ACTION OF A POLYSACCHARIDE FROM *Thuja occidentale* L. ON STROMAL PRECURSOR CELLS OF THE HEMATOPOIETIC MICROENVIRONMENT IN MICE

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High-molecular-weight subfractions of a polysaccharide isolated from *Thuja occidentale* (TPS) possess a mitogenic action on peripheral blood mononuclears, induce proliferation of $CD4^+$ T-cells [7], modulate hematopoiesis, and stimulate hematopoietic precursor cells for 10 days after sublethal irradiation. It has been suggested that the action of TPS is mediated through IL-1 and IFNγ, for in certain cases it is blocked by antibodies against these factors. It is not known whether TPS affects the proliferation of hematopoietic precursor cells in radiation damage to the hematopoietic system directly, or whether it acts on stromal cells, which in turn, regulate hematopoietic stem cells.

The aim of the investigation was to study the action of TPS on precursor cells of the stromal microenvironment during "steady-state" hematopoiesis and after sublethal irradiation. As the model with which to study stromal hematopoietic precursor cells we used their ability to transfer the hematopoietic microenvironment and to construct a focus of ectopic hematopoiesis under the renal capsule of syngeneic mice [9].

EXPERIMENTAL METHOD

Female (CBA × C57BL/6)F_1 and (DBA/2 × C57BL/6)F_1 hybrid mice aged 16-22 weeks were used.

Mice donating bone marrow were irradiated on the IPK apparatus in doses of 3 or 6 Gy and with a dose rate of 0.17 Gy/min.

As recipients, intact mice and chimeras were used. To obtain chimeras the mice were irradiated (12.5 Gy) with two equal doses, separated by an interval of 3 h. The mice were given an intravenous injection of cells of intact syngeneic bone marrow from one-third of a femur 2 h after irradiation. Another group of animals was irradiated in a dose of 6 Gy. All recipient mice were irradiated 2 months before the beginning of the experiment.

On day zero of the experiment the donor mice received one intravenous injection of TPS, dissolved in nutrient medium RPMI 1640. Concentrations of 0.1, 0.5, 1.0, 2.5, and 5.0 mg dry substance per mouse were used. Animals of the control group were given an injection of medium RPMI 1640. Some of the control and lectin-treated animals were irradiated 24 h later in doses of 3 Gy or 6 Gy. The animals were killed 4 and 7 days after receiving the injection of TPS by cervical dislocation, and their femoral bone marrow was implanted beneath the renal capsule of syngeneic intact mice or chimeras, as described in [5]. Each experimental group included five animals. The dimensions of the foci of ectopic hematopoiesis produced by bone marrow were determined 6 weeks later by counting the number of nucleated cells in them. The weight of the bony shell formed also was measured.

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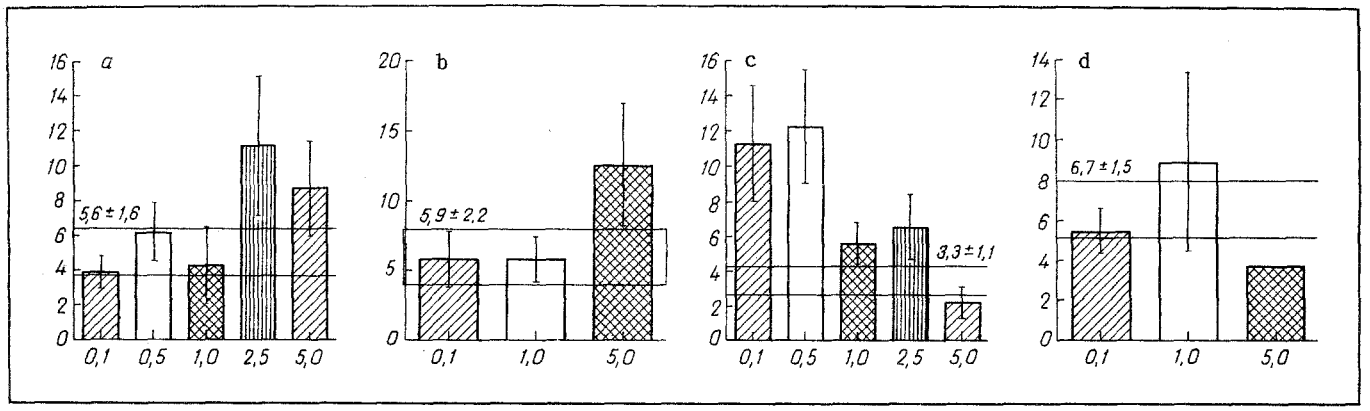

Fig. 1. Dimensions of foci of ectopic hematopoiesis formed by bone marrow of unirradiated mice. a, b) Four days after injection of TPS: a) unirradiated recipients; b) chimeras; c, d) 7 days after injection of TPS; c) unirradiated recipients, d) chimeras. Region between lines represents dimensions of focus formed by bone marrow of control mice not receiving injections (M ± m). Abscissa, dose of TPS injected (mg/mouse). Ordinate, cell content of foci of ectopic hematopoiesis (· 10^{-6}).

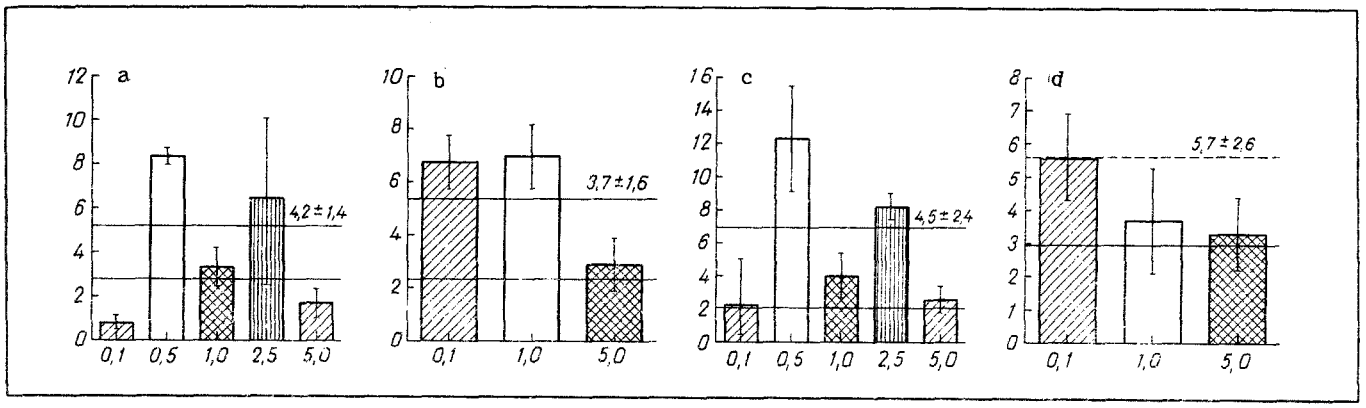

Fig. 2. Dimensions of foci of ectopic hematopoiesis formed by bone marrow of mice irradiated in a dose of 3 Gy. a, b) Four days after injection of TPS: a) unirradiated recipients, b) chimeras, c, d) 7 days after injection of TPS: c) unirradiated recipients, d) chimeras. Region between lines represents dimensions of focus formed by bone marrow of control mice not receiving injections (M ± m). Abscissa, dose of TPS injected (mg/mouse), ordinate, cell content of foci of ectopic hematopoiesis (· 10^{-6}).

EXPERIMENTAL RESULTS

After implantation of bone marrow beneath the renal capsule the stromal precursor cells in the developing foci of ectopic hematopoiesis were of donor origin whereas the majority of the hematopoietic cells belonged to the recipient [8]. It must be pointed out that the focus in intact recipients was formed mainly on account of early stromal precursor cells, whereas in the chimeras or irradiated recipients the size of the focus was determined by more highly differentiated inducible precursor cells [1, 2]. Thus implantation of bone marrow of experimental animals beneath the renal capsule of unirradiated recipients or chimeras enables two subpopulations of stromal precursor cells to be studied.

The Action of TPS on Stromal Precursor Cells of Unirradiated Mice. Four days after injection of TPS in doses of up to 2.5 mg per mouse, the polysaccharide had no appreciable effect on the dimensions of foci of ectopic hematopoiesis formed by their bone marrow, either in intact recipients or in chimeras (Fig. 1a, b). When bone marrow was implanted on the 7th day after injection of low doses of TPS the dimensions of the foci of ectopic hematopoiesis formed beneath the renal capsule in intact recipients were greater than those of foci formed by the bone marrow of animals not receiving TPS

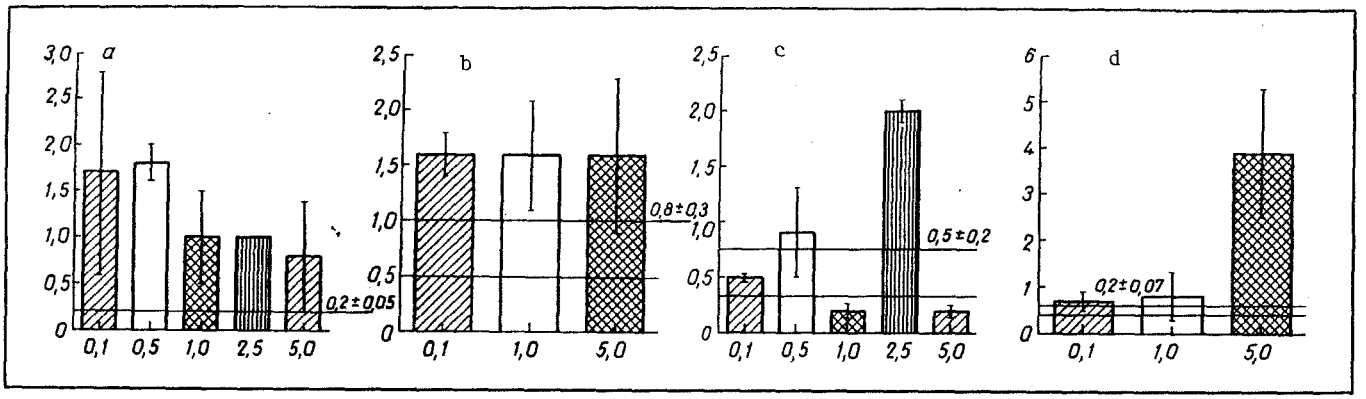

Fig. 3. Dimensions of foci of ectopic hematopoiesis formed by bone marrow of mice irradiated in a dose of 6 Gy. a, b) Four days after injection of TPS: a) unirradiated recipients; b) chimeras; c, d) 7 days after injection of TPS: c) unirradiated recipients; d) chimeras. Region between lines represents dimensions of focus formed by bone marrow of control mice not receiving injections (M ± m). Abscissa, dose of TPS injected (mg/mouse); ordinate, cell content of foci of ectopic hematopoiesis (· 10^{-6}).

(Fig. 1c). The dimensions of the foci in the chimeras did not differ in any of the groups studied (Fig. 1d). These findings indicate that TPS is not a toxic agent for stromal precursor cells and does not have a stimulating action on early and inducible precursor cells in the early stages after its injection; however, a weak stimulating action of the lectin can be detected on early precursors of the hematopoietic microenvironment 7 days after injection of TPS.

Action of TPS on Stromal Precursor Cells of Irradiated Mice. The dimensions of foci of ectopic hematopoiesis formed by bone marrow of mice irradiated in a dose of 3 Gy varied greatly in intact recipients into which bone marrow was transplanted on the 4th and 7th days after injection of TPS (Fig. 2a, c). The mean dimensions of the foci from all doses of TPS used did not differ significantly from those of control foci formed by the bone marrow of irradiated animals not receiving injections. Similar results also were obtained in chimeras (Fig. 2b, d). The dose of irradiation was 3 Gy less than that for precursor cells of the hematopoietic microenvironment, for which the dose was 4.44 ± 0.05 Gy [4, 5], whereas for the construction of a focus of ectopic hematopoiesis it was 3.25 ± 0.24 Gy [3]. It can be tentatively suggested that TPS has no significant effect on stromal precursors in radiation damage caused by similar doses.

However, after irradiation of the donor mice in a dose of 6 Gy, a significant increase was found in the size of foci of ectopic hematopoiesis obtained after implantation of bone marrow from animals receiving an injection of TPS 4 days before the beginning of the experiment (Fig. 3a). The dimensions of the focus of ectopic hematopoiesis in the chimeras also were almost doubled after transplantation of bone marrow from mice receiving injections of the polysaccharide (Fig. 3b). The action of TPS 7 days after irradiation on stromal precursor cells was weaker in the intact recipients (Fig. 3c); in chimeras into which bone marrow from mice receiving the largest dose of TPS (5 mg/mouse) was implanted, the dimensions of the focus were greatest and differed significantly from the control (Fig. 3d). Thus, with a dose of irradiation of 6 Gy, TPS has a protective action on stromal precursor cells at different levels of differentiation.

The study of the weight of the bony shell showed virtually no change in its value in animals of all experimental groups, i.e., TPS probably had no effect on osteogenic precursors in mice.

The results indicate that modulation of hematopoiesis by TPS after radiation damage may be directly connected with the effect of the polysaccharide on stromal precursor cells. However, the possibility cannot be ruled out that TPS stimulates more mature cells of the hematopoietic microenvironment in relation to expression of various growth factors, such as granulocytic-macrophagal and granulocytic growth factors and interleukins 1 and 6.

Nevertheless, it is evident that TPS has no toxic effect on precursor cells of the hematopoietic stroma and it has a protective action against severe radiation damage.

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