EXPERIMENTAL BIOLOGY

Megakaryocyte Colony-Forming Units in Peripheral Blood of Patients with Hyperlipoproteinemia

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Megakaryocyte colony-forming units with high proliferative activity (6-7 mitoses) were detected in peripheral blood of patients with type IIa and IIb hyperlipoproteinemia. These cells are present in 30-40% patients, their occurrence being higher in patients with type IIb hyperlipoproteinemia.

Key Words: *hyperlipoproteinemia; megakaryocyte colony-forming units; mononuclears; peripheral blood*

Hyperlipoproteinemia (HLP) promotes atherosclerotic processes in human blood vessels. Previously, hemopoietic and stromal precursors cells, granulocyte-macrophage, basophil-mast cell and fibroblast colony-forming units (CFU) were identified in atherosclerotic lesions in human aortic intima [2,5]. Moreover, in patients with primary HLP (types IIa and IIb according to WHO classification) CFU fibroblast were found in the mononuclear fraction of peripheral blood [4]. Megakaryocytes and platelets produce growth factors (platelet-derived and transforming growth factor-β) that modulate the intensity of sclerotic processes and regulate proliferative activity and synthesis of glycosaminoglycans and collagen in fibroblasts and myoblasts. Therefore, it was interesting to study megakaryocyte stem cell in different HLP. In the present study we compare megakaryocyte CFU (CFU-Meg) in peripheral blood of normolipidemic donors and patients with type IIa and IIb HLP.

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MATERIALS AND METHODS

Blood samples were obtained from 20th HLP patients aged 6-60 and 7 donors (control) aged 30-50 Table 1).

Heparin (40 U/ml) was used as anticoagulant. Mononuclear cells were isolated from leukocyte-rich plasma by centrifugation on a Ficoll-Verografin gradient for 30 min at 400*g*. To remove platelets, mononuclear cells were suspended in phosphate-buffered physiological saline (pH 7.2) containing 15% citrate, layered onto embryonic bovine serum, and centrifuged at 100 rpm for 10 min. The cells were then resuspended in Iscove medium (Flow Lab) supplemented with 20% embryonic bovine serum (Hy Clone) and 2 mM glutamine (Gibco), and adherent cells were removed by an overnight incubation in plastic flasks at 37°C, 5% CO_2 and 100% humidity and. The presence of CFU-Meg in adherent cell-depleted mononuclear (ACDM) fraction was de-

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TABLE 1. Lipid and Lipoprotein in Patients with HLP and Donors*

Group	Total cholesterol	Triglycerides	High-density lipoprotein cholesterol
	mmol/liter		
Donors (*n*=7)	4.3-6.3	0.80-1.94	1.05-1.86
Type IIa HLP (*n*=10)	8.4-16.0	0.81-1.90	0.92-1.90
Type IIb HLP (*n*=10)	6.9-18.9	2.4-14.8	0.8-1.48

Note. *Total cholesterol, triglycerides, and high-density lipoprotein cholesterol were enzymatically measured in Technikon-Tex analyzer. Low-density lipoprotein cholesterol calculated from the Friedewald formula was 4.9-9.8 mmol/liter for HLP patients and below 4.1 mmol/liter for donors.

TABLE 2. CFU-Meg in Peripheral Blood of Patients with Primary HLP and Normolipidemic Donors (*M±m*)

HLP type, No. of patients	CFU-Meg per 10^6 ACDM	Mean number of cells in colony	CFU-Meg per 10^6 ACDM, number of cells in colony		
			3-20	21-40	>40
Normolipidemia (*n*=7)					
1	4.40	8.00	4.40	0	0
2	8.11	9.70	7.86	0.25	0
3	7.02	11.50	6.27	0.75	0
4	0.87	5.75	0.87	0	0
5	1.00	7.67	1.00	0	0
6	2.00	15.00	1.67	0.33	0
7	3.75	11.10	3.25	0.50	0
Mean...	3.88±1.08*	9.8±1.15*			
Type IIa HLP (*n*=10)					
1	4.66	17.70	4.04	0.31	0.31
2	2.00	6.25	2.0	0	0
3	2.50	7.60	2.50	0	0
4	0.72	6.00	0.72	0	0
5	16.50	14.00	13.70	2.40	0.40
6	2.50	8.00	2.25	0.25	0
7	0	0	0	0	0
8	0	0	0	0	0
9	11.30	10.15	10.40	0.90	0
10	2.10	15.30	1.87	0	0.23
Mean...	4.23±1.71*	8.5±0.48*			
Type IIb HLP (*n*=10)					
1	8.97	33.60	5.13	1.28	2.56
2	8.00	23.10	5.75	1.00	1.25
3	5.45	31.40	2.42	0.92	2.12
4	2.22	4.60	2.22	0	0
5	0	0	0	0	0
6	1.37	5.25	1.37	0	0
7	0.68	9.00	0.68	0	0
8	17.40	14.40	13.80	2.60	1.00
9	6.95	13.54	5.60	1.35	0
10	0.69	12.00	0.69	0	0
Mean...	5.17±1.72*	14.7±3.6*			

Note. *p>0.1 for any two values.

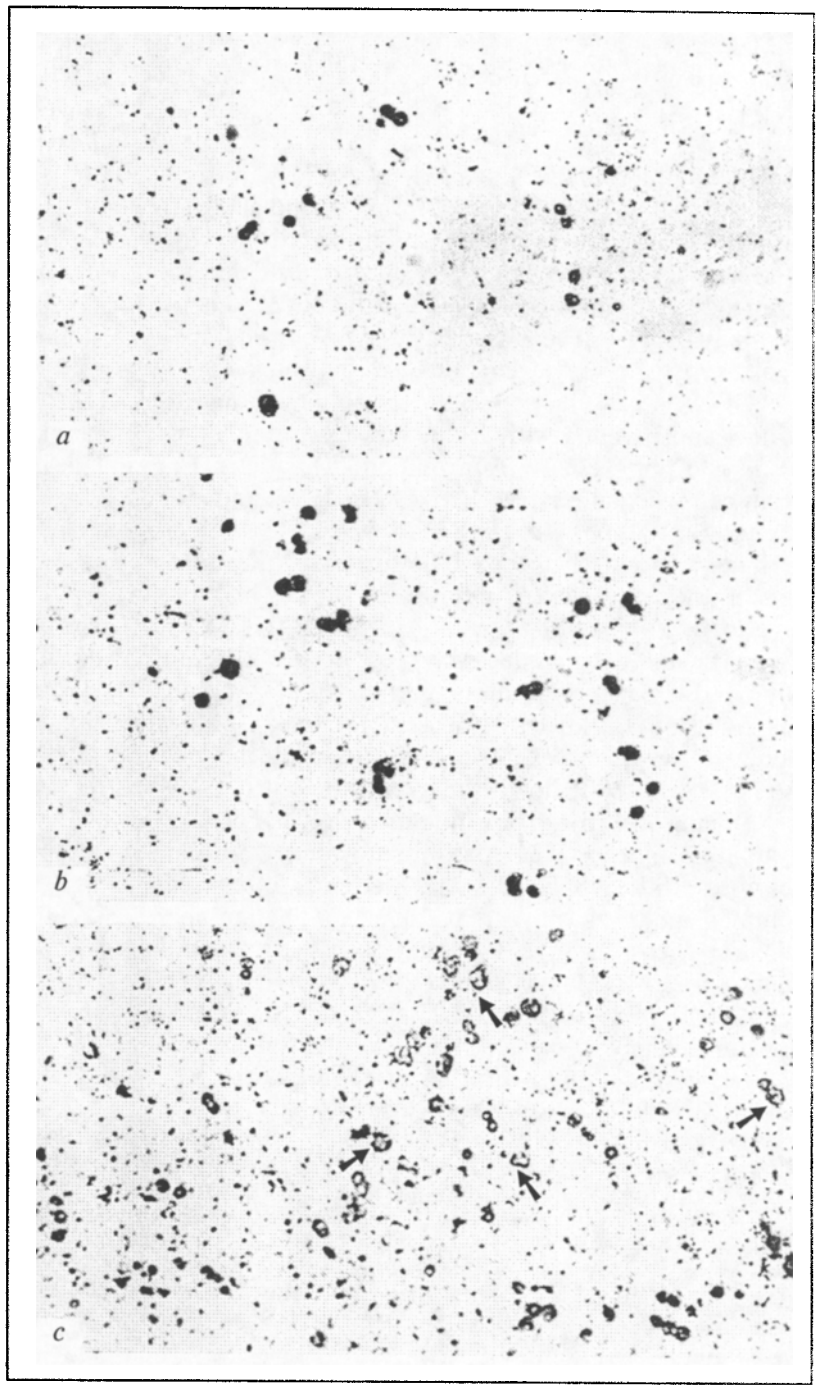

Fig. 1. Megakaryocyte colonies in a 12-day-old culture of peripheral blood adherent-cell-depleted mononuclear fraction in fibrin clot. ×160. Cells were stained by the immunoperoxidase method using monoclonal antibodies to VM 16a glycoprotein IIb-IIIa. All colonies contain cells of different size. Platelets are seen in all microphotograms. 12- (*a*) and 30-cell (*b*) megakaryocyte colonies; *c*) a fragment of megakaryocyte colony containing more than 40 cells. Large cells with segmented nuclei are showed with arrows.

termined by culturing in a fibrin clot. The culture medium consisted of Iscove medium supplemented with 30% embryonic bovine serum, 5% leukocyte-conditioned medium [3], 2-mercaptoethanol in a final concentration of 5×10^{-5} M, 10%, fibrinogen (5 mg/ml in 0.1 M phosphate buffer, pH 7.1), and 10% 2-fold Iscove medium. Fibrinogen was converted into fibrin with 1 U/ml thrombin; ACDM in 1 ml culture medium (10^6) were cultured for 12 days in 35-mm plastic dishes (Flow) at 37°C, 5% CO_2, and 100% humidity (4-5 dishes for each patient).

The cultures were fixed with methanol-acetone (1:3) for 20 min, washed with 0.15 M NaCl and 0.01 M Tris-buffer (pH 7.2), air-dried, and stored at -20°C. Megakaryocyte colonies were identified by the immunoperoxidase test using monoclonal VM 16a antibodies (MonA, Russian Cardiology Research-and-Production Center) to IIb-IIIa glycoprotein [1] in a working concentration of 12.5 μg/ml. Peroxidase-positive cells were visualized with 0.04% 4-chloro-1-naphthol and 0.03% H_2O_2, cell aggregates containing no less than three cells were considered as colonies.

The data were processed statistically using the Student *t* test.

RESULTS

Cells containing endogenous peroxidase looked deep-blue to black, while cells of the megakaryocyte lineage and platelets were brown. In fibrin clot, megakaryocyte colonies usually consisted of diffuse-arranged small and evidently low-differentiated cells. In many colonies cells varied in size considerably. Typical megakaryocyte colonies are shown in Fig. 1.

CFU-Meg were present in peripheral blood of donors and patients with HLP; however, their number varied considerably from patient to patient. For instance, two patients with type IIa HLP and one patient with type IIb HLP had no CFU-Meg in peripheral blood. In other HLP patients, the number of megakaryocyte precursors varied in close ranges (0.72-16.5 and 0.68-17.4 per 10^6 ACDM for types IIa and IIb, respectively); in normolipidemic donors this range was more narrow (0.87-8.11 per 10^6 ACDM, Table 2). However, the mean values were similar for these three groups and the differences were statistically insignificant.

During culturing, peripheral blood CFU-Meg from donors and HLP patients formed 3-20-cell colonies. In some donors and patients, individual colonies included more that 20 cells. The number of CFU-Meg forming 21-40-cell colonies was similar in patients with type IIa HLP (except 1 patient) and donors (0.25-0.75 and 0.25-0.87 per 10^6 ACDM for donors and type IIa HLP, respectively) and differed considerably from that of patients with type IIb HLP (0.92-2.6 per 10^6 ACDM).

CFU-Meg forming colonies of 40 and more cells were found only in patients with HLP. Some of these colonies consisted of 41-185 cells, which suggests the presence of CFU-Meg with high proliferative potential (6-7 mitoses) in peripheral blood of patients with HLP. In 3 patients with type IIa HLP, the number of these cells varied from 0.23-0.4 per 10^6 ACDM, while 4 patients with type IIb HLP had 1.0-2.56 such cells per 10^6 ACDM (Table 2).

Thus, megakaryocyte precursors with high proliferative activity (up to 7 mitoses during *in vitro* culturing) appeared in the blood of patients with HLP. This phenomenon was observed in 30-40% patients, being more pronounced in type IIb HLP. The mechanisms inducing the appearance of CFU-Meg with high proliferative potential in peripheral blood of patients with disturbed lipid metabolism remain to be investigated.

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