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INCREASED NUMBER OF PRECURSORS OF ROSETTE-FORMING CELLS SENSITIVE TO THYMUS HORMONE IN THE SPLEEN OF MICE VACCINATED WITH SMALLPOX VACCINE

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Injection of smallpox vaccine into female C57BL/6j mice aged 2 months led on the first few days to a sharp increase in the number of precursor cells of rosette-forming lymphocytes sensitive to the differentiating effect of thymus extract. By the tenth day after vaccination the normal number of these cells was restored.

KEY WORDS: thymosine; smallpox vaccine; precursor cells; rosette-forming cells.

The immunologic response of the body to particular antigens is connected with cooperative interaction between different subpopulations of T- and B-leucocytes and macrophages. In order to understand the mechanism of the immunologic cooperative response it is essential to know the quantitative content of the various populations of immunocompetent cells reflecting the response of the host to injection of antigen.

In the investigation described below the number of precursor cells of lymphocytes capable of forming spontaneous rosettes with sheep's red blood cells (so-called E-rosettes) *in vitro* was determined in the spleens of mice vaccinated with smallpox vaccine. For this purpose a method based on the ability of thymus hormone to induce differentiation of precursor cells into rosette-forming lymphocytes was used.

EXPERIMENTAL METHOD

Female C57BL/6j mice aged 2 months, bred in the writers' laboratory, were used. The mice were obtained initially from the Stolbovaya Nursery, Academy of Medical Sciences of the USSR.

Thymus extract (TE) was prepared by Goldstein's method [4] as far as fraction 3 (TE-3). The extract contained 9 mg protein/ml, determined by Lowry's method. The TE-3 was kept at −196°C, and was thawed and used on the day of the experiment. Lyophilized bovine serum albumin (BSA, from Biomed, Cracow, Poland; batch 1/04/74) was diluted to the required concentration with physiological saline. The protein content in the thymosine and BSA preparations was the same, namely 9 mg/ml.

A commercial preparation of smallpox vaccine from the L-IVI strain, produced by the Mechnikov Research Institute of Vaccines and Sera (batch No. 0444, control No. 1388), was used. The vaccine was diluted with physiological saline.

The animals were divided into three groups: control — intact or animals receiving BSA (0.2 ml), and experimental, vaccinated subcutaneously with smallpox vaccine in a dose of 10^6 pock-forming units (PFU)/ml. On the 2nd, 7th, 14th, and 30th days after vaccination four mice in each group were killed, the spleen was removed, and a cell suspension was prepared in medium 199 in the proportion of 10^7 cells/ml. The suspension (0.1 ml) was incubated with thymosine solution in a volume of 0.15 ml at 37°C for 90 min. The lymphocytes were then

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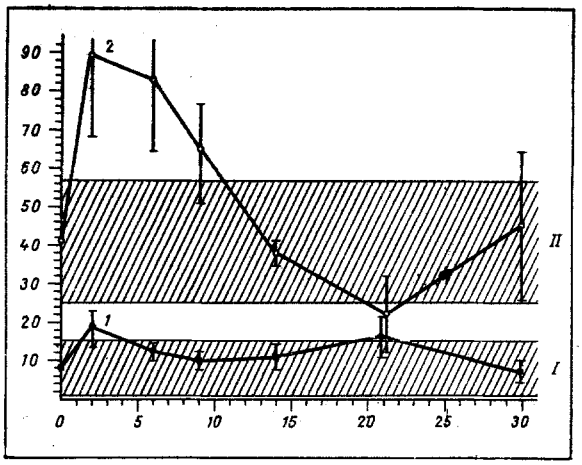

Fig. 1. Effect of thymus extract on RFC formation by spleen cells of mice immunized with smallpox vaccine. Abscissa, time after vaccination (in days); ordinate, number of RFC per 10,000 spleen cells; vertical lines of graph denote standard error of mean. 1) smallpox vaccine; 2) smallpox vaccine + TE-3. I) confidence interval (P = 0.01) of number of RFC in suspension of normal spleen cells; II) the same after incubation with TE-3.

tested for spontaneous rosette formation. The controls to the rosette-formation test consisted of lymphocytes not treated with thymosine, but incubated with medium.

The rosette-formation test was carried out by the method of Bach et al. [2] with slight modifications. To each sample of lymphocyte taken in a volume of 0.4 ml of a suspension containing $1 \cdot 10^7$ cells/ml, 0.1 ml of a suspension of sheep's red blood cells in physiological saline, in the proportion of $4 \cdot 10^7$ red cells to 1 ml, was added. The tubes were allowed to stand for 30 min in a refrigerator at 4°C, after which they were centrifuged at 800 rpm for 5 min. After careful shaking the number of rosette-forming cells (RFC) was counted and the total number of cells determined in four grids on a Goryaev's chamber. Cells to whose surface at least four erythrocytes adhered were classed as RFC. Knowing the total number of RFC and the total number of cells, the number of RFC per 10,000 spleen cells was counted.

EXPERIMENTAL RESULTS

Incubation with TE-3 led to an increase in the number of RFC in a suspension of spleen cells from intact mice. The number of spleen cells of the control mice sensitive to TE-3 showed no change throughout the period of investigation. Incubation of lymphocytes from the spleen of mice immunized with smallpox vaccine with TE-3 caused a sharp increase in the number of RFC. The study of this process over a period of time showed that the greatest increase in the number of cells sensitive to TE-3 was observed in the spleen of the animals during the first 9 days after vaccination. Attention is also drawn to two small peaks of RFC above the normal level in the spleen of the second and 21st days after inoculation with smallpox vaccine. Administration of BSA did not affect the number of cells sensitive to TE-3 and, indeed, actually reduced the number of RFC a little (Fig. 1). Cells sensitive to TE-3 differentiated into RFC only under the influence of the thymus extract, and incubation with BSA *in vitro* did not cause them to differentiate.

Thymus hormone can induce differentiation of precursor cells of T-lymphocytes *in vitro*. The simplest method of testing this effect is by the reaction of formation of so-called direct E-rosettes. Human peripheral blood lymphocytes forming E-rosettes belong to the T-population. The lymphocyte population to which mouse RFC belong is less clear. By means of treatment with azathioprine and antiserum [1] it has been shown that RFC formed under the influence of thymus extract prepared by Goldstein's method are T-cells.

The results described above show that vaccination of mice with smallpox vaccine leads to a marked increase in the number of RFC precursor cells in the spleen (from 0.9 to 9%). This increase is perhaps connected with the specific pattern of immunogenesis against smallpox vaccine. If E-rosettes in mice do in fact correspond to the population of T-lymphocytes, the increase in their number under the influence of smallpox vaccine must be a fact of great

importance in the understanding of the mechanisms of immunity to smallpox. The resistance and insusceptibility of an animal or vaccinated person to smallpox infection are largely dependent on the state of their cellular immunity. Some demonstrative investigations from this point of view have been carried out by Downie and McCarthy [3], who showed that the presence of smallpox antibodies in high titers in the blood does not completely reflect the state of resistance of the body to smallpox infection.

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INVESTIGATION OF IMMUNOGLOBULIN-POSITIVE CELLS IN MOUSE BONE MARROW

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The number of immunoglobulin- (Ig-) positive lymphocytes and of their precursors in mouse bone marrow was investigated 6 and 36 h after treatment with hydroxyurea. The number of Ig-positive B-cells in bone marrow so treated was increased a little, whereas dividing and nondividing precursors of B-lymphocytes were virtually absent, with the exception of stem cells.

KEY WORDS: bone marrow; B-cells; precursors of B-cells; stem cells.

The problem of fractionation of bone marrow cells in order to isolate early precursors of hematopoiesis and, in particular, stem cells is a problem of continually increasing urgency [4, 8-10]. After treatment of bone marrow with hydroxyurea passage of the cells from the G_1-period into the S-phase is blocked [11, 12] and DNA synthesis is inhibited and the cells die in the S-period [12, 14, 15]. Considerable exhaustion of the pool of proliferating precursors of hematopoiesis has been achieved by means of hydroxyurea [5], so that the subsequent development of these cells takes place almost entirely on account of previously nonproliferating stem cells.

The object of the present investigation was to study the following problems: How does the relative proportion of mature immunoglobulin- (Ig-) positive B-lymphocytes and of colony-forming units (CFU) in bone marrow change under the influence of hydroxyurea; under the same conditions how does the number of proliferating bone marrow cells decrease and, in particular, what is the degree of exhaustion of the pool of early precursers of B-lymphocytes? Finally, what is the quantitative contribution of early (stem cells) and late precursors to the formation of Ig-positive lymphocytes after transplantation of bone marrow into lethally irradiated recipients?

EXPERIMENTAL METHOD

Experiments were carried out on male CBA mice weighing 18-22 g obtained from the Stolbovaya nursery, Academy of Medical Sciences of the USSR.

Hydroxyurea (Serva) was injected intraperitoneally in a dose of 500 mg/kg body weight 4 times at intervals of 5 h between injections [5]. Cell suspensions were prepared from the

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