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REGULATION OF IMMUNOGLOBULIN PRODUCTION BY MYELOPIDE IN PERIPHERAL BLOOD LYMPHOCYTE CULTURE FROM NORMAL INDIVIDUALS AND PATIENTS WITH A SECONDARY IMMUNODEFICIENCY STATE

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Myelopide (MP) is an immunocorrective agent based on myelopeptides — a group of low-molecular-weight peptides secreted by bone marrow cells, and discovered because of their ability to intensify antibody production in the productive phase of the immune response [2]. Addition of MP to a culture of immune mouse lymph node cells or injection of MP into animals at the peak of the secondary immune response leads to an increase in the number of antibody-forming cells and in the titers of antibodies both to soluble and to corpuscular antigens in the blood [3, 7]. Moreover, the antibody-stimulating effect of MP is more marked against the background of immunodeficiency states. For instance, in mice of the MRL/1 pr line with genetically determined disturbance of the immune system, injection of MP restored the secondary immune response to sheep's red blood cells (SRBC) [4]. The study of antibody production by peripheral blood lymphocytes (PBL) from patients with agammaglobulinemia [6] showed that MP increases the IgG and IgM levels in cultures stimulated by pokeweed mitogen.

In this investigation we studied the effect of MP on secretion of immunoglobulins (Ig) in vitro in PBL of healthy individuals and of patients for heart surgery. It was shown previously that operations for rheumatic diseases of the heart valve in these patients lead to the development of an immunodeficiency state [5].

It has been shown that MP has a stimulating effect on mitogen-induced IgA and IgM production.

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TABLE 1. Effect of MP (in μg/ml) on Ig Production by Healthy Human PBL

PWM, μg/ml		Control (without MP)	Injection of MP
IgG	0,05	4,5	4,9
	0,125	5,3	5,1
IgA	0,05	3,3	4,3*
	0,125	4,8	5,4
IgM	0,05	5,9	6,0
	0,125	8,6	7,8

Legend. Here and in Table 2, data given in the form of mean geometric IS; $*p < 0.05$ denotes significant stimulation by MP.

TABLE 2. Effect of MP (in μg/ml) on Ig Production by PBL from Patients for Heart Surgery

PWM, μg/ml		Time of investigation							
		before operation		1-2 days after operation		7-8 days after operation			
						comparison group		MP therapy after operation	
		w/o MP	with MP	w/o MP	with MP	w/o MP	with MP	w/o MP	with MP
IgG	0,05	2,7*	2,7	1,2	1,1	1,7*	1,7	1,3	1,3
	0,125		2,4	1,2	1,2	1,6*	1,7	1,7*	1,7
IgA	0,05	2,4*	2,6	1,0	0,9	1,1	1,3	1,2	1,0
	0,125	2,6*	2,6	1,0	1,0	1,1	1,4	1,8*	1,5
IgM	0,05	3,6*	3,6	1,4*	1,5	3,7*	3,5	5,8*	5,8
	0,125	4,7*	4,7	1,4*	1,5	4,5*	6,3	8,1*	8,9

Legend. Significance of stimulation by MP underlined ($p < 0.05$).

EXPERIMENTAL METHOD

Peripheral blood lymphocytes (PBL) were isolated on a Ficoll-Hypaque gradient. The cells were cultured in round-bottomed microplanchets for 8 days at 37°C in an atmosphere with 5% CO_2. The concentration of the cell suspension was 10^6/ml and the volume of each individual culture was 0.2 ml. The culture medium, based on RPMI 1640, contained 10% of embryonic calf serum ("Flow Laboratories," Great Britain), 2 mM gluamine, 5×10^{-5} M 2-mercaptoethanol, 10 mM HEPES buffer, and 50 μg/ml of gentamicin. Spontaneous Ig synthesis was determined in a cell culture without the mitogen. Ig synthesis was stimulated by the use of pokeweed mitogen (PWM) ("Sigma," USA) in optimal (0.125 μg/ml) and suboptimal (0.05 μg/ml) concentration. MP in a concentration of 0.5 μg/ml was added to the culture at the beginning of cultivation [1]. The concentrations of IgG, IgA, and IgM in the supernatants was determined by ELISA [9]. Flat-bottomed planchets made of polyvinyl chloride were activated by γ-fractions of monospecific goat antisera against human IgG and IgM or sheep antiserum against human IgA, diluted with 0.1 M phosphate buffer (pH 7.4) to a concentration of 12 μg/ml. The test supernatants and also standard solutions of Ig, which were used to obtain calibration curves, were diluted with 0.05% Tween-20 with 0.5% bovine serum albumin in phosphate buffer. Conjugates of horseradish peroxidase with goat antibodies against γ-, α-, and μ-heavy chains of human Ig ("Sigma," USA) were used in a dilution of 1:1000. The substrate mixture consisted of 0.04% orthophenylenediamine and 0.012% H_2O_2 in 0.5 M citrate-phosphate buffer (pH 5.0). The optical density of the specimens was measured at a wavelength of 492 nm. Indices of stimulation (IS) were calculated as the ratio of the Ig level in the experiment (with or without MP) to the Ig level in the control. The results were subjected to statistical analysis by Wilcoxon's paired test.

EXPERIMENTAL RESULTS

Ig production in a culture of PBL was studied in healthy individuals (27 subjects), in patients before their operation (34 subjects), on the 1st-2nd day after the operation (37 subjects), and on the 7th-8th day after the operation, in two groups of

patients: after immunocorrective treatment by MP (17 subjects) and patients not receiving MP (15 subjects) – the comparison group.

A comparative study of Ig production induced by mitogens in a culture of PBL from healthy individuals and patients revealed the following general pattern. In the preoperative period, the level of Ig production of all classes was lower in the patients than in the healthy subjects ($p < 0.05$; Table 1, Table 2).

On the 1st-2nd day after the operation even more intensive suppression of the response to PWM was observed: the mitogen did not stimulate production of IgA or IgG, and the index of stimulation of IgM production was lower than before the operation.

On the 7th-8th day after the operation the response to the mitogen in patients of the comparison group was partially restored – a significant increase in secretion of IgG and IgM was observed in the culture of PBL, but IgA production was unchanged as before. In the group receiving MP postoperatively, restoration of the response in the case of IgA also was observed, but IgM production was higher than in the comparison group and indistinguishable from the normal level.

The sensitivity of mitogen-induced antibody production to MP in healthy individuals and patients tested at different times varied. In normal individuals MP increased IgA production in culture stimulated by a suboptimal dose of PWM. In patients in the preoperative and early postoperative periods MP did not change mitogen-induced antibody production.

On the 7th-8th days after the operation, MP stimulated IgA production in the comparison group, but also increased IgM production by 1.4 times against the background of stimulation by PWM in the optimal dose. In a culture of PBL from patients receiving immunocorrective treatment with MP, the preparation did not change the level of Ig production (Table 2).

Thus, the stimulating effect of MP on Ig production in vitro is exhibited only in a culture of PBL activated by mitogen, both in the case of normal individuals and patients for heart surgery. The absence of effect of MP in vitro on patients before the operation and in the early postoperative period may be due to weakening of the ability of PBL to respond to PWM. In the postoperative period this weakening is probably connected with the considerable depression of T-cell function [5], for these cells are known to play an important role in the development of the responses of PBL to mitogen [8].

It was shown that immunocorrective treatment with MP in the postoperative period leads to more complete restoration of the response to PWM in vitro and it is accompanied by abolition of its sensitivity to MP. The most interesting aspect of MP is in connection with IgA and IgM antibody production.

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