 expression in monocytes (A) and neutrophils (B) after an acute myocardial infarction. Intensity is expressed in mean channels. Statistical significance of difference: A, p<sub>group</sub> < 0.001; p<sub>day</sub> = 0.023; interaction = 0.95; B, p<sub>group</sub> and p<sub>day</sub> = 0.001. Abbreviations and symbols as in Figure 1.

Figure 3. Intensity changes of VLA-4 expression in monocytes after an acute myocardial infarction. Intensity is expressed in mean channels. Statistical significance of difference: p<sub>group</sub> < 0.001; p<sub>day</sub> = 0.01; interaction = 0.42. Abbreviations and symbols as in Figure 1.
neutrophils in patients with an acute myocardial infarction. Intensity is expressed in mean channels. Statistical significance of difference: $p_{\text{group}} < 0.001$; $p_{\text{day}} = 0.005$; interaction = 0.005. Abbreviations and symbols as in Figure 1.

change and day of occurrence of LFA-1, Mac-1, VLA-4, ICAM-1 and CD14 as recorded were 22% on day 7, 67% on day 4, 13% on day 4, 44% on day 4 and 29% on day 4, respectively.

CD14, the monocyte-specific cell surface marker, displayed a pronounced difference between patients and normal control subjects ($p < 0.001$) but without a temporal variation throughout the study period ($p \leq 0.08$). This cell surface receptor responded as a preformed cellular component that reached its maximal expression very soon after the onset of myocardial infarction.

No significant changes in percent distribution of gated neutrophils or monocytes throughout the study period were recorded with respect to adhesion molecules studied. Therefore, it can be concluded that only the fluorescence intensity (corresponding to receptor density) increased, but not the fraction of leukocytes expressing the adhesion molecules studied.

The average monocyte counts for the patients were $599 \pm 268$, $772 \pm 319$ and $778 \pm 309$ cells/mm$^3$ for days 1 (on admission), 2 and 3, respectively. Conversely, the average monocyte counts for the control group were $459 \pm 154$, $420 \pm 116$ and $474 \pm 175$ cells/mm$^3$ for their three measurements. The difference between the groups was statistically significant ($p < 0.0025$). No statistically significant difference between monocyte counts obtained on disparate sampling days in both study groups was demonstrated.

The average creatine kinase (CK) values for postinfarction patients were $1,133 \pm 2,031$, $2,128 \pm 2,100$ and $906 \pm 1,200$ U/liter for days 1, 2 and 3, respectively (normal range $<190$ U/liter); CK values were not obtained for normal subjects. There was no correlation between CK levels in patients and the magnitude of change in the expression of any of the adhesion molecules evaluated.

**Discussion**

The principal finding in the present study is that circulating neutrophils in patients with an acute myocardial infarction express higher densities of the cell surface adhesion molecule Mac-1 than control subjects, and monocytes display significantly more LFA-1, Mac-1, VLA-4 and ICAM-1, as well as the monocyte-specific marker CD14. In addition, we confirmed previous reports that after myocardial infarction, there is an increase in monocyte count (4). The patients were age and gender matched to the control subjects. Most patients (76%) were treated with thrombolytic agents, a fact that can be accounted for by the study design, which enlisted patients with a short duration of symptoms before admission, but this did not affect the extent of change in the expression of adhesion molecules evaluated.

**Adhesion molecule interactions.** LFA-1 and Mac-1 are beta$_2$-integrins that promote the adherence of neutrophils and monocytes to ICAM-1 on the endothelial cell and facilitate their spread on its surface as the second step in the adhesive cascade (5,8). LFA-1 is found on all leukocytes, whereas Mac-1 is present in myeloid cells and monocytes. ICAM-1, as well as vascular cell adhesion molecule-1 (VCAM-1), are members of the immunoglobulin supergene family, but the former serves as a ligand for the beta$_2$-integrins, LFA-1 and Mac-1. ICAM-1 is moderately expressed on endothelial cells and is also localized on lymphocytes and monocytes (5). Expression of ICAM-1 on endothelial cells is upregulated by the cytokines interleukin (IL)-1, tumor necrosis factor-alpha (TNF-alpha) and interferon-gamma (9,10). Hence, its presence on vascular endothelial cells enables the adherence of leukocytes and their transmigration into the tissue.

VLA-4 is a beta$_1$-integrin leukocyte adhesion molecule that binds to VCAM-1. The latter is an adhesion molecule that resides on endothelial cell membranes but is mainly expressed after activation with the cytokines IL-1, IL-6 and TNF-alpha, which are increased in myocardial infarction and feasibly more in the vicinity of myocardial necrosis. Monocyte VLA-4 interaction with endothelial VCAM-1 induces leukocyte “rolling” on lumen surface (11) and augments the effect of the beta$_2$-integrin–ICAM-1 interaction on monocyte endothelium adherence. Hence, the finding of significantly increased expression of the adhesion molecules LFA-1, Mac-1 and VLA-4 on monocytes clearly attests to their enhanced tendency to adhere to activated endothelial cells.

The expression of monocyte ICAM-1 in our study was significantly increased after myocardial infarction. Despite the fact that the role of ICAM-1 on the leukocyte membrane has not been fully elucidated, this may indicate its significance in mediating leukocyte–leukocyte adhesion as a means of intercellular communication.

**Proposed mechanism for monocyte activation.** A plausible mechanism that accounts for leukocyte activation after an acute myocardial infarction can be based on T cell activation by cardiac antigens that are normally contained within the intact myocyte and that are released from necrotic cells after infarction. Activation of T cells generates cytokines, such as interferon-gamma, IL-3 and granulocyte–monocyte colony stimulating factor (GM-CSF). These cytokines cause leukocyte activation and, subsequently, the secretion from monocytes of IL-1 and TNF-alpha, which further induces synthesis and
release of GM-CSF and IL-6 by fibroblasts and endothelial cells (12), along with augmentation of monocyte activation as an autocrine effect (13). Indeed, TNF-alpha concentrations have been reported to be increased in myocardial infarction (14,15), as well as the levels of IL-1 (16). These lymphocyte- and monocyte-derived cytokines are probably instrumental not only in increasing peripheral monocyte count but also in inducing their activation, as manifested by the elevated cell surface density of adhesion molecules, in keeping with the findings of the present study.

**Clinical implications.** Evidence for changes in the expression of leukocyte cell surface adhesion molecules is important and clinically relevant. It may provide the pathogenesis for the “no-reflow” or “slow-reflow” phenomenon that seems to occur often in the context of myocardial infarction after reperfusion by thrombolysis, as described by Gibson et al. (17). This phenomenon may also account for some postinfarction events, such as ongoing ischemia and infarction extension. Activated leukocytes exhibiting increased expression of cell surface adhesion molecules could adhere to altered coronary endothelium downstream to culprit lesion and modify local vasomotor function (6), thus compromising runoff flow. Ma et al. (18) showed that ICAM-1–dependent neutrophil adherence plays an important role in reperfusion injury and that neutrophil adherence and infiltration contribute significantly to coronary endothelial dysfunction. Monocyte adhesion, if compounded by enhancement of monocyte tissue factor expression due to engagement of Mac-1 receptor, as reported by Fan and Edginton (7) and Lo et al. (19), could add a local procoagulant effect as well. Such a mechanism may explain the occasional occurrence of persistent postinfarction ischemia despite recanalization of occluded infarct-related artery or the slow flow observed in the culprit artery (17).

The finding that activated neutrophils and monocytes manifest increased density of Mac-1, whereas monocytes also express significantly enhanced density of LFA-1 and their ligand ICAM-1, suggests that after myocardial infarction, leukocytes may adhere to each other and form microaggregates. These may form locally at sites of adherence to dysfunctional endothelium or in the vicinity of the infarct-related coronary lesion and may embolize distally to small vessels, causing microvascular plugging and impeding perfusion, as depicted schematically in Figure 5. Engler et al. (20) actually demonstrated, by histopathologic and electromicroscopic examination, leukocyte plugging in capillaries of ischemic myocardium after reperfusion in a canine model, which lends credence to this hypothesis.

In theory, the interruption of leukocyte–leukocyte and leukocyte–endothelium interactions in acute myocardial infarction may be clinically salutary. Tanaka et al. (21) evaluated the effect of anti-CD18 monoclonal antibody infusion on myocardial neutrophil accumulation and infarct size in a dog model of ischemia and reperfusion. They found that neutrophil accumulation contributed to reduced postischemic microvascular perfusion, in keeping with the observation of Engler et al. (20); however, anti-CD18 monoclonal antibody infusion did not affect infarct size in that model. In rabbits, in contrast, it was demonstrated that neutrophils do exacerbate tissue injury after a 30-min period of ischemia (22). Infusion of an anti-CD18 monoclonal antibody before reperfusion resulted in a reduction of myocardial infarct size.

Acute myocardial infarction is a convenient model for the study of changes in leukocyte adhesion molecule expression secondary to ischemia and infarction because the magnitude of the response is such that local coronary effects overflow into the systemic circulation where they can be probed and quantitated by flow cytometry. Whether this represents saturation of the trapping capacity of the coronary microcirculation or some alteration of leukocyte adhesion molecules rendering them nonfunctional yet still recognizable as an antigenic entity by specific monoclonal antibodies cannot be presently determined. A similar process, although of a smaller extent and not discernible systemically, may be operating in unstable angina and non-Q wave myocardial infarction as well. Indeed, increased expression of neutrophil and monocyte adhesion molecule Mac-1 across the coronary circulation, but not in the systemic circulation, has been documented in patients with unstable coronary artery disease (23). In contrast to the finding in postmyocardial infarction patients, we have not recorded similar changes measured in systemic blood in patients with stable angina or in others admitted to the intensive care unit for other indications. It is worthwhile to note with regard to the specificity of this phenomenon that there have been a few reports on changes in the expression of adhesion molecules in the context of brain ischemia and infarction (24) and during clinical cardiopulmonary bypass (25). Most of the studies describing alterations of adhesion molecule expression in brain pathology reported local changes by endothelial cells or on infiltrating leukocytes. Nonetheless, one report (24) described...
changes in beta_2-integrin expression in circulating leukocytes from patients with ischemic stroke. It is possible that similar changes to those documented in the present study may be encountered in other conditions involving tissue necrosis. However, this is not a crucial point because all other pathologic processes can be easily differentiated from acute myocardial infarction on the basis of clinical signs and symptoms, as well as laboratory tests.

Conclusions. The present study confirms the hypothesis that after acute myocardial infarction, circulating neutrophils and monocytes display an increased cell surface concentration of the adhesion molecules LFA-1 and Mac-1 and also monocyte ICAM-1 and VLA-4, which indicate their activation with respect to leukocyte endothelial adherence. This expression signifies an enhanced propensity of leukocytes to adhere to endothelium and provides the mechanism for endothelial transmigration required for myocardial infarction by leukocytes, as documented after infarction. In addition, the significantly increased expression of monocyte ICAM-1 points to a possible role in leukocyte microaggregate formation through association with its upregulated ligands LFA-1 and Mac-1 on neutrophils and monocytes. Altered expression of leukocyte adhesion molecules may be of clinical importance because it provides a theoretical basis for changes in the coronary microvascular bed during myocardial infarction. This mechanism could serve as an explanation for the “no-reflow” phenomenon or postinfarction sequelae as ongoing ischemia despite patency of the culprit coronary artery. Because full elucidation of these changes may yield novel therapeutic interventions, further study in this field is warranted.

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References

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