Monoclonal Anti-CD18 Antibody Prevents Transcellular Biosynthesis of Cysteinyl Leukotrienes In Vitro and In Vivo and Protects Against Leukotriene-Dependent Increase in Coronary Vascular Resistance and Myocardial Stiffness

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Background—Cysteinyl leukotrienes (cys-LT) can constrict small and large vessels and increase vascular permeability. Formation of cys-LT arising from polymorphonuclear leukocytes (PMNL) and endothelial cell cooperation (transcellular synthesis) led to the hypothesis that PMNL–endothelial cell adhesion may represent a key step toward the formation of vasoactive cys-LT.

Methods and Results—We studied the effect of pretreatment with a monoclonal antibody directed against the CD18 subunit of PMNL β2-integrin on the synthesis of cys-LT in a PMNL-perfused isolated rabbit heart in vitro and in a model of permanent ligature of the left descending coronary artery in the rabbit in vivo. Challenge of PMNL-perfused rabbit hearts with formyl-met-leu-phe (0.3 μmol/L) caused synthesis of cys-LT and increase in coronary perfusion pressure that were prevented by the anti-CD18 antibody. Similar results were obtained with the use of A-23187 (0.5 μmol/L) as a challenge. Persistence of PMNL-associated myeloperoxidase activity in the perfusion buffer was observed in the presence of the anti-CD18 antibody, indicating decreased PMNL infiltration. Coronary artery ligature in vivo increased urinary excretion of leukotriene E₄, supporting the activation of the 5-lipoxygenase pathway during experimental acute myocardial infarction. Pretreatment with the anti-CD18 antibody (1 mg/kg) prevented the increase in leukotriene E₄ excretion.

Conclusions—These data support the importance of adhesion in promoting cys-LT formation, originating from PMNL–endothelial cell cooperation, and contributing to myocardial stiffness and increased coronary resistance. (Circulation. 2000;101:1436-1440.)

Key Words: leukocytes ■ endothelium ■ cell adhesion molecules ■ prevention
EC in processing the reactive intermediate LTA₄ into biologically active LTC₄ has been demonstrated. This process has been termed “transcellular biosynthesis” and suggests that the cellular environment (ie, cell-cell interactions) represents an important control mechanism in the production of eicosanoids, which may ultimately affect organ function. Indeed, challenge of PMNL within the coronary vasculature causes coronary vasoconstriction associated with PMNL extravasation and widespread perivascular edema, both dependent on endogenous cys-LT formation. PMNL-EC adhesion is regulated by several cell-surface adhesion molecules; among them PMNL β₂-integrins are known to play a significant role in firm adhesion of PMNL to EC.

In the present study, we provide evidence that a monoclonal antibody (mAb) directed against the CD18 subunit of PMNL β₂-integrins (a) inhibits cys-LT generation decreasing PMNL-dependent tissue edema and coronary resistance in the isolated heart of the rabbit in vitro and (b) inhibits the increased urinary leukotriene excretion occurring after acute myocardial infarction of the left ventricular wall in the rabbit in vivo.

**Results**

Intravascular challenge of granulocyte macrophage-colony stimulating factor (GM-CSF)–primed PMNL in the isolated perfused heart with the chemotactic peptide formyl-met-leu-phe (fMLP, 0.3 μmol/L) in the presence of a murine nonbinding mAb (MOPC-21, 5 μg/mL) resulted in a significant increase of coronary resistance to perfusion (coronary perfusion pressure, CPP), causing the arrest in systole in 3 of 4 isolated hearts within 30 to 45 minutes after challenge. Basal left ventricular end-diastolic pressure (LVEDP) values were very stable (5±0.2 mm Hg, n=4) and increased markedly after challenge (at 20 minutes, 55±14.6 mm Hg, n=4, P<0.01 vs basal).

High-performance liquid chromatography (HPLC) analysis of the total volume of the circulating perfusate (44 to 47 mL) collected at the end of the experiment allowed positive identification of cys-LT by on-line UV-spectrum analysis. Pretreatment with the anti-CD18 antibody (6.5E, 5 μg/mL) resulted in a significant inhibition of the increase in coronary perfusion pressure (CPP), allowing survival of all isolated hearts throughout the observation period of 60 minutes, and was accompanied by a significant decrease in cys-LT formation (Figure 1). LVEDP values did not differ from basal values (5±0.2 mm Hg, n=4). The assay of cell-associated myeloperoxidase (MPO) enzyme activity in the recirculating buffer confirmed a rapid disappearance of MPO in the presence of control mAb, whereas pretreatment with the anti-CD18 mAb resulted in a significantly inhibited adhesion of PMNL (Figure 2).

To test whether the observed effect of the anti-CD18 antibody could be reversed by a more sustained activation of the 5-lipoxygenase (5-LO), PMNL-perfused, isolated hearts were challenged with A-23187 (0.5 μmol/L). As previously reported, challenge with A-23187 induced the PMNL-dependent formation of cys-LT, together with a significant increase in CPP, which resulted in arrest in systole in 3 of 4 isolated hearts within 20 to 30 minutes after challenge. As observed with fMLP, pretreatment with the anti-CD18 mAb significantly reduced the increase in CPP, and all isolated hearts survived throughout the observation period of 30 minutes (Figure 3, left) and resulted in a marked suppression of the formation of cys-LT (Figure 3, right), suggesting the pivotal role of adhesion in their production.

The assay of circulating PMNL provided evidence of efficacy of the pretreatment with the anti-CD18 antibody in inhibiting PMNL adhesion. After A-23187 activation, a rapid disappearance of PMNL from the recirculating buffer was observed, suggesting intravascular adhesion. However, pre-
treatment with the anti-CD18 mAb resulted in persistence of PMNL-associated MPO activity in the recirculating buffer as a result of inhibited adhesion (Figure 4).

Challenge with fMLP (0.3 μmol/L, 60 minutes) of GM-CSF–primed PMNL preparations in suspension showed a substantial release of LTA4 metabolites, which was not affected by pretreatment with anti-CD18 mAb 6.5E (25.5 ± 3.4 vs 31.1 ± 2.9 pmol/10^6 PMNL in control and anti-CD18–treated cells, respectively; n = 3). Similarly, production of LTA4 metabolites on challenge with A-23187 (0.5 μmol/L, 30 minutes) was not affected by pretreatment with the anti-CD18 mAb (5 μg/mL) (291.3 ± 13.7 vs 265.3 ± 10.3 pmol/10^6 PMNL in control and anti-CD18–treated cells, respectively; n = 3).

In Vivo Studies
Excretion of LTE4 in urine was evaluated during the 3 hours after permanent ligature of the left descending coronary artery (coronary artery ligature, CAL) in the rabbit, resulting in acute myocardial infarction of the left ventricular wall, and was compared with the values obtained in sham-operated animals. Urinary excretion of LTE4 was significantly higher in the CAL group, treated with the nonbinding IgG1 mAb MOPC-21 (1 mg/kg IV, 15 minutes before ligature), indicating endogenous production of cys-LT during the ischemia associated with the coronary ligature. Treatment with the anti-CD18 mAb 6.5E (1 mg/kg IV, 15 minutes before ligature) fully prevented the increase in LTE4 excretion (Figure 5).

Discussion
In the present study, we report that a mAb against the functional epitopes of leukocyte CD18 complex of adhesive glycoproteins prevents the generation of cys-LT taking place through the interaction of PMNL with coronary EC. Cell adherence may therefore represent an important mechanism regulating leukotriene generation in situ and is in line with our current understanding of cys-LT as paracrine hormones.

Neither PMNL nor EC can synthesize cys-LT from the precursor AA; however, the former have been shown to
produce predominantly LTA₄, whereas the latter possess a remarkably effective metabolic capacity for cys-LT from the epoxide precursor LTA₄. It is therefore likely that during adhesion, a privileged interface between the donor PMNL and the acceptor EC is formed, creating the necessary conditions to transfer the unstable intermediate LTA₄. LTC₄-activated endothelium may then become adhesive for PMNL through the surface expression of platelet-activating factor and P-selectin, providing a self-amplifying loop that may result in increased transcellular synthesis of cys-LT.

The mechanism that explains the increase in coronary vascular resistance and myocardial stiffness involves activated PMNL attaching to the vascular endothelium and triggering transcellular biosynthesis of cys-LT; local formation of cys-LT results in edema formation and extravascular compression of coronary microvessels, as previously shown by scanning electron microscopy. Local production of cys-LT also may contribute to active coronary vasoconstriction; in fact, the increase in coronary perfusion pressure evoked by PMNL activation is partially reversible after intracoronary injection of sodium nitroprusside. The inhibition of PMNL-EC adhesion by the anti-CD18 mAb, reducing cell-cell contact and making transcellular biosynthesis events much less efficient, exerts protective effects against cardiac inflammation and its functional outcomes. We used the presence of cell-associated MPO activity as an indirect tool to quantitatively evaluate the extent of PMNL adhesion to its target cells and obtained evidence that anti-CD18 mAb effectively blunted PMNL sequestration through the coronary bed. A significant body of evidence supports the notion that the inflammatory tissue damage that accompanies ischemia or ischemia-reperfusion is mediated to a large extent by PMNL. Accordingly, prevention of leukocyte-EC interaction, through the use of mAbs directed against adhesion molecules, has proven successful in limiting ischemic damage in experimental models. A study with isolated PMNL-glomerular EC cocultures showed that transcellular synthesis of cys-LT was inhibited by pretreatment with an anti-CD18 mAb. Our work extends these findings to a functional organ system and provides a link between adhesion of PMNL, synthesis of cys-LT, and functional modifications.

The model of in vitro PMNL-dependent cardiac damage used for this study is different from more complex in vivo models of ischemia-reperfusion injury. Recently, a 54% reduction in PMNL accumulation and a 57% decrease of myocardial necrosis after ischemia-reperfusion was observed in CD18-deficient mice and intracellular adhesion molecule-1–deficient mice, supporting a critical role of these cell adhesion molecules in myocardial cell injury of the reperfused myocardium. The more significant functional protection observed in our study (>80% inhibition of increase in CPP and LVEDP) is not unexpected given the fact that our model is uniquely PMNL dependent, whereas it is conceivable that in vivo other cells and factors may contribute to the development of the cardiac injury.

Measurement of LTE₄ in urine has been largely adopted as a noninvasive, time-integrated index of cysteiny1 leukotriene synthesis in vivo. Evaluation of urinary LTE₄ excretion showed a significant increase after permanent coronary ligation in the rabbit, in agreement with the results of 2 independent groups reporting increased urinary LTE₄ excretion in patients with coronary artery disease and in patients after myocardial infarction. The observed inhibition after pretreatment with anti-CD18 antibody supports the hypothesis that pathophysiologically relevant cys-LT formation within an ischemic myocardium may represent the outcome of transcellular biosynthetic events. The increased urinary excretion of LTE₄ associated with the CAL observed in the present study is also clearly complementary with our previous results with the same model, in which we showed a significant decrease of the mortality rate by pretreatment with a specific leukotriene synthesis 5-lipoxygenase-activating protein (FLAP) inhibitor.

Although it may seem difficult to hypothesize the infiltration of neutrophils into the infarcted area in the time course described, we must point out that under our working hypothesis there is no need to have actual neutrophil infiltration, but it would be sufficient for them to adhere to EC to achieve local leukotriene formation associated with altered vascular permeability and tone. In fact, it has recently been shown that increased endothelial permeability occurs even in the absence of neutrophil infiltration.

The leukocyte count has been originally proposed as a valuable routine index for the assessment of risk for myocardial infarction. Since then, a number of epidemiological studies have shown the existence of a significant relation between blood white cell count and the occurrence of coronary heart disease (eg, angina pectoris and myocardial infarction). Furthermore, enhanced neutrophil expression of CD11b/CD18 adhesion receptors has been recently reported in patients with unstable angina. Our data, supporting the functional relevance of CD18-mediated, PMNL-dependent synthesis of cys-LT, provide a link between PMNL implication in the natural history of coronary heart disease and increased urinary LTE₄ levels in patients with cardiac ischemia, two observations apparently uncorrelated.

In conclusion, we propose that among the PMNL-dependent factors contributing to the development of cardiac damage associated with ischemia, the production of cys-LT through transcellular biochemical mechanisms may have a significant role and may represent a potential therapeutic target.

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