Macrophage-Granulocyte Colony-Stimulating Factor and Fibroblast Growth Factor in the Blood of Patients with Hyperlipoproteinemia

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Solid-phase ELISA revealed increased content of macrophage-granulocyte colony-stimulating factor and basic fibroblast growth factor in the serum of hyperlipoproteinemic patients.

Key Words: *atherosclerosis; hyperlipoproteinemia; colony-stimulating factor; macrophage granulocytes; fibroblast growth factor*

Cytokines regulate proliferation and differentiation of target cells by reacting with complementary receptors on the cell surface and transmitting the signal through elements of intracellular transduction systems into the nucleus, where the appropriate genes are activated. Proteins (products of gene-activated cytokines) are produced by cells and regulate these processes.

The macrophage-granulocyte colony-stimulating factor (MG-CSF, 18-22 kDa) belongs to the class of early hemopoietic CSF. Along with Steel factor and interleukins-1, 3, 6, and 11, promoting differentiation from stem to precursor cells, MG-CSF participates in the granulocyte/macrophage colony formation in semisolid cultures. This factor is produced by macrophages, endothelial and mast cells, T lymphocytes, granulocytes, fibroblasts, and smooth muscle cells (SMC) [8].

The family of fibroblast growth factors (FGF) includes about 20 homologous polypeptide factors with similar characteristics. The basic FGF (FGF-2, 18 kDa) is best studied. FGF stimulate the proliferation and differentiation of mesodermal stromal cells, *e.g.* fibroblasts, osteoblasts, chondroblasts, myoblasts, and endothelial cells, and induce the formation of fibroblast colonies in a semisolid culture *in vitro* [3]. These factors are secreted by macrophages, fibroblasts, endotheliocytes, and SMC. FGF participate in many pathological processes associated with intensive angiogenesis or excessive cell proliferation [5]. During inflammation activated macrophages produce platelet growth factor and FGF-2, thus activating fibroblasts which actively proliferate, migrate to the inflammation focus, and actively produce extracellular matrix substances.

The concept of atherosclerosis as a chronic inflammation caused by accumulation of lipids in the vascular wall suggests the involvement of blood cells (lymphocytes, macrophages), intimal cells (endothelial, SMC), and growth factors produced by these cells (including MG-CSF and FGF-2) in the initiation, formation, and progress of atherosclerotic lesions in the intima [9]. We previously demonstrated the presence of bone marrow stem hemopoietic and stromal CFU, along with monocytes/macrophages and lymphocytes, in the atheromatous aortic intima and accumulation of stromal fibroblast CFU in the blood of patients with primary hyperlipoproteinemia (HLP) and coronary sclerosis [1,14]. Here we measured the content of MG-CSF and FGF-2 in the blood of patients with HLP, the most important risk factor of atherosclerosis.

MATERIALS AND METHODS

The sera from 31 patients with primary HLP and 7 normal subjects with normolipemia (cholesterol <5.2

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mmol/liter; triglycerides <2.1 mmol/liter) were analyzed. The blood was collected from the ulnar vein after overnight fast. Serum cholesterol and triglycerides and the content of HDL cholesterol were measured using an Express Plus biochemical autoanalyzer (Chiron/Diagnostics) in the supernatant after precipitation of other lipoproteins with a mixture of phosphotungstic acid and magnesium chloride. LDL cholesterol was calculated using the Friedwald formula.

HLP type (according to Frederickson classification) was identified by electrophoresis of serum proteins in agarose gel using Lipogel plates on a Paragon system (Beckman) with subsequent densitometry.

Serum levels of MG-CSF and FGF-2 were measured using Amersham kits (sensitivity <2 pg protein/ml). The system is based on solid-phase ELISA including the sandwich enzyme immunoassay. Horseradish peroxidase bound to antibodies to the detected protein was developed with tetramethylbenzidine. The intensity of developed peroxidase staining, corresponding to the concentration of measured CSF, was measured on a Multiscan spectrophotometer with vertical beam at 450 nm. The amount of bound protein in experimental serum samples was estimated using a calibration curve.

The significance of differences between the means was estimated using unpaired Student's *t* test. The relationships between the parameters were evaluated using analysis of correlations. The differences were considered significant at $p<0.05$.

RESULTS

We detected MG-CSF and FGF-2 in the sera of normolipidemic donors and patients with HLP (Table 1) despite the opinion of some scientists that normally serum MG-CSF is undetectable because of its extremely low concentration (<2 pg/ml) [12]. It is noteworthy that 10^{-11} mol/liter is a concentration sufficient for manifestation of mitogenic activity of cytokines.

Zero levels of FGF-2 were detected in 3 subjects (one per group: controls, type IIa HLP, and type III HLP).

Comparison of the concentrations of MG-CSF and FGF-2 revealed a relationship between these factors (Fig. 1). No significant relationship between the levels of cholesterol, triglycerides, or HDL cholesterol and levels of FGF-2 or MG-CSF were detected ($r<0.14$).

Analysis of our findings with consideration for HLP types showed that the mean concentrations of MG-CSF and FGF-2 in patients with different types of HLP surpassed the control (Table 1). The highest increase in both MG-CSF and FGF-2 concentrations (by 71.19 and 117.34%, respectively, compared to normal) was detected in patients with type IV HLP, characterized by elevated blood concentration of VLDL; a less pronounced increase was detected in patients with type IIb HLP (by 54.58 and 73.41%, respectively) and with type IIa HLP (by 28.81 and 9.05%). Type IIa HLP was characterized by high blood concentration of LDL, type IIb by high LDL and VLDL levels. The only exclusion was type III HLP (dysbetalipoproteinemia) characterized by the presence of intermediate-density lipoproteins: the concentration of MG-CSF slightly differed from the control (15.25%), while the mean concentration of FGF-2 was below the normal (by 3.40%). However, the group of patients with type III HLP was very small (the incidence of this HLP type is 1:5000).

High serum level of MG-CSF in patients with HLP can be due to hyperfunction of the monocyte/macrophage stem. Macrophages (foam cells) are present in the vascular intima. The need in scavenger macrophages during lipid accumulation in vascular wall requires intense proliferation and differentiation of bone marrow colony-forming macrophage granulocyte precursors. This process is mediated by the corresponding cytokines, primarily Steel factor, interleukins, and MG-CSF. Recent studies showed that growth factors, including Steel factor, are produced by human aortic intima cells (endothelial and SMC) and

TABLE 1. Serum Concentrations of MG-CSF and FGF-2 in Patients with HLP and Normolipidemic Donors (*M*±*m*)

Parameter	Control (*n*=7)	HLP IIa (*n*=11)	HLP IIb (*n*=9)	HLP III (*n*=3)	HLP IV (*n*=8)	HLP, mean (*n*=31)
FGF-2						
pg/ml	2.95±0.42	3.80±0.36*	4.56±0.41*	3.40±0.72	5.05±0.80*	4.20±0.57*
%	100.00±14.24	128.81±12.20	154.58±13.89	115.25±24.40	171.19±27.11	142.40±19.32
MG-CSF						
pg/ml	19.67±5.04	21.45±4.15*	34.11±9.09*	19.00±10.21	42.75±15.90	29.40±4.50*
%	100.00±25.62	109.05±21.09	173.41±46.13	96.60±51.90	217.34±80.85	149.47±22.88

Note. *$p<0.05$ compared to the control.

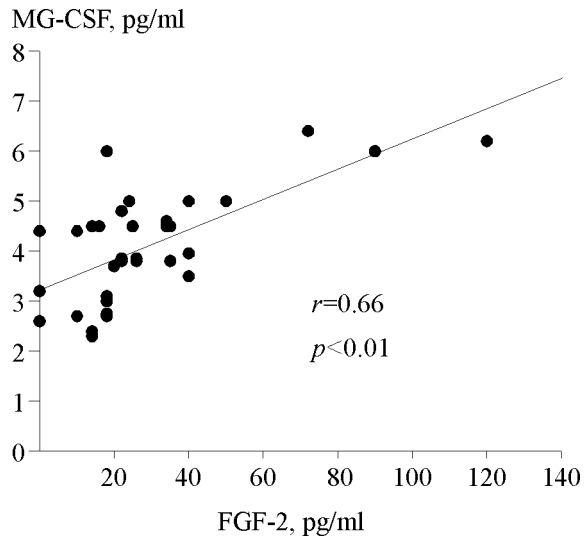

Fig. 1. Correlation between concentrations of macrophagal-granulocytic colony-stimulating factor (MG-CSF) and fibroblast growth factor-2 (FGF-2) in human serum (n=38).

by blood cells (monocytes and lymphocytes) [8], which can influence the content of CSF in the blood. Injection of modified LDL to animals induced expression of MG-CSF and macrophagal CSF in vascular endothelium *in vitro* and *in vivo* [10]. Early-acting MG-CSF and late-acting macrophagic CSF activate the monocytic/macrophagal bone marrow stem, while the increase in macrophage population promotes lipoprotein absorption by cells in the vascular wall, which can lead to regression of atherosclerotic lesions in animals with experimental atherosclerosis injected with CSF [13].

The increase in serum FGF-2 concentration can correlate with the appearance of bone marrow stromal CFU (absent in normolipidemic donors) in patients with HLP and with the presence of stromal stem precursor cells in human aortic intima [14,15]. Accumulation of modified LDL in the intima induces the expression of FGF by intimal cells [2]. However not only FGF-2, but also MG-CSF modulates proliferation of bone marrow fibroblast precursors [4]. The content of MG-CSF also notably increased in HLP patients in comparison with healthy donors.

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