Defensin of Human Neutrophils Modulates the Functional Activity of Monocytes

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It is known that activated neutrophils secrete a vast array of peptide transmitters into the bloodstream [3,6,12]. Of particular interest among them are defensins, which are released from neutrophil granules and exhibit a broad spectrum of antibacterial, antiviral, and cytotoxic activities [8,10]. Previously we have shown that human defensin modulates agonist-induced platelet activation [1,13]. Based on this finding, we decided to further investigate the role of defensins in the regulation of the functional activity of other peripheral blood cells, namely monocytes. The objective of this study was to examine the effect of human defensin on the aggregation of monocytes isolated from donor blood.

MATERIALS AND METHODS

Defensins were extracted from donor blood by the method of V. N. Kokryakov [2]. The total defensin fraction was used. Immunophoretically, this fraction contained three major components: HNP-1, HNP-2, and HNP-3. Venous blood was obtained from healthy donors in 5 mM EDTA (1:9). Monocytes were isolated by the method of Boyum [5]. Whole blood (5-6 ml) was layered over 3 ml of NycoPrep 1.068 Medium (Nycomed Pharma AS, Norway) with a constant density gradient and centrifuged at 600 g for 15 min at 20°C. Monocytes sedimented as an opalescent ring were readily removed and washed by two subsequent centrifugations: in bovine serum albumin (Chrono-Par, USA) solution (7 min, 600 g) and in Hank's medium (10 min, 50 g). The resultant cell suspension contained not less than 90% monocytes. Aggregation of monocytes in the suspension was estimated by photometry in a PICA lumiaggregometer (Chrono-Log, Howertown, PA, USA). The amplitude and maximum aggregation rate were measured in an Aggro-Link apparatus (Chrono-Log). Agonists were added to the cell suspension at the 10th min of incubation with defensin. Platelet aggregation was induced with arachidonic acid (AA, 40 μM, Sigma) or phorbol 12-myristate 13-acetate(PMA, 10 nM, Sigma). The data were analyzed using Student's t test and regarded as statistically significant at $p \leqslant 0.05$.

RESULTS

Within the concentration range of 0.01-100 μg/ml, defensin induced dose-dependent changes in the functional activity of monocytes. The addition of defensin (100 μg/ml) resulted in irreversible aggregation of monocytes which reached a maximum (27±4%) by the 5th min of the experiment at an aggregation rate of 36±2%/min (Table 1). A decrease in the defensin concentration to 1 μg/ml

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TABLE 1. Effect of Human Defensin (100 μg/ml) on Aggregation of Monocytes Activated with Arachidonic Acid (AA) or Phorbol Ester (PMA) ($M \pm m$)

Parameter	Defensin	AA, 40 μM		PMA, 10 nM	
		control	defensin	control	defensin
Aggregation amplitude, %	27±4	65±5	93±3*	33±3	48±5*
Maximum aggregation rate, %/min	36±2	136±14	130±15	44±8	81±6

Note. *: $p \leq 0.05$ compared with the control.

induced no statistically significant changes in these parameters; however, cell disaggregation was observed by the 7th min after addition of the peptide. At a concentration of 0.01 μg/ml defensin induced a slight (12±4%) increase in the light transmittance of the monocyte suspension, followed by a drop to the original value. It should be mentioned that changes in the light transmittance were observed 10-20 sec after the addition ofdefensin, irrespective of its concentration.

Thus, human defensin (1-100 μg/ml) induces monocyte aggregation. At lower concentrations (0.01 μg/ml) it causes only a short-term activation of monocytes.

After activation of monocytes with 1-100 μg/ml defensin, additional stimulation with the same concentrations did not change the aggregation activity of monocytes. On the other hand, the addition of AA or PMA to monocytes activated with 100 μM/ml defensin increased their aggregation to 93±3% (aggregation rate 130±15%/min) and 48±5% (aggregation rate 81±6%/min), respectively (Table 1). It is noteworthy that the amplitudes of aggregation under these conditions were significantly higher compared with those of AA- (65±5%) or PMA-induced (33±3%) aggregation in the absence of defensin. It follows that defensin not only induces, but also potentiates AA- or PMA-induced aggregation of monocytes.

The structure and functions of defensins as compounds with a broad spectrum of antibacterial, antiviral, and cytotoxic activities have been studied and described in detail [3,8,10]. Further investigations revealed novel properties of defensins. For example, it has been reported that an intravenous injection of defensin alters the activity of the anticoagulant system in the rat [4]. Earlier, we showed that human defensin (0.1-40 μg/ml) inhibits thrombin-induced platelet aggregation [1,13]. A strong chemoattractant activity of defensin was revealed in a study of its effect on monocyte function [12]. Defensin was used here in a concentration of 0.04 μg/ml. In our experiments, in a concentration of the same order of magnitude (0.01 μg/ml) defensin also induced aggregation of monocytes, registered as a short-term increase in the light transmittance of the cell suspension after the peptide was added. The mechanisms of monocyte aggregation remain unclear, although possible pathways have been proposed in the literature [9,11]. It was demonstrated that AA-induced aggregation of mononuclear leukocytes is inhibited by aspirin and is notaffected by lipoxygenase inhhibitors or luekotrienes B_4, C_4, and D_4. On the basis of these observations it was suggested that the main pathway of monocyte involvement in aggregation is associated with the activation of cyclooxygenase. At the same time, we observed no changes in the aggregation of activated monocytes upon their stimulation with defensin, whereas the addition of AA or PMA to a suspension of defensin-activated monocytes increased the amplitude and rate of aggregation. This observation may indicate that the aggregation of defensin-activated monocytes proceeds not only via the cyclooxygenase pathway and that the mechanisms underlying this aggregation may be different from those operating in a suspension of AA- or PMA-stimulated cells. According to published data, defensins account for about 25% of granular and 5-7% of the cellular proteins of human neutrophils. It can therefore be assumed that human defensins are the major physiological neutrophil transmitter regulating the functional activity of monocytes.

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Cholesterol Level in Peripheral Blood Lymphocytes: Relationship with Ischemic Heart Disease in Patients with Various Forms of Hyperlipidemia

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Even though a correlation between the incidence of ischemic heart disease(IHD) and the plasma level of "atherogenic" cholesterol has been demostrated in a number of extensive population studies [1-3], the mechanism underlying this phenomenon remains unclear. It is generally accepted that a rise of plasma cholesterol (Ch) level above 6.5 mmol/liter increases the risk of atherosclerotic plaque formation [4]. Cholesterol accumulation in the vascular intima is due to the transformation of macrophages and smooth muscle cells into foam cells [5,6], which does not reflect the Ch content of other cells in the body. Cholesterol accumulation in tissues may occur without pronounced hypercholesterolemia. Yu. M. Lopukhin has described "cholesterosis" of red blood cells in patients with hypercholesterolemia [7]. However, these non-nucleated cells do not have their system for own controlling Ch balance, and the Ch concentration in their plasma membrane simply reflects the intensity of the concentration gradient transport. The mechanism responsible for the coupling and balance between the Ch content of the vessel wall and other tissues and circulating plasma lipids is unknown.

The lymphocytes circulating in human blood share a considerable number of parameters with vascular endothelium because these cells function at the blood-tissue interface and receive physiological stimuli from the blood and tissues. In addition, peripheral blood lymphocytes are a more convenient tool for biological investigations as compared with fibroblasts and endothelial cells.

This study is an attempt to compare the changes in the Ch content of peripheral blood lymphocytes with plasma concentrations of total Ch, triglycerides and Ch derived from low-density lipoprotein (LDL).

MATERIALS AND METHODS

Eighty-five patients (55 women and 30 men) aged 29-62 years were included in the study. Ischemic heart disease (IHD) with typical clinical signs and electrocardiological symptoms was identified in 66 patients; in 40 of these atherosclerotic lesions of the coronary arteries were documented angiographi-

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