COMPARISON OF THE PROLIFERATIVE AND IMMUNOGLOBULIN-SECRETING ABILITY OF PERIPHERAL BLOOD LYMPHOCYTES OF HEALTHY SUBJECTS AND PATIENTS WITH B-CELL IMMUNOPROLIFERATIVE DISEASE

A. E. Kopylov, L. V. Koval'chuk, R. N. Stepanenko, A. K. Golenkov, and I. N. Nazarova

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Much attention is currently being paid to the study of the mechanism of activation, proliferation, and differentiation of immunocompetent cells and, in particular, of B lymphocytes [4]. On the basis of these key processes of the immune response new principles of evaluation of the human immune system are being developed [1]. In this connection the study of these processes under normal and immunopathological conditions and, in particular, in multiple myeloma, is assuming great importance. This disease is characterized by uncontrolled proliferation of malignant plasma cells in the bone marrow together with accompanying changes in the quantitative composition and functional activity of the immunocompetent cells of the peripheral blood.

In this investigation we compared mitogen-induced proliferative ability and immunoglobulin synthesis in vitro by peripheral blood lymphocytes from patients with multiple myeloma and healthy controls.

EXPERIMENTAL METHOD

Of 12 untreated patients with monoclonal gammopathy 11 had a diagnosis, according to the classification, of multiple myeloma (IgG type) and one had a diagnosis of Bence–Jones proteinuria. Blood from 15 healthy donors was used as the control.

Mononuclear peripheral blood cells were isolated on a Ficoll-PAK gradient. The cells were washed twice with phosphate buffer and once with medium 199, after which they were resuspended in complete medium RPMI 1640, containing 10% fetal calf serum, 2 mM L-glutamine, $5 \cdot 10^{-5}$ M 2-mercaptoethanol, 50 mg/ml of gentamicin, and 10 mM HEPES-buffer. To assess their proliferative capacity the cells in a concentration of $0.5 \cdot 10^6$/ml were cultured in the presence of phytohemagglutinin (PHA) 5 μg/ml for 3 days, and with pokeweed mitogen (PWM) 0.25 μg/ml for 3, 4, and 5 days in round-bottomed planchets at 37°C in an atmosphere with 5% CO_2. ^{3}H-thymidine was added in a dose of 1 μCi per well 6 h before the end of culture. The cells were transferred to filters and incorporation of the label determined in CPM on a scintillation counter. To assess immunoglobulin production, cells in a concentration of $1 \cdot 10^6$/ml were cultured for 8 days under the same conditions, the supernatant was collected, and the quantity of IgA, IgM, and IgG was determined by ELISA [5]. Monospecific antisera and conjugates were provided by Candidate of Biological Sciences N. V. Sorokina (Department of Chemical Enzymology, M. V. Lomonosov Moscow University).

The results were subjected to statistical analysis by Student's test, Mann–Whitney nonparametric test, and Pierson's test.

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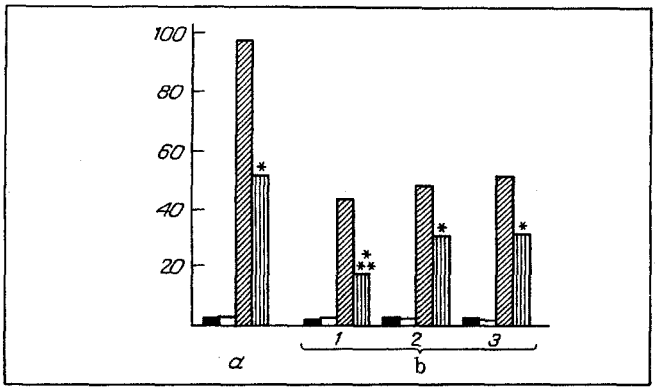

Fig. 1. Ordinate, cpm · 10^{-3}; abscissa: a) response to PHA on 3rd day; b) response to PWM (on 3rd day – 1, on 4th day – 2, on 5th day – 3); *p < 0.01 indicates significant differences compared with response of healthy controls; **) significant differences compared with response of patients' lymphocytes on 4th and 5th days; shaded columns – response of healthy controls, unshaded columns – response of patients; small columns denote spontaneous proliferation.

TABLE 1. In Vitro Production of Immunoglobulins IgA, IgM, and IgG (in ng/ml)

Groups	Spontaneous production			Induced by PMW			Index of stimulation		
	IgA	IgM	IgG	IgA	IgM	IgG	IgA	IgM	IgG
Healthy (n = 15)	154±63	186±117	237±134	392±148	459±249	532±265	3,9	4,0	3,3
Patients (n = 12)	133±78	59±29*	188±102	253±101	615±301	657±376	5,1	4,4	3,5

Legend. *p < 0.01 denotes significant difference.

EXPERIMENTAL RESULTS

Proliferation of lymphocytes of healthy blood donors, stimulated by PWM reached a maximal on the 5th day, although differences on individual days were not significant. The response to PHA on the 3rd day was significantly higher than that to PWM (Fig. 1). Meanwhile proliferation of lymphocytes from patients with multiple myeloma in response to these mitogens was significantly inhibited ($p < 0.01$), and amounted to about 50% of the response of the healthy subjects. Statistically significant differences also appeared between the response on different days. For instance, the level of proliferation on the 3rd day on the patients was significantly lower than on the 4th and 5th days. However, despite the differences revealed, spontaneous proliferation of the peripheral blood lymphocytes was similar in patients and healthy controls.

These results are in agreement with those obtained by other workers [3] and indicate reduced ability of peripheral blood lymphocytes to give a proliferative response to stimulation by polyclonal activators.

The concentration in vitro of immunoglobulins secreted by healthy human lymphocytes after their stimulation by PWM was 532 ± 265 ng/ml for IgG, 459 ± 249 ng/ml for IgM, and 392 ± 148 ng/ml for IgA. According to Pierson's nonparametric paired test, significant positive correlation ($p < 0.05$) exists between synthesis of the individual classes of immunoglobulins. No such correlation could be found between proliferative activity and immunoglobulin synthesis, and in our opinion this may due to the existence of different systems for proliferation and differentiation of B lymphocytes [2].

Similar results of PWM-induced immunoglobulin production (Table 1) and a significant difference in the level of spontaneous IgM production (59 ± 29 ng/ml, $p < 0.01$) were obtained in patients with multiple myeloma. Correlation between synthesis of the three classes of immunoglobulins also was found.

It can thus be concluded from these results that in patients with multiple myeloma, if spontaneous proliferation is preserved the proliferative response to PHA and PWM is depressed, but if PWM-induced immunoglobulin production is normal, spontaneous IgM production is depressed.

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