TABLE 3. Change in Number of Endogenous CFUs in Mice Tolerant to SRBC in Response to Antigenic Prining

Preliminary treatment of animals	Number of animals	Test injection	Number of CFUs per spleen
—	14	—	3±0,3
—	15	SRBC	6,7±0,7
SRBC + CP	11	—	2,7±0,4
SRBC + CP	22	SRBC	5,6±0,6

The mechanism of activation of PHSC in response to antigenic priming thus differs for the bone marrow and spleen. In bone marrow it evidently takes place with the participation of T lymphocytes, but in the spleen with the participation of macrophages.

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STIMULATION OF PHAGOCYTIC FUNCTION BY LEUKOCYTE AND MACROPHAGE MIGRATION INHIBITION FACTORS IN MAN

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An important trend in immunocorrection is the search for preparations with selective action on different cell populations. Recent years have seen a beginning of the use of biological factors secreted by lymphocytes, namely lymphokines, for the treatment of certain immunodeficiency states. These substances include a group of factors selectively influencing macrophages and polymorphonuclear leukocytes, such as macrophage migration inhibition factors (MMIF), leukocyte migration inhibition factor (LMIF), and macrophage activating factor. The effective use of these factors in the treatment of small-cell forms of cancer spontaneous metastases of melamonas, and so on, under experimental conditions, has been reported [4, 5]. With the opening up of these prospects for the widespread clinical use of these mediators, the task of their isolation and purification had the more detailed study of the mechanism of their action on target cells are of great urgency.

This paper describes the simultaneous isolation of MMIF and LMIF from supernatants of lymphocyte cultures stimulated by phytohemagglutinin (PHA), and the effect of the isolated factors on migration and phagocytic activity of target cells was studied.

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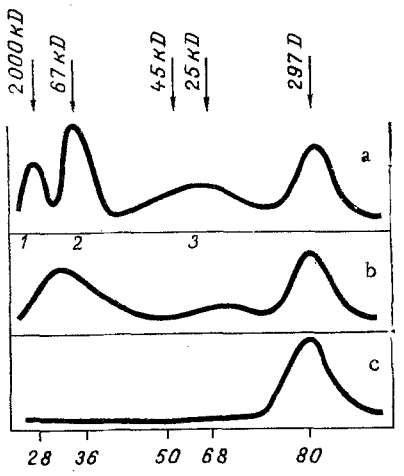

Fig. 1. Charomatograms of experimental (a) and control (b) supernatants and of medium 199 (c) obtained by gel-filtration on Sephadex G-200. Abscissa, elution volume (in ml); ordinate, absorbance of fractions at wavelength of 280 nm. 1) Peak 1, region of elution of substances with mol. wt. of 100 kD; 2) peak 2, region of elution of substances with mol. wt. of 60-70 kD; 3) peak 3, region of elution of substances with mol. wt. of 20-30 kD.

EXPERIMENTAL METHOD

A pure suspension of mononuclear cells was obtained from healthy human peripheral blood by the method in [3]. Lymphocytes were isolated and $5 \cdot 10^6$ of these cells were stimulated by PHA (Difco, USA) in a concentration of 10 µg/ml for 3 h. The cells were then washed to remove mitogen, cultured for 20-24 h in medium 199 with antibiotics (100 U/ml of penicillin and 100 µg/ml of streptomycin), and the supernatant retained after centrifugation. Supernatant obtained after culture of intact lymphocytes served as the control.

The experimental and control supernatants were fractionated on a column with Sephadex G-200 (1 × 100 cm, "Whatman" type). The column was calibrated with dextran blue, bovine serum albumin, ovalbumin, chymotrypsinogen, and dinitrophenol-leucine. The experimental or control lyophilized supernatant, 100 ml in volume, was dissolved in 3 ml of Hanks' solution and applied to a column equilibrated with standard Hanks' solution (rate of elution 10 ml/h at 4°C). The protein concentration in fractions obtained after gel-filtration was determined spectrophotometrically ($1.45\ E_{280} - 0.74\ E_{260}$).

The functional activity of the isolated fractions was estimated by their effect on mobility of various targets (macrophages from mouse peritoneal exudate and human peripheral blood leukocytes) and also on phagocytic activity of peripheral neutrophils.

Migration activity was determined in the capillary migration inhibition test [1]. To compare the results, migration inhibition indices were calculated: $MII_1 = (1 - P_1/P_2) \times 100\%$ to estimate spontaneous MIF production; $MII_2 = (1 - P_3/P_1) \times 100\%$, to estimate induced factor production, where P_1 is the mass of the migration zone in the control fraction, P_2 the mass of the migration zone in Hanks' solution, and P_3 the mass of the migration zone in the experimental fraction.

Fc-receptor-dependent phagocytic activity of the neutrophils was estimated in a microtest [2]. A 1% suspension of EA complex (bovine erythrocytes, opsonized with rabbit antireythrocytic IgG) served as the object of phagocytosis. The fractions for testing or Hanks' solution (in the control) were applied to a monolayer of neutrophils on a coverslip in a volume of 0.1 ml and incubated for 1 h at 37°C, after which 0.1 ml of the 1% EA complex was added. After 30 min, phagocytosis was stopped by washing the coverslip in Hanks' solution, after which it was dried, fixed with methanol, and stained with Carazzi's hematoxylin and eosin. The ingestive capacity of the neutrophils was estimated in relation to the following parameters: phagocytic number, the percentage of neutrophils with ingested EA particles; and the phagocytic index, the number of ingested particles per neutrophil. Monocytes and eosinophils were disregarded because of their small numbers.

EXPERIMENTAL RESULTS

Elution profiles of the experimental and control supernatants and of medium 199 and also the protein concentration in the fractions are given in Fig. 1. As a result of gel-chromatography the following fractions were obtained: high-molecular-weight, with mol. wt. of 100 kilodaltons (kD) (peak 1), with mol. wt. of 60-70 kD (peak 2), and with mol. wt. of 20-30 kD (peak 3). Similar but smaller peaks also were obtained on fractionation of the control supernatant, the protein content of which was about one-tenth of that of the experimental supernatant.

TABLE 1. Effect of Experimental and Control Fractions of Mobility of Target Cells

Fraction for testing	Stimulation by PHA during cell culture	MII, %	
		leukocytes	macrophages
Over 100 kD	Experiment	42,1±11,3	
	Control	31,2±13,9	—
	Experiment	91,2±8,7*	15,0±6,3
60—70 kD (LMIF)	Control	20,4±10,1	0,1±7,2
		—15,4±8,0	
20—30 kD (MMIF)	Experiment	14,3±8,4	91,3±8,5*
	Control	—5,6±7,2	25,2±10,3

Legend. *$P \leq 0.001$ compared with control.

TABLE 2. Effect of Isolated Fractions on Phagocytic Activity of Neutrophils

Fraction for testing	Stimulation by PHA during cell culture	Phagocytosis	
		phagocytic number, %	phagocytic index
60—70 kD	Experiment	70,9±3,2*	1,63±0,10*
	Control	24,0±2,0	0,34±0,30
30—60 kD	Experiment	37,2±7,0	0,71±0,18
	Control	45,5±7,5	0,72±0,19
20—30 kD	Experiment	75,1±2,8*	1,78±0,14*
	Control	34,5±4,5	0,56±0,11
Hanks' solution (normal)	—	27,3±6,1	0,52±0,15

Legend. *$P \leq 0.001$ compared with normal.

The isolated fractions were tested for functional activity in the migration inhibition test (Table 1). High-molecular-weight fractions of the experimental and control supernatants inhibited mobility of the target cells equally, and their protein concentrations also were equal. This effect of the high-molecular-weight fraction can evidently be explained by the fact that it contains products of lumphocyte metabolism which, as a result of gel-filtration, were concentrated in a small volume and had a nonspecific action. The presence of leukotoxin in this fraction likewise cannot be ruled out.

The experimental fraction with mol. wt. of 60-70 kD caused almost total inhibition of migration of leukocytes, but not of macrophages ($P \leq 0.05$). An inhibitory effect (35%) was observed when the fraction was diluted 500 times. The fraction with mol. wt. of 20-30 kD inhibited migration of mouse peritoneal exudate macrophages, but not of human peripheral blood leukocytes ($P \leq 0.05$). Spontaneous LMIF production was not found in all the donors tested (Table 1): In 70% of those tested MII was 20.4 ± 10.1, whereas in the rest spontaneous mediator production was absent (MII = −15.4 ± 8.0). Under the conditions described, gel-filtration on Sephadex G-200 can thus yield two mediators simultaneously: one acting on the locomotor functions of macrophages (MMIF), the other on these functions of leukocytes (LMIF).

The results of estimation of the action of the various lymphokine fractions on phagocytic activity of neutrophils are summarized in Table 2. As the data show, both LMIF and MMIF stimulate phagocytic activity equally. Incubation of the cells with the test fractions for 1.5 h caused an almost threefold increase in both phagocytic number and phagocytic index. The fact will be noted that although only macrophages were used as target cells from MMIF in the migration inhibition test, and not polymorphonuclear leukocytes (Table 1), MMIF was also to influence phagocytosis of neutrophils, which it greatly potentiated.

Incubation of neutrophils with fractions obtained in the same ranges of molecular weight as MMIF and LMIF, but from supernatants of mononuclear cells cultured without PHA (control) had no stimulating action on phagocytosis. Fractions within the range of molecular weights between MMIF and LMIF, i.e., 30-60 kD, taken together had no effect on phagocytosis (either control or experimental). However, additional investigations showed that within the range 30-60 kD fractions with different molecular weights differed in their effect on phagocytosis.

The model of phagocytosis of EA particles can therefore be used successfully to assess the activity of different lymphokines, from the point of view of their possible future therapeutic use. The stimulating effect of lymphokines on phagocytosis is evidently realized through an incerase in the number of Fc-receptors expressed by the phagocyte, although a stimulating action of the fractions on the intensity of transmission of the phagocytic signal from the Fc-receptor to the contractile system of the cell, leading to activation of the ingestive process itself, cannot be ruled out.

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IMMUNOSTIMULATING PROPERTY OF ASPARTIC ACID

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Not only thymus peptides, but also brain peptides and some of their synthetic analogs have immunostimulating properties [3, 12]. An important role is ascribed to them in the regulation of higher nervous activity [5, 12], in which not only peptides but also individual amino acids, such as aspartic acid (AA) participate [4]. The question arises whether individual amino acids and, in particular, AA can act on processes of immunogenesis. The aim of this investigation was to compare the effect of AA and functionally different oligopeptides containing it (thymopentin [11, 13], pentagastrin [6, 7]) on differentiation of bone marrow precursor T cells into T cells and on the immune response in mice.

EXPERIMENTAL METHOD

Experiments were carried out on 406 male CBA mice weighing 14-16 g. The substance tested included AA (from Sigma, USA), the pharmacopoieal preparation panangin, which is a mixture of potassium and magnesium aspartates, and pentapeptides, including AA in their composition: thymopentin (HArg-Lys-Asp-Val-Tyr-OH), synthesized in the Department of Natural Compounds, Scientific-Research Institute of Chemistry, A. A. Zhdanov Leningrad University, and pentagastrin (Boc-HAla-Trp-Met-Asp-Phe-NH_2 [6, 7], from Sanitas, Kaunas).

Bone marrow precursor T cells were differentiated as described previously [2], by a modified method [8]. after treatment of the bone marrow cells *in vitro* with the test preparations at 37°C for 1.5 h. The number of T lymphocytes in the bone marrow cell population was determined with the aid of rabbit antibrain serum in the complement-dependent cytotoxicity test [1]. The antiserum was used in a dilution of 1:50, in which, in the presence of fresh guinea pig complement (1:3), and after absorption by liver homogenate and mouse and sheep red blood cells [1], it caused death of 85 ± 2.5% of thymocytes and did not interact with bone marrow cells of CBA mice. In each test at least 200 cells, whose viability was estimated with a 0.2% aqueous solution of trypan blue, were counted. The experiment was repeated no fewer than 4-5 times.

The preparations were injected subcutaneously into the animals, dissolved in pyrogen-free physiological saline, daily for 10 days. The AA and panangin, like the other amino acids composing the test peptides, were injected in a dose of 1 μg, and thymopentin and pentagastrin in a dose of 5 μg daily. The control animals received pyrogen-free physiological saline by a

TABLE 1. Stimulation of Differentiation of T Precursor Cells *in Vitro* by AA, Panangin, Thymopentin, and Pentagastrin (M ± m)

Dose of preparation, μg/ml	Cytotoxicity index of antibrain serum (in %) after treatment of bone marrow cells with				
	AA	panagin	thymopentin	pentagastrin	Hanks' solution
1	20,7±2,8	6,9±1,8	0	17,5±2,7	
0,1	19,5±2,7	9,4±2	0	16,1±2,1	
0,01	19,7±2,8	9,7±2	0	16,8±1,9	0
0,001	13,5±2	3,0±0,8	0	2,8±1,2	
0,0001	1,3±0,8	0	6,9±1,8	0	
0,00001	0	0	6,5±1,7	0	

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