Mechanism Underlying the Effect of Myelopeptides on Lymphocyte Proliferation *in Vitro*

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We studied the role of monocytes in the effect of myelopid and myelopeptides MP-1, MP-3, MP-5, and MP-6 on functional activity of peripheral blood lymphocytes in the reaction of blast transformation. Myelopeptides MP-1, MP-3, and MP-6 suppressed blast transformation of lymphocytes. The effect depended on the presence of monocytes in a cell culture.

Key Words: *myelopid; myelopeptides; monocytes; lymphocyte blast transformation*

Myelopeptides (MP) belong to the group of bioregulatory bone marrow peptides with neurotropic, differentiation, and immunocorrecting properties [6]. The mixture of MP exhibits a wide range of biological activities, which is associated with directed effect of individual peptide fractions on various stages of immunogenesis. This effect is mediated by the interaction of MP with specific cell populations. MP-1 has specific binding sites on T lymphocytes [3,7]. MP-3 activates monocytes and macrophages [1]. MP-6 stimulates differentiation of grafted human promyelocytic leukemia cells (HL-60) [4]. The mechanisms for the action of individual MP on the interaction of immune cells during progression of the immune response are poorly understood.

Here we studied the role of monocytes in the effect of myelopid, MP-1, MP-3, MP-5, and MP-6 on functional activity of peripheral blood lymphocytes in the reaction of blast transformation (RBTL).

MATERIALS AND METHODS

Peripheral blood leukocytes were isolated from healthy donors (men, 22-30 years). These cells were cultured with phytohemagglutinin P (PHA, Sigma) in optimal and suboptimal concentrations (20 and 2.5 μg/ml, respectively) in 96-well round-bottom plates. Each culture contained 2×10^5 cells in 0.2 ml complete nutrient medium consisting of medium 199, 10 mM HEPES (Sigma), 2 mM L-glutamine (Sigma), 100 μg/ml gentamicin, and 10% autoplasma.

Culturing was performed in a humid atmosphere at 5% CO_2 and 37°C for 72 h. Methyl-^{3}H-thymidine (2 μCi, 10 μl) was added to each well 18 h before the end of culturing. The content of each well was subsequently pelleted on a filter paper at a vacuum pump pressure of 0.5-1.0 atm. Each well was washed 4-5 times with 0.85% NaCl. The filters were washed with 5 ml 0.85% NaCl, 5 ml cold 5% trichloroacetic acid (4°C), and 5 ml 0.85% NaCl to remove unbound thymidine.

Radioactivity of samples was measured on a Guardian liquid scintillation counter (Wallac). MP-1 (Phe-Leu-Gly-Phe-Thr) and MP-3 (Leu-Val-Cys-Tyr-Pro-Gln) were used in concentrations of 10^{-6}, 10^{-8}, and 10^{-10} g/ml. Myelopid, MP-5 (Val-Val-Tyr-Pro-Asp), and MP-6 (Val-Asp-Pro-Pro) were applied in a concentration of 10^{-7} g/ml [5]. The test preparations were added immediately after the start of culturing. MP were presented by A. A. Mikhailova (Institute of Bioorganic Chemistry, Russian Academy of Sciences).

The fraction of mononuclear cells was isolated on a Ficoll-Verografin density gradient (d=1.077). To this end, 4 ml heparinized blood was layered onto 2 ml Ficoll-Verografin and the tubes were centrifuged at 400*g* (1500 rpm) and room temperature for 40 min.

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The fraction of mononuclear cells was washed 2 times with cold medium 199 and resuspended in cold complete nutrient medium containing 10% autoplasma. The cell suspension was maintained at 4°C for 1 h to suppress activation associated with isolation. The suspension of mononuclear cells was cooled, resuspended in 5 ml complete nutrient medium, and placed in a sterile 9-cm Petri dish to remove monocytes. The dish was placed in a thermostat at 37°C for 1 h. Nonadherent cells were shaken, transferred into another Petri dish, and repeatedly incubated for 1 h. Nonadherent cells were collected into a glass tube. Each culture contained 2×10^5 cells in 0.2 ml complete nutrient medium.

The effects of MP-1 and MP-3 were analyzed by one-way analysis of variance for paired samples and Fischer's test for least significant difference. The effects of myelopid, MP-5, and MP-6 were analyzed by pairwise Student's *t* test.

RESULTS

Myelopid did not modulate spontaneous and PHA-induced proliferation of lymphocytes. Similar results were obtained in experiments with monocyte-depleted cultures (Table 1). Published data show that myelopid can stimulate or suppress proliferation of peripheral blood lymphocytes and phagocytic activity of peripheral blood cells from healthy donors [2]. The absence of the effect of myelopid in our experiments had can be explained by the presence of admixtures of α-endorphins, β-endorphins, and γ-endorphins in femtomolar concentrations (apart from 6 major myelopeptide fractions) producing opposite immunoregulatory effects [4].

MP-1 had a significant effect on cell proliferation induced by 20 μg/ml PHA (F=4.6, p<0.01). MP-1 in a concentration of 10^{-10} g/ml inhibited ^{3}H-thymidine incorporation into lymphocytes. MP-1 did not modulate RBTL in the presence of 2.5 μg/ml mitogen. MP-1 had no effect on spontaneous proliferation of cells. After removal of monocytes from the fraction of mononuclear cells, MP-1 had no effect on induced and spontaneous proliferation of cells. These data show that the peptide produced no direct effects on lymphocyte proliferation. A minor inhibitory effect of PM-1 on RBTL was mediated by monocytes. Our previous studies showed that MP-1 in a concentration of 10^{-8} g/ml *in vitro* decreases phagocytic activity of neutrophils [2]. It should be emphasized that the key immunoregulatory effect of this peptide are recovery of antibody production after exposure to inhibitory factors due to specific low-affinity binding to the surface of CD4$^+$ lymphocytes [3,7] and liquidation of CD4$^+$/CD8$^+$ imbalance caused by concanavalin A. The abi-

TABLE 1. Effects of Myelopid and MP on Proliferation of Peripheral Blood Lymphocytes (cpm, $M\pm m$, n=9)

Experimental treatment	Dose, g/ml	Non-fractionated cell culture			Monocyte-depleted cell culture		
		without mitogen	PHA, μg/ml		without mitogen	PHA, μg/ml	
			20	2.5		20	2.5
Control		314.72±45.75	91,136.35±14,847.67	30,280.70±5718.16	374.01±82.01	8627.42±4813.17	4455.19±1469.33
Myelopid	10^{-7}	349.62±70.81	86,768.92±10,009.57	28,263.71±6927.96	351.40±53.88	11,366.43±6047.17	10,327.12±5171.92
MP-1	10^{-6}	313.61±30.50	104,289.32±18,837.12	27.027.06±6329.32	329.70±47.75	2935.26±1184.23	6795.19±2807.04
	10^{-8}	285.81±26.72	66,964.02±12 633.18	24,105.9±7535.88	262.87±37.53	2625.99±1186.74	7750.14±3483.98
	10^{-10}	260.71±28.41	52,991.40±15,505.92*	17,935.79±5135.51	245.56±37.91	5049.02±2137.00	8489.64±3705.10
MP-3	10^{-6}	309.57±36.20	83,176.62±17,025.91	23,621.19±4148.02*	278.68±50.98	4314.78±2061.03	3918.92±1489.31
	10^{-8}	277.62±26.83	90,939.63±17,451.68	16 649.26±3324.34***	236.96±84.94	4043.70±1902.45	3637.63±1268.56
	10^{-10}	321.14±27.37	66,280.63±14,126.00	20,515.60±5437.23***	320.77±72.24	4814.19±2725.41	3548.63±1289.58
MP-5	10^{-7}	273.84±39.96	71,156.39±13,408.61	23,985.23±5704.37	250.49±51.32	11,935.66±6627.46	5866.14±2288.89
MP-6	10^{-7}	368.78±30.41	79,392.38±14,782.59	20,605.41±3666.33*	188.62±22.42	3648.83±1897.42	3988.45±2042.98

Note. *p<0.05, **p<0.01, and ***p<0.001 compared to the control.

lity of MP-1 to normalize immune parameters contributes to its inhibitory effect on proliferation in the presence of high concentration of the mitogen.

MP-3 significantly modulated lymphocyte proliferation in the non-fractionated culture containing PHA in the suboptimal dose of 2.5 μg/ml (F=9.3, p<0.001). This peptide in different concentrations inhibited RBTL. This effect was less pronounced after removal of monocytes from the fraction of mononuclear cells. MP-3 had no effect on spontaneous proliferation of cells in the non-fractionated and monocyte-depleted culture containing PHA in an optimal concentration of 20 μg/ml. Previous experiments demonstrated that MP-3 stimulates phagocytosis, increases cytotoxicity, promotes adhesion of monocytes, and activates expression of Ia-antigens. However, this peptide did not modulate the synthesis of interleukin-1 and tumor necrosis factor-α the major growth factors initiating lymphocyte proliferation [1]. Our results are consistent with published data that monocytes and macrophages serve as the major target for MP-3.

The effect of MP-6 was similar to that observed after treatment with MP-3. MP-6 inhibited RBTL in a non-fractionated culture containing PHA in the suboptimal concentration. After removal of monocytes from the culture MP-6 did not modulate proliferation of cells. The inhibitory effect of MP-6 on RBTL is probably associated with differentiation activity of this peptide [4] and modulation of lymphocyte proliferation realized via the interaction with cultured monocytes.

MP-5 had no effect on spontaneous and mitogen-induced proliferation of lymphocytes.

Our findings indicate that monocytes play an important role in the effects of MP-1, MP-3, and MP-6. They can produce a direct or indirect effect on the immune response.

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