Accumulation of Membrane-Bound Urokinase on Monocytes in Atherosclerotic Patients is Accompanied by Suppression of Urokinase-Induced Monocyte Migration

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Membrane expression of β_2-integrins Mac-I, urokinase receptors, and membrane-bound urokinase on peripheral blood monocytes is studied in healthy donors and patients with stable angina pectoris. Spontaneous, formylpeptide- and urokinase-induced monocyte migration is assessed. Monocytes from patients are characterized by a higher content of membrane-bound urokinase. In contrast to peripheral blood monocytes from healthy donors, exogenous urokinase does not enhance migration of monocytes from patients with angina.

Key Words: *monocytes; angina pectoris; urokinase; urokinase receptor; migration*

Peripheral blood monocytes participate in atherogenesis by migrating through damaged vascular wall and synthesizing bioactive substances such as growth factors, cytokines, coagulation and fibrinolytic factors [13], in particular, urokinase-type plasminogen activator or urokinase (UR). Monocytes also express membrane urokinase receptor (rUR) [12] which promotes local proteolytic UR activity and participates in cell adhesion and migration [6,10,15]. The exact mechanism underlying these processes remains unknown. It is assumed that activated rUR interacts with integrins that modulate their functional state [11]. Monocyte rUR is presumably coupled to β_2-integrin Mac-1 [1], mediating monocyte adhesion to activated endothelium and extracellular matrix.

Immunohistochamical studies have demonstrated a higher content of rUR and UR on atherosclerotic plaques compared with unaffected vessel, primarily due to accumulation of monocyte/macrophages in this zone [9]. We have previously observed an increased plasma UR content in patients with atherosclerosis and simultaneously reduced number of free rUR on monocytes [1]. The peculiarities of UR binding to monocytes and UR-induced monocyte migration in coronary atherosclerosis have not yet been studied. Taking into account the role of monocytes in atherogenesis, we studied membrane expression of rUR and the content of membrane-bound UR and β_2-integrin Mac-1 on monocytes from patients with coronary atherosclerosis; monocyte migration in response to UR as chemoattractant was also evaluated.

MATERIALS AND METHODS

Experiments were carried out on peripheral blood monocytes from 6 healthy donors and 5 patients with stable angina pectoris. All the patients were examined in accordance with the Cardiology Center Ethical Committee protocol and had coronary heart disease — stable angina functional class III and angiographically documented coronary atherosclerosis. The patients received standard therapy which

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included aspirin, β-blocker, or calcium antagonists and hypolipidemic agents (lovastatin or simvastatin). Control group comprised healthy donors without cardiovascular risk factors and receiving no drugs. Patients and donors had no diabetes mellitus, neoplasms, immune and infectious diseases, and surgical interventions one month before experiment.

Blood was taken after an overnight fast using heparin as an anticoagulant.

Peripheral blood was diluted 1:4 with RPMI-1640 and incubated for 2 h at 37°C and 5% CO_2 in the presence of 10 mg/ml lipopolysaccharide (Sigma) to activate leukocytes. Analysis of antigen expression on activated cells seems to be most important, since cell adhesion and migration both *in vitro* and *in vivo* are necessarily associated with activated state characterized by maximum expression of the activation-dependent antigens, in particular, β_2-integrins and pUR.

The expression of surface monocyte receptors was analyzed by immunofluorescence using monoclonal antibodies against rUR (American Diagnostica), Mac-1 α-subunit (CD11b), CD14 monocyte marker (FITC and phycoerythrin conjugates) and UR (Laboratory of Immunochemistry, Institute of Experimental Cardiology, Cardiology Research-and-Production Center) on a FACScan flow cytometer (Becton Dickinson). To prevent additional cell activation and dissotiation of the UR-rUR complex, all samples were fixed for 3 min in 0.4% paraformaldehyde before immunostaining.

Erythrocytes were lysed in the presence of NH_4Cl, the cells were washed with phosphate-buffered saline containing 1% bovine serum albumin and incubated with monoclonal antibodies for 30 min at 4°C. When using unlabeled antibodies, the cells were additionally incubated with FITC-labeled rabbit antimouse antibodies and fixed in 1% paraformaldehyde in phosphate-buffered saline. The data were expressed in fluorescence units. It should be noted that anti-rUR antibodies bind both free and occupied rUR, while anti-UR antibodies recognized UR in UR/rUR and UR/rUR/PAI-1 (plasminogen activator inhibitor-1) complexes.

Mononuclear monocytes were isolated using modified Boyum method [2]. Monocyte migration was assessed by a modified Boyden chamber technique (Neuro Probe Inc.) using polycarbonate membranes with 5-μm pores (Poretics) according to a standard protocol [8]. Synthetic formylpeptide formyl-Met-Leu-Phe (PMLP, Sigma) and recombinant pro-UR (Laboratory of Gene Engineering, Institute of Experimental Cardiology) were used as chemoattractants. Migration was assessed by scanning Diff-Quick-stained membranes and expressed in relative units. The data were processed statistically using the Student *t* test.

RESULTS

Table 1 shows the expression of surface antigens on monocytes from healthy donors and patients with angina pectoris before and after activation. The migration of peripheral blood monocytes from a patient with atherosclerosis and a healthy donor is illustrated by Fig. 1 (taking into account great variability of individual parameters, we present data of a representative experiment).

Expression of CD11b and rUR on monocytes from donors and patients increased about 2-fold upon activation, while the content of membrane-bound UR remained unaffected in both these groups (data not shown).

There were no differences between donors and patients in spontaneous and stimulated expression of CD11b and rUR. However, the content of membrane-bound UR on resting peripheral blood monocytes from patients was significantly increased ($p<0.05$). This agrees with our previous data on reduced content of free rUR on monocytes in stable angina pectoris [1].

Spontaneous and FMLP-induced monocyte migration was similar in donors and patients, but pro-UR practically did not stimulate migration of monocytes from patients. It has been previously reported [3,4,15] that UR acts on smooth muscle cells, neutrophils, and monocytes as a chemoattractant. Moreover, rUR participates in FMLP-induced migration

TABLE 1. Membrane Expression of β_2-Integrins Mac-1 α-Subunit (CD11b), rUR, and UR on Monocytes from Healthy Donors and Patients before and after Whole-Blood Stimulation

Parameter	Healthy donors		Patients	
	before activation	after activation	before activation	after activation
CD11b	29±7	58±13	33±10	63±11
rUR	32±3	56±2	36±10	52±2
UR	10±10		33±17	

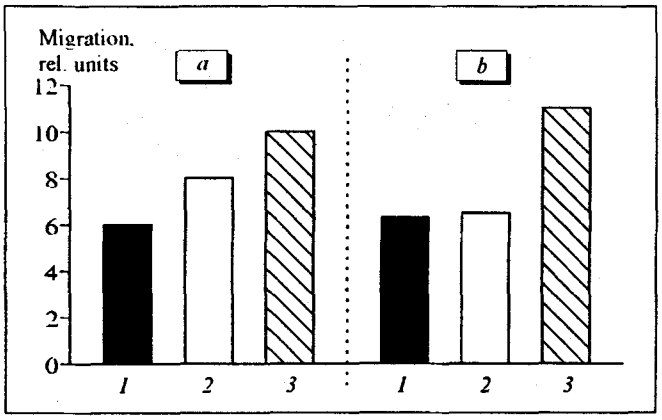

Fig. 1. Spontaneous (*1*) and urokinase- (10 nM, *2*) and formylpeptide- (10 nM, *3*) induced migration of peripheral blood monocytes of healthy donor (*a*) and patient with stable angina pectoris (*b*).

of peripheral blood monocytes [5]. Our findings suggest that spontaneous and FMLP-induced monocyte migration depend on the expression of adhesion molecules, in particular, β_2-integrins Mac-1 and probably rUR, but not on the content of membrane-bound UR. It can be hypothesized that the migration-stimulating effect of UR is proportional to the number of free rUR on cell membranes. The increased number of occupied UR on peripheral blood monocytes from patients with coronary atherosclerosis is not accompanied by stimulation of these cells with pro-UR.

The fact that UR does not stimulate monocyte migration in patients with stable angina pectoris is at variance with modern views on the role of monocytes in atherosclerotic plaques [13]. This can be due to different properties of activated peripheral blood monocytes and monocytes localized in plaques.

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