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Proliferative Response of Lymphocyte to Pokeweed Mitogen Depends on the Concentration of Endogenous Cortisol in the Early Post-Traumatic Period in Patients with Penetrating Eye Injury

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The intensity of lymphocyte proliferation in response to pokeweed mitogen depends on cortisol level in the peripheral blood in the early post-traumatic period of penetrating eye injury. Lymphocyte proliferation in 72- and 96-h cultures from patients with high levels of endogenous hormone was suppressed. In 120-h cultures, the intensity of proliferation remains unchanged. Lymphocyte blast transformation was increased in 120-h cultures from patients with normal cortisol concentration and remained unchanged in case of low cortisol level.

Key Words: *lymphocytes; cortisol; penetrating eye injury*

Immune system plays an important role in the pathogenesis of the inflammatory process in the injured eye [1,5,7,8,12,13]. Specific features of eye structure and systemic mechanisms of antigen response contribute to the immune privilege of this organ and its resistance to damage [15]. Penetrating eye injury (PEI) is an example of local injury inducing not only local responses with impaired immunosuppression in the organ, but also systemic stress-induced changes in the immune system in response to the threat of organ loss [6].

In the early post-traumatic period before therapy (days 1-3 after injury), pronounced suppression of lymphocyte proliferation response to pokeweed mitogen (PWM) and phytohemagglutinin was noted in 72-h cultures from patients with PEI, which attested to inhibition of T cell activation and proliferation [9,10]. Similar changes in the immune responses to thymus-dependent antigen were described in experimental PEI [2]. Suppression of delayed-type hypersensitivity response and antibody production was detected in rats after administration of the sensitizing dose of sheep erythrocytes 7 h after injury. Blast transformation of lymphocytes cultured with PWM is the experimental model for evaluation of the thymus-dependent response of human B-cells *in vitro* and reflects *in vivo* events [11,14].

Here we studied the dependence of lymphocyte proliferative response to PWM on the levels of endogenous cortisol in patients with PEI in the early post-traumatic period.

MATERIALS AND METHODS

The study included 20 men (age 19-49 years, mean 35 years) with severe PEI. The patients were examined at admission to the hospital before the start fo gluco-

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corticoid therapy (days 1-3 after injury). Ten healthy age-matched male volunteers served as the control.

Lymphocyte proliferative response was assessed in cultures with PWM, a thymus-dependent polyclonal B-cell activator (L8777, Sigma), in concentrations of 0.625, 1.25 and 2.5 μg/ml in medium 199 supplemented with 2 mM L-glutamine, 10 mM HEPES, 100 μg/ml gentamicin sulfate, and 10% autoplasma (2×10^5 cells per well in a round-bottom 96-well plate, total volume of culture 0.2 ml). The cells were cultured in a humidified atmosphere with 5% CO_2 for 72, 96, and 120 h at 37°C. Methyl-^{3}H-thymidine (1 μCi, 37 kBq; Izotop Ltd.) was added to each well 18 h before the end of culturing. Radioactivity of acid-insoluble fraction was measured on a Guardian WinSpectral DSA 1414-03 Wallac liquid scintillation counter.

Blood cortisol levels were measured by competitive ELISA (Khema Medika test systems).

Statistical analysis was performed taking into account the log-normal distribution of the parameters of lymphocyte proliferation using post-hoc Duncan's test for multiple comparisons between the groups and *F* ratio.

RESULTS

The patients exhibited significant heterogeneity in *F* ratio of individual serum levels of endogenous cortisol

TABLE 1. Effect of Blood Serum Levels of Endogenous Cortisol in Patients with PEI during the Early Post-Traumatic Period on *in Vitro* Lymphocyte Proliferative Response to PWM

PWM concentration, μg/ml	Control group (*n*=10)	Subgroups examined		
		high cortisol level (*n*=6)	normal cortisol level (*n*=9)	low cortisol level (*n*=5)
72-h cultures				
0	3.5508±0.0734 (3554)	3.2854±0.1017* (1929)	3.3900±0.0724 (2455)	3.2278±0.2115 (1690)
0.625	4.0481±0.1498 (11,171)	3.8261±0.1405+ (6701)	4.4122±0.1240 (25,837)	3.9533±0.3197 (8980)
1.25	4.3230±0.0903 (21,039)	4.0329±0.1336 (10,786)	4.5282±0.0945 (33,743)	4.3155±0.0818 (20,677)
2.5	4.4418±0.0852 (27,658)	4.0907±0.1257*+ (12,322)	4.5124±0.1097 (32537)	4.3135±0.0666 (20,584)
96-h cultures				
0	3.4424±0.1030 (2769)	3.2758±0.0758 (1887)	3.2968±0.0714 (1980)	3.2049±0.1837 (1603)
0.625	4.0585±0.1636 (11,443)	4.0852±0.1505 (12,167)	4.5973±0.1092 (39,568)	4.1113±0.3408 (12,922)
1.25	4.3147±0.1341 (20,640)	4.1027±0.1368+ (12,667)	4.7328±0.0805 (54,054)	4.3228±0.0888 (21,028)
2.5	4.5640±0.0901 (36,645)	4.1475±0.1213*+ (14,045)	4.7721±0.0620 (59,176)	4.3508±0.1874 (22,427)
120-h cultures				
0	3.4682±0.0915 (2939)	3.3298±0.0402 (2137)	3.1681±0.1208 (1473)	3.2601±0.1291 (1820)
0.625	4.0955±0.1365 (12,460)	4.2761±0.1467 (18,884)	4.5697±0.0994* (37,131)	4.4103±0.1948 (25,721)
1.25	4.3216±0.1383 (20,969)	4.2825±0.1451 (19,166)	4.4913±0.1960 (30,995)	4.5014±0.1293 (31,724)
2.5	4.5860±0.0905 (38,548)	4.3379±0.1643 (21,770)	4.7388±0.0580 (54,808)	4.5852±0.1314 (38,475)

Note. The values *M*±*m* are given for the parameters $\log_{10}$ cpm; in parentheses: geometric means of cpm. $p<0.05$ in comparison with: *control group (Duncan's test), +subgroup with normal cortisol level.

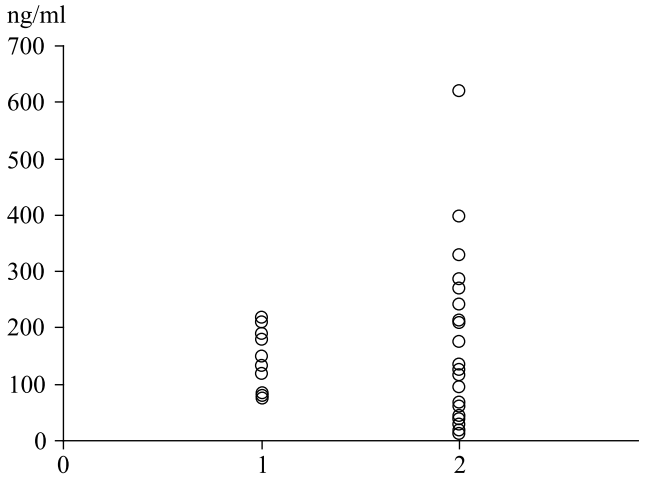

Fig. 1. Individual serum levels of cortisol in the early post-traumatic period. *1*) control group; *2*) patients.

in the early post-traumatic period in comparison with the control group (F=8.43; p=0.003; Fig. 1). Based on individual hormone levels, the patients were divided into 3 subgroups: with high (>216 ng/ml; subgroup 1), normal (50-216 ng/ml, subgroup 2), and low (<50 ng/ml, subgroup 3) levels of endogenous cortisol.

In 72-h cultures from individuals with high levels of endogenous cortisol, the lymphocyte proliferative response to optimal PWM concentration (2.5 μg/ml) and spontaneous proliferation of lymphocytes were suppressed in comparison with those in the control group (Table 1). In patients with normal or low cortisol levels, no significant changes in lymphocyte proliferation were detected. The involvement of increased endogenous cortisol in the suppression of lymphocyte proliferative response was confirmed by lower intensity of lymphocyte proliferative response in patients with high cortisol levels in comparison with that in patients with normal hormone concentration.

In 96-h cultures, reduced lymphocyte proliferative response in comparison with the control group was found only in patients with high levels of endogenous cortisol (Table 1). The intensity of lymphocyte proliferation in cultures containing PWM in concentrations of 1.25-2.5 μg/ml in these patients was also lower than in individuals with normal cortisol levels.

In 120-h cultures with suboptimal PWM concentration (0.625 μg/ml), lymphocyte proliferative response surpassed the control level only in patients with normal cortisol concentration (Table 1).

It should be noted that enhanced immunoglobulin production associated with thymus-dependent B cell activation in cultures with PWM was recorded not earlier than after 96 h and was most pronounced after 120 h and later, while increased production of IL-4 and IFN-γ by T cells was observed as soon as after 24 h [11,14]. It can be assumed that different changes in lymphocyte proliferation in response to PWM in 72-, 96-, and 120-h cultures found by us are linked with temporal characteristics of T-and B-cell proliferation. This is confirmed by the fact that changes in the proliferative response in 72-h cultures with PWM and the response to phytohemagglutinin, the classic T-cell mitogen, were co-directed, while the changes in lymphocyte proliferation in 120-h cultures with PWM had the opposite character [9,10].

Two adaptive strategies of the organism are currently considered, namely the resistant (classical manifestation, sympathoadrenal stress and general adaptation syndrome) and the tolerant strategy (concession to the environment, minimization of body functions in strain and extremely dangerous situations) [3,4]. From this perspective, we can assume that the first strategy was implemented in the patients with elevated levels of endogenous cortisol and the second in patients with low concentrations of hormone, and therefore the latter display no changes in the lymphocyte proliferative response.

The results confirm that lymphocyte proliferative response to PWM in patients with penetrating eye injury depends on the concentration of endogenous cortisol in the early post-traumatic period.

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