Effect of Hydrocortisone on Spontaneous and Mitogen-Dependent Activity of Peripheral Blood Lymphocytes in Some Vertebrates

P. R. Alimkhodzhaeva, A. S. Usmanova*, and B. S. Gil'dieva

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 133, No. 5, pp. 544-547, May, 2002
Original article submitted December 19, 2001

The effect of hydrocortisone on functional activity of peripheral blood lymphocytes in different vertebrates during ontogeny was studied by the blast transformation test. Hydrocortisone in a dose of 100 mg/kg inhibited functional activities of T and B lymphocytes *in vitro* stimulated by mitogens. This inhibitory effect was observed in both young and adult animals. However proliferative activity of concanavalin A-dependent blood T suppressors in rats increased, especially in adult animals (by 3.5 times). Hence, hydrocortisone produced different effects on functional activity of peripheral blood lymphocytes in the studied species, and these effects are dose- and species-dependent.

Key Words: *lymphocytes; mitogens; vertebrates; hydrocortisone*

Capacity to transformation into blast cells under the effects of antigens and mitogens is a most important functional characteristic of lymphocytes. Evaluation of functional activity of immunocompetent cells *in vitro* helps to evaluate the status of cell immunity *in vivo*, and therefore alteration of lymphocyte blast transformation in response to different stimulators in comparison with spontaneous transformation is an important criterion of the immune status in clinical practice. The method is based on stimulation of minor lymphocytes, which in the presence of mitogens and antigens are transformed into blast-like cells, capable of mitosis and proliferation [4]. Such transformation is characteristic of the greater part of human and animal circulating lymphocytes.

Few reports on functional activity of vertebrate immunocompetent cells by the blast transformation method are available [5,7]. Data on blast transforming activity of animal lymphoid cells in the phylo- and ontogeny are scanty [2,6]. We investigated functional activity of peripheral blood lymphocytes in vertebrates of different classes in the ontogeny in response to stimulation with mitogens and sheep erythrocytes (SE) and under the effect of hydrocortisone.

Department of Biology, Russian State Medical University, Moscow; *Department of Medical Biology and Genetics, First Tashkent State Medical Institute

MATERIALS AND METHODS

Experiments were carried out on young (15-20 g) and adult (45-50 g) *Rana ridibunda* frogs (*n*=50), young (15-20 g) and adult (45-50 g) *Bufo viridis* toads (*n*=40), this year's broods (200-250 g) and adult (400-500 g) *Testudo horsfieldi* turtles (*n*=24), *Gallus gallus* chicken (*n*=30) aged 1, 3, 5, 7, and 20-30 days, and *Rattus norvegicus* albino rats (*n*=24) aged 3, 10, and 30 days kept in vivarium in spring-summer.

The animals were sacrificed by decapitation. Functional activity of lymphocytes was evaluated by incorporation of [^{3}H]-thymidine in cell DNA under the effect of mitogens, immunization with SE, and hydrocortisone injection. Phytohemagglutinin (PHA), concanavalin A (ConA), and pokeweed mitogen (PWM) were used.

Lymphocyte sensitivity to different doses of mitogens was studied in preliminary experiments. Optimal

0007-4888/02/1335-0471$27.00

doses of mitogens, inducing the greatest stimulation of peripheral blood lymphocyte blast transformation, were selected for each animal species. The optimal dose of PHA for frog and turtle lymphocytes was 30 μg/ml, for birds and rats 60 μg/ml; optimal doses of ConA were 20 and 40-60 μg/ml, respectively, of LPS 50 μg/ml, and of PWM 1 μg/ml for all animals.

Immunization with SE was carried out 1 week before sacrifice. Hydrocortisone (Richter) was injected intramuscularly in a single dose of 100 mg/kg. Controls were injected with normal saline. Stimulation index (SI) was estimated as the ratio of mitogen-stimulated to spontaneous proliferative activity.

We showed that the highest lymphopenic reaction to hormone manifested on days 5-7 after injection in amphibians, on day 10 in turtles, on days 1-3 in birds, and during the first hours in mammals; therefore the material was collected in these periods.

RESULTS

Study of spontaneous and mitogen-stimulated activity of peripheral blood lymphocytes of different vertebrates showed that these mitogens selectively modulated functional activity of lymphocytes in different animals, depending on their status on the evolution scale and period of individual development. PHA and ConA increased proliferative activity of peripheral blood lymphocytes of toads, turtles, chicken, and rats, while in frogs PHA inhibited DNA synthesis (Table 1).

Immunization with SE increased functional activity of T lymphocytes. Turtle lymphocytes in 5-fold diluted blood exported to PHA stimulation by more than 8-fold increase of functional activity, while ConA-dependent T suppressors virtually did not exported to ConA stimulation. B lymphocytes exported to PWM by more than 2-fold increase of proliferative activity. Ten-fold dilution of turtle blood resulted in a decrease of T lymphocyte blast transformation, while B lymphocytes remained sensitive even in 10-fold diluted blood.

The dose of PHA inducing the greatest stimulation of peripheral blood lymphocytes in chicken aged 1 week and 1 month (SI—1.27 and 2.45, respectively) was 60 μg/ml, while in 1-day-old chicken the sensitivity of T lymphocytes to this mitogen was very low. The sensitivity of T lymphocytes to PHA increased in 1-month-old chicken: even low doses of the mitogen (5-10 μg/ml) stimulated lymphocyte proliferation. As for the sensitivity of ConA-dependent T suppressors to ConA, functional activity of lymphocytes increased more than 2-fold at a dose of 10 μg/ml (SI—2.25).

LPS in a dose of 50 μg/ml increased proliferation of B lymphocytes 1.5 times. Increasing of the mitogen dose to 100 μg/ml suppressed proliferative activity of B lymphocytes.

Immunization of 5-7-day-old chicken with SE caused a 4-fold increase of functional activity of T lymphocytes at PHA dose of 60 μg/ml, which indicates a sufficient functional maturity of cellular immunity in birds of this age. However peripheral blood T suppressors of chicken of this age did not react to immunization. Moreover, T suppressors responded to ConA by a 2-fold increase of functional activity despite its dose. No appreciable changes in functional activity of chicken B lymphocytes in response to LPS stimulation were observed after immunization with SE.

In rats even low doses of PHA, ConA, and PWM stimulated proliferative activity of lymphocytes, SI depending on the mitogen dose. Immunization of animals with SE caused an increase in functional activity of total pool of T lymphocytes but did not modulate activity of T suppressors and B lymphocytes.

A single injection of hydrocortisone (100 mg/kg) to frogs and toads suppressed mitogen activity of PHA, particularly in toads (Table 2).

T suppressors belong to regulatory cells of the immune system; however hydrocortisone increased their functional activity in amphibians. In frogs ConA stimulated proliferative activity of T suppressors, while hydrocortisone attenuated this effect (Table 2). Proliferative activity of B lymphocytes decreased under the effect of PWM, but hydrocortisone 1.75 times increased blast transformation. Increased activity of immunocompetent cells was observed in evaluation of hydrocortisone effect on immune response to SE in amphibians (toads): the number of antibody-producing cells in the spleen increased 2-fold, while the number of nucleus-containing cells decreased 2.1 times. It seems that hydrocortisone suppressed other types of

TABLE 1. Mitogen-Sensitive Activity of Peripheral Blood Lymphocytes in Different Vertebrates

Animal	PHA, 60 μg/ml	ConA, 40 μg/ml	PWM, 1 μg/ml	LPS, 50 μg/ml
Rana ridibunda	-1.2	2.19	-1.2	—
Bufo viridis	13.1	2.10	-1.38	—
Testudo horsfieldi	8.6	0.9	2.1	—
Gallus gallus	1.27	2.45	—	1.5
Rattus norvegicus	1.5	2.45	—	2.6

TABLE 2. Spontaneous and Mitogen-Sensitive Activity of Peripheral Blood Lymphocytes of Frogs and Toads under the Effect of Hydrocortisone (*M*±*m*)

Experiment conditions	Rana ridibunda		Bufo viridis	
	control	hydrocortisone	control	hydrocortisone
Series I				
No mitogen	372±38	664.2±8.0	540.5±34.0	630±31
PHA	308±30 (-1.2)	356.2±2.5 (-1.86)	7096.2±156.0 (13.1)	2583±125 (4.1)
Con A	815±52 (2.19)	854±32 (1.29)	1183.7±131.5 (2.19)	1524.6±110.5 (2.42)
Series II				
No mitogens	781±91	417.2±6.9	782.2±22.8	572.0±56.7
PWM	624±110 (-1.2)	719±96 (1.79)	565.7±30.0 (-1.38)	1534±132 (2.68)

Note. Stimulation or suppression (for negative values) indexes are presented in parentheses.

nuclear cells in the spleen and did not affect the immunocompetent cells.

Injection of hydrocortisone to turtles stimulated peripheral T lymphocytes in the presence of PHA more than 2-fold in 5-fold-diluted blood and 7.5 times in 10-fold-diluted blood. The same effect was observed after stimulation of peripheral blood T lymphocytes with ConA. Five-fold dilution of the peripheral blood led

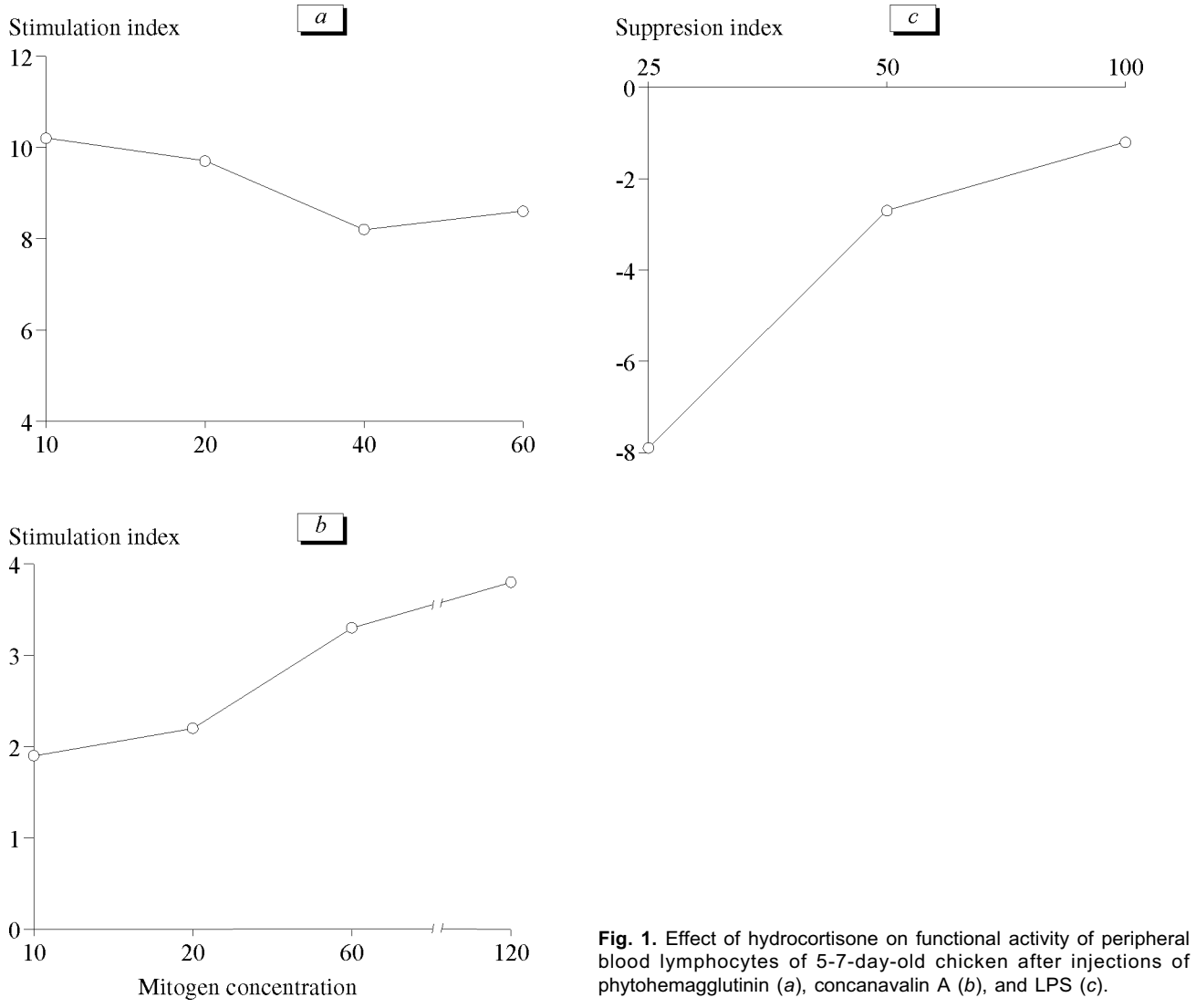

Fig. 1. Effect of hydrocortisone on functional activity of peripheral blood lymphocytes of 5-7-day-old chicken after injections of phytohemagglutinin (*a*), concanavalin A (*b*), and LPS (*c*).

to an almost 4-fold increase in proliferative activity of ConA-dependent T suppressors, and 10-fold dilution to its 6.5 times increase. The increase of functional activity of peripheral blood lymphocytes in turtles was paralleled by an increase in the number of antibody-producing cells in the spleen [1]. Hence, hydrocortisone increased the mitogen sensitivity of peripheral and splenic lymphocytes in turtles *in vitro* and *in vivo*.

A single injection of hydrocortisone to 5-7-day-old chicken 3 days before sacrifice stimulated the reaction to PHA and ConA, but suppressed DNA synthesis in B lymphocytes (Fig. 1).

T and B lymphocytes in rats of different age differently reacted to hydrocortisone. The intensity of response depended on lymphocyte subpopulation. ConA-dependent T suppressors reacted to stimulation by 2-3-fold increase of functional activity in both young and adult rats. The stimulatory effect of ConA in the presence of hydrocortisone decreased 2.5 times in 3-day-old rats and 1.4 times in adult rats. PHA stimulation suppressed T lymphocyte proliferation in rats of all age groups; 3-day-old rats were the most sensitive: hydrocortisone decreased functional activity of T lymphocytes more than 4.5 times in them.

Presumably, the age-associated changes in the lymphocyte blast transformation result from shift in the ratio of lymphocyte subpopulations differing by sensitivity to different doses of mitogens and the hormone towards the more sensitive subpopulations. Some lymphocyte subpopulations are sensitive to both PHA in different doses and to hydrocortisone.

Hence, the capacity of the peripheral blood lymphocytes to react adequately to mitogens in the studied animal species seems to be not congenital, but acquired in the course of ontogeny and presumably depends on animal species and age, as well as on the degree of cell differentiation, culturing conditions, and, the last but not the least, on the mitogen concentration.

REFERENCES

1. P. R. Alimkhodzhaeva, B. S. Gil'dieva, B. U. Ibragimkhodzhaev, *et al.*, *Dokl. Akad. Nauk Rep. Uzbekistan*, No. 4, 52-53 (1994).
2. G. I. Allayarova, *Morphofunctional Studies of the Chicken Thymus in the Ontogenesis* [in Russian], Abstract of Cand. Biol. Sci. Dissertation, Tashkent (1993).
3. R. Z. Inogamova, *Morphofunctional Study of Humoral Immunity in the Ontogenesis* [in Russian], Abstract Synopsis of Cand. Biol. Sci. Dissertation, Tashkent (1993).
4. N. R. Ling, *Lymphocyte Stimulation* [in Russian], Moscow (1971).
5. D. J. Roeder, M. J. Lei, and D. C. Morrison, *Infect. Immunol.*, **57**, No. 4, 1054-1058 (1989).
6. F. Salvadori and A. Tournefier, *Immunology*, **88**, No. 4, 586-592 (1996).
7. W. T. Weber, *Exp. Cell. Res.*, **46**, No. 1, 464-472 (1967).