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EFFECT OF INJECTION OF *Cryptococcus* HETEROPOLYSACCHARIDE INJECTION INTO BONE MARROW DONORS AND RECIPIENTS ON STRUCTURE OF A HETEROTOPIC FOCUS OF HEMATOPOIESIS

I. N. Ivasenko, S. V. Gurina, M. A. Kashkina, A. Yu. Zaritskii, and G. E. Arkad'eva

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The number of known preparations with an inhibitory or, still less, a stimulating action on hematopoiesis in vivo is still extremely limited. However, glycosaminoglycans, which have an appreciable effect on hematopoietic cell proliferation [3-5], are one of the components of the hematopoietic microenvironment.

It was accordingly decided to study the action of a *Cryptococcus* heteroglycan on the formation of a heterotopic focus of hematopoiesis.

EXPERIMENTAL METHOD

The extracellular polysaccharide (PS) formed by the yeast *Cryptococcus luteolus* strain 228, is a branched heteropolymer containing α-1,3-bound mannan in its main chain and xylose and glucuronic acid residues in the side chain, connected to the main chain by β-glycoside bonds [1].

Experiments were carried out on 120 male (CBA × C57B1)F_1 mice weighing 18-20 g. Heterotopic transplantation of mouse bone marrow was carried out by the method in [2, 3]. The polysaccharide was injected intraperitoneally into the mice which had received the bone marrow in the course of 30 days after the operation in doses of 25 mg/kg once or twice a week and of 200 mg/kg once a week. Retransplantation of the ectopic focus was carried out 7 days after the first implantation. In the course of these 7 days the polysaccharide was injected into intermediate recipients once or twice in a dose of 25 mg/kg. Control animals received injections of physiological saline. The donor mice were given intraperitoneal injections of solutions of the polysaccharide (25 mg/kg) parallel with physiological saline twice a week with an interval of 3 days, and daily for 7 days immediately before implantation. Bone marrow, isolated from the femora of these animals, was then implanted into intact recipients. The mice were killed 30 days after primary implantation and after retransplantation, by compression in the suboccipital region, and the dimensions of the heterotopic foci of hematopoiesis which had formed were estimated on the basis of the number of cells and the weight of the bony capsule.

EXPERIMENTAL RESULTS

Injection of PS into the experimental animals in a dose of 25 mg/kg once a week for 30 days after implantation of bone marrow caused a significant increase in the number of cells and in the weight of the bony capsule of the ectopic foci compared with the control. When the dose of polysaccharide was increased to 200 mg/kg the dimensions of the ectopic foci in the experimental and control animals were identical (Fig. 1a). To find out why the number of cells and the weight of the bony capsule increased, PS was injected once into intermediate recipients of the transplanted bone marrow. In this case the dimensions of the

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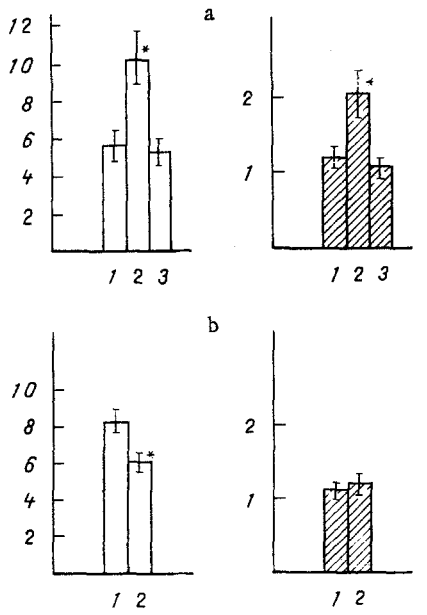

Fig. 1. Effect of one (a) and two (b) injections of PS on formation of a heterotopic focus of hematopoiesis. Abscissa: experimental conditions; 1) injection of physiological saline (control); 2, 3) injection of PS in dose of 25 and 200 mg/kg respectively. Ordinate, dimensions of focus: number of cells $\cdot 10^6$ (unshaded columns), weight of bony capsule (in mg, shaded columns). Asterisk indicates that difference from control is significant ($p < 0.05$).

TABLE 1. Structure of Focus of Ectopic Hematopoiesis after Premilinary Injection of PS into Mice Donating Bone Marrow (M ± m)

Parameters of size of focus	Injection of physiological saline (control)	Injection of PS	
		in a dose of 25 mg/kg twice a week	in a dose of 25 mg/kg daily
Number of cells × 10^6	3,47±0,70	3,9±1,3	3,58±0,66
Weight of bony capsule, mg	0,66±0,17	0,7±0,24	0,5±0,2

secondary hematopoietic focus were indistinguishable from the control. Preliminary injection of polysaccharide into the mice donating the bone marrow for 1 week before implantation caused no change in size of the foci in the recipient mice (Table 1). Consequently, enlargement of the heterotopic focus could be explained by the stimulating effect of PS on untransplantable, more mature cells of the stroma and bone tissue.

When PS was injected into the recipient mice twice a week for 30 days, a significant reduction was observed in the number of hematopoietic cells in the ectopic focus ($p < 0.01$), but the weight of its bony capsule was the same as in the control (Fig. 1b). Injections of PS into intermediate recipients in accordance with the same schedule for 1 week were accompanied, on retransplantation, by construction of a focus in which the number of cells was smaller than in the control but the weight of the bony capsule did not differ from that in the control animals. Consequently, when PS was given in accordance with this schedule it had an inhibitory effect on the cells forming the focus of ectopic hematopoiesis.

It can accordingly be concluded that *Cryptococcus* PS had a dual action, which depended on the dose and schedule of administration (stimulating or inhibitory), on the hematopoietic and osteogenic tissue. This provides the basis for preclinical studies, with the aim of assessing the effect of PS on excessive or deficient hematopoiesis, on disturbances of osteogenesis, and also to elucidate the mechanism of action of this heteropolymer.

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