Immunomodulating, Antianemic, and Adaptogenic Effects of Polysaccharides from Plaster Clover (*Melilotus officinalis*)

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Daily oral administration of polysaccharides extracted from plaster clover plants increased physical endurance (as determined in swimming tests) and body weight in mice and rats, stimulated the recovery of hematopoiesis in rats with experimentally produced lead anemia, and boosted the immune response to rat erythrocytes in mice, causing pronounced changes in the erythrocytic and leukocytic series and particularly in immunocompetent organs such as the thymus and spleen.

Key Words: *plaster clover; polysaccharides; lead anemia*

Broad-spectrum immunomodulating preparations have been shown to be able to produce a diversity of beneficial effects in the host (e.g., to enhance regeneration, mitigate anemia, and promote adaptive responses) and are therefore of considerable practical interest. Such effects appear to stem from the ability of certain T-lymphocyte populations to act directly on cell growth, as has been discussed at length in reports on studies with various animal models [1-7].

Much promise in this regard seems to lie in research into the immunomodulating, antianemic, and adaptation-promoting potentials of preparations made from the plant plaster clover, whose polysaccharides are endowed with valuable biological properties while being virtually nontoxic.

In the present study we tested plaster clover polysaccharides (PCP) for their effects on lead anemia in rats, on immune plaque formation in the spleen of mice, and on the general physical activity of both mice and rats.

MATERIALS AND METHODS

PCP were obtained from plaster clover (*Melilotus officinalis* D.) plants by extraction with hot water, followed by precipitation with 96% ethanol and purification by electrodialysis. The resulting preparations of light gray color were soluble in hot water and contained 76-82% of the active principle.

A total of 55 random-bred male rats (body weight 85-95 g) and 120 female BALB/c mice (body weight 18-20 g) were used. Test animals were administered PCP by the oral route once daily at 50 or 500 μg/kg body weight for 30 days.

At 3-5-day intervals during the 30-day treatment period, the animals were weighed, their physical work capacity was evaluated in a swimming test by noting how long they could swim, and samples of their peripheral blood were taken from the retroorbital sinus and assayed for hemoglobin and erythrocyte, leukocyte, and differential blood counts using Romanowsky's stain; in addition, several animals were sacrificed at each 3-5-day interval to measure thymus and spleen weights and determine the number of peripheral blood lymphocytes after their isolation by centrifugation in a Ficoll density gradient [8].

In rats, lead anemia was produced by the standard method using lead acetate.

In mice, the immune response to rat erythrocytes was assessed in a plaque assay in monolayers [9] by counting the number of antibody-producing cells (APC) in their spleens on day 4 or 5 after in-

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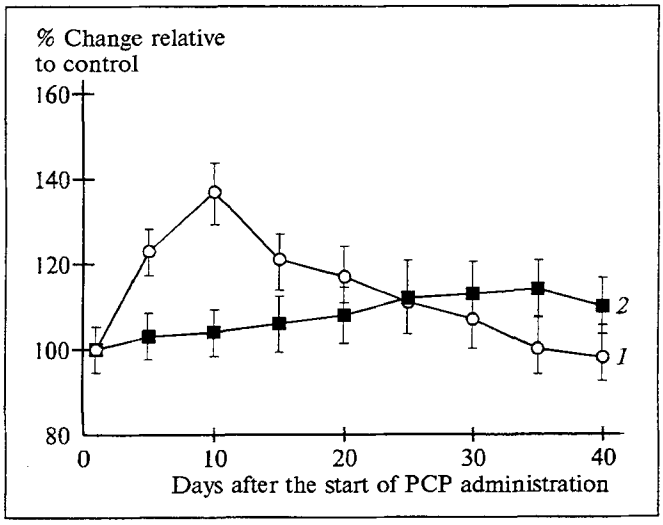

Fig. 1. Stimulation of physical work capacity and body weight gains by PCP (50 μg/kg) in rats. *1*) physical endurance (as evaluated in the swimming test); *2*) body weight. Intact (PCP—untreated) rats were used as controls.

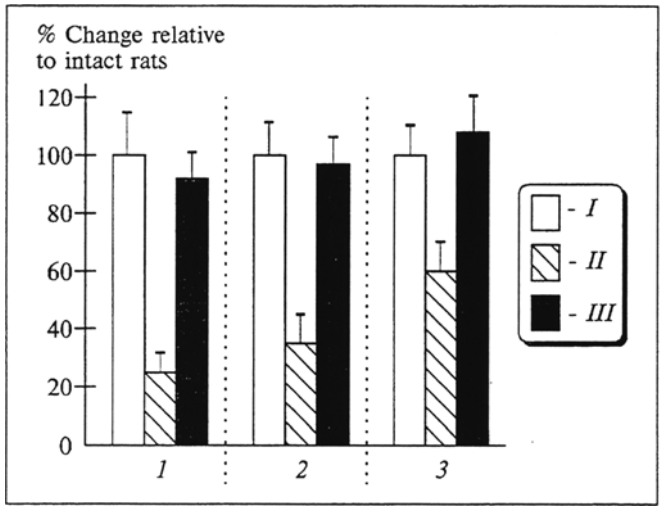

Fig. 2. Antianemic effects of PCP in rats. *1*) hemoglobin; *2*) erythrocytes; *3*) leukocytes. *I*) intact rats; *II*) rats given lead acetate; *III*) rats given lead acetate and treated with PCP.

traperitoneal injection of 1×10^6 washed rat erythrocytes per mouse.

The test results were expressed either in percent of control values or as the means and standard deviations; the significance of intergroup differences was evaluated by Student's *t* test.

RESULTS

Rats treated with PCP showed increases in physical work capacity, their swimming time increasing by 38.5±0.2% ($p<0.001$) as compared to untreated controls (being longest on day 10 after the start of treatment), and in body weight throughout the 30-day treatment period, at the end of which they weighed 19.8±0.3% more than the controls ($p<0.01$) (Fig. 1). In addition, the treatment improved the appetite and external appearance of the animals; their coat became glossier and they were calmer. Similar effects were observed in the PCP-treated mice.

In mice, the state of the peripheral blood and immunocompetent organs (spleen and thymus) was examined in greater detail. Both PCP doses (50 and 500 μg/kg) led to significant decreases in spleen weight and significant rises in thymus weight and in erythrocyte, leukocyte, and particularly lymphocyte counts in the peripheral blood (Table 1).

The changes in hematopoiesis were at their peak in mice on day 3 after the start of PCP treatment. Differential counts on that day revealed greatly increased (by factors of 1.7 to 2.2) numbers of large lymphocytes as compared to the untreated control group and a substantially lowered proportion of neutrophils (12.4% *vs.* 21.5% in the control group; $p<0.05$); later, the intergroup differences became insignificant. Such changes are characteristic of situations where the growth of the lymphoid series is actively stimulated with a resultant rapid differentiation of activated large lymphoid cells. Erythrocyte counts were also markedly elevated in the treated mice (by 26-32% on average; $p<0.001$), particularly on days 3-7 of the treatment period.

The antianemic and immunoprotective effects of PCP were best expressed in rats made anemic with lead acetate (Fig. 2). Whereas the anemic control rats exhibited 3- to 3.5-fold falls in hemoglobin and erythrocytes, the rats given 10 PCP injections had

TABLE 1. Effects of Plaster Clover Polysaccharides (PCP) on Immunocompetent Organs in Mice

PCP dose, μg/kg	Weight			Peripheral blood	
	body, g	spleen, mg	thymus, mg	erythrocytes, $\times10^9$ cells/ml	lymphocytes, $\times10^4$ cells/ml
Control	25.7±0.6	183.0±16.5	21.5±2.7	6.42±0.43	31.2±5.4
50	24.3±0.7	137.0±20.1*	30.8±1.3**	7.74±0.42**	39.4±6.6
500	26.2±1.5	150.0±22.9	36.7±5.6*	7.33±0.35**	41.3±8.2

Note. *$p<0.05$, **$p<0.01$ relative to the control (intact) mice.

almost normal hemoglobin and erythrocyte values while their leukocyte counts were even above normal (the differences from control values were all significant at $p<0.01$ or $p<0.001$).

PCP treatment exerted a similar stimulatory effect on the immune response of mice to rat erythrocytes: on day 4 postimmunization, the mice treated with PCP at 50 μg/kg had, on average, 57.7±9.3 APC per 10^6 spleen cells *vs.* 19.2±3.3 APC in the untreated controls ($p<0.01$). Raising the PCP dose to 500 μg/kg did not lead to any appreciable further increase in the immune response, which suggests that the minimal dose (50 μg/kg) was sufficient for stimulating this response to the maximum.

In summary, PCP have been shown to exert well-defined antianemic, immunostimulating, and general adaptogenic effects. Such diverse effects of these polysaccharides can be explained only in the light of our concept that lymphocytes are universal growth regulators for cells of various types and stimulate regeneration, immune responses, and other adaptive processes [4,5].

REFERENCES

1. A. G. Babaeva, *The Cellular Basis of Regeneration in Mammals* [in Russian], Moscow (1984).
2. V. I. Dontsov, *Byull. Eksp. Biol. Med.*, **100**, No. 7, 65-67 (1985).
3. V. I. Dontsov, *Eksp. Onkol.*, No. 5, 48-50 (1989).
4. V. I. Dontsov, *Immunologiya*, No. 5, 94-96 (1989).
5. V. I. Dontsov, *Immunobiology of Postnatal Development* [in Russian], Moscow (1990).
6. A. A. Podkolzin and V. I. Dontsov, *Immunity and Trace Elements* [in Russian], Moscow (1994).
7. L. Ya. Shilova, V. S. Poltorakin, and A. G. Suslov, *Byull. Eksp. Biol. Med.*, **93**, No. 6, 20-25 (1982).
8. A. Boyum, *Lymphology*, **10**, 71 (1977).
9. A. J. Cunningham, *Nature*, **207**, 1106 (1965).

The Effect of Various Incorporated Doses of ^{131}I on Immunological Reactions of the Organism

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Administration of radioisotope ^{131}I at 148 kBq/g body weight results in an inhibition of the primary immune response and in a decrease of proliferating activity of mouse lymphocytes in response to alloantigen stimulation. The number of antibody-producing spleen cells for immunization of mice with sheep erythrocytes diminished after administration of ^{131}I at 74 kBq/g animal weight.

Key Words: *lymphocyte; immunity; immune system;* ^{131}I

^{131}I enters the environment as a result of nuclear and thermonuclear tests, nuclear accidents, and industrial wastes [2]. With its high mobility the ^{131}I radioisotope easily enters the human organism with food when environmental pollution is present [3].

The high probability of ^{131}I entry into the human body calls for a study of its effect on different systems of the organism. There are few published data on the reaction of the immune system to various ^{131}I doses.

In addition, ^{131}I is widely used clinically, in particular, in the treatment of thyroid cancer [4]. It is thus necessary to study immune reactions to ^{131}I administration, which have a strong impact on the results of treatment in cancer patients.

This study was undertaken to examine immunological reactions of the organism in one-trial administration of the radioactive ^{131}I isotope at different doses.

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