STIMULATION OF POSTRADIATION REGENERATION OF ERYTHROPOIESIS IN MICE BY *Bordetella pertussis* VACCINE

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Irradiation of mice in a sublethal dose, damaging more than 90% of hematopoietic stem cells (HSS), is accompanied by temporary prohibition of differentiation [7, 9-11]. This is evidently due to the fact that those HSS which remain begin to undergo intensive compensatory proliferation, without suicidal differentiation. The latter begins only when the HSS population reaches a certain "threshold" value, simultaneously with the acquisition of ability to react to differential stimuli [6, 9]. Disturbing influences such as blood loss [7], injection of *Salmonella typhosa* endotoxin [7, 13], and *Mycoplasma arthritidis* [4, 5] accelerates the regeneration of erythropoiesis after sublethal irradiation of mice.

The aim of the present investigation was to determine the effect on postirradiation restoration of erythropoiesis of a possible increase in the demand for differentiation due to injection of killed whooping cough vaccine. We know that *B. pertussis* vaccine, widely used as an adjuvant, stimulates endogenous colony formation if injected before irradiation [1], and also increases the recirculation of HSS [12].

EXPERIMENTAL METHOD

Male CBA and BALB/c mice weighing 20-23 g, obtained from the "Stolbovaya" nursery, Academy of Medical Sciences of the USSR, and also $(C57Bl/6 \times A/Sn)F_1$ $(B6AF_1)$ hybrid mice obtained from the "Rappolova" nursery, Academy of Medical Sciences of the USSR, were used (Table 1).

For immunization, standard killed vaccine, washed 3 times to remove formalin, from *B. pertussis* strain 305, obtained from the Laboratory of Microbial Physiology and Biotechnology, I. I. Mechnikov Moscow Research Institute of Vaccines and Sera, was used for immunization. The vaccine was injected intravenously into mice in a dose of 10^7 or 10^{10} microbial cells per mouse.

Rauscher leukemia virus (RLV), in the form of virus-containing plasma, obtained from BALB/c mice, was injected intraperitoneally into the mice in a dose of 10 IU in 0.1 ml.

To study endocolonization, mice were irradiated in a dose of 5.5 Gy and killed on the 9th day after irradiation. The spleens were fixed in Bouin's solution and the number of macroscopically visible colonies was counted.

In experiments to study degeneration of erythropoiesis the mice were irradiated in a dose of 6.0 Gy. *B. pertussis* vaccine was injected 24 h before irradiation. At various times after irradiation, 0.5 μCi of ^{59}Fe (iron citrate, specific radioactivity 0.2 mCi/ml) in 0.5 ml physiological saline was injected into the immunized and control irradiated mice. The mice were killed by cervical dislocation 6 h later and radioactivity was counted in the spleen and hind limb on a gamma-spectrometer (Nuclear Chicago, USA). Parallel with this, at the same times the number of cells was determined in the bone marrow and spleen.

The significance of the numerical differences was estimated by Student's test.

EXPERIMENTAL RESULTS

Incorporation of ^{59}Fe in the spleen of the control mice 24 h after irradiation in a dose of 6.0 Gy was 5% of normal (Fig. 1, I). During the next 6 days, uptake of the isotope by the

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TABLE 1. Effect of Killed Whooping Cough Vaccine, Injected at Different Times after Irradiation (5.5 Gy) on Development of Endogenous Colonies in Spleen of CBA Mice (M ± m)

Time of injection of vaccine after irradiation	Dose of vaccine (microbial cells/mouse)			
	10^7	number of mice	10^{10}	number of mice
4 h	2,4±0,3 (1,9—2,9)	27	3,1±0,6 (1,9—4,3)	24
1 day	3,0±0,3 (2,3—3,7)	24	2,8±0,6 (1,6—4,0)	22
3 days	2,5±0,3 (1,9—3,1)	24	2,8±0,4 (3,0—3,6)	34
4 »	5,2±0,5* (4,3—6,1)	29	5,4±0,5* (4,3—6,5)	24
5 »	5,3±0,5* (4,1—6,5)	29	5,4±0,7* (4,0—6,8)	30
6 »	2,5±0,4 (1,6—3,4)	17	2,6±0,3 (2,0—3,2)	20
7 »	2,5±0,4 (1,7—3,3)	30	3,6±0,6 (2,4—4,8)	27
Control	2,8±0,2 (2,3—3,3)		(n=35)	

Legend. Confidence interval calculated at $P = 0.05$ level shown in parentheses; *$P < 0.05$ compared with control.

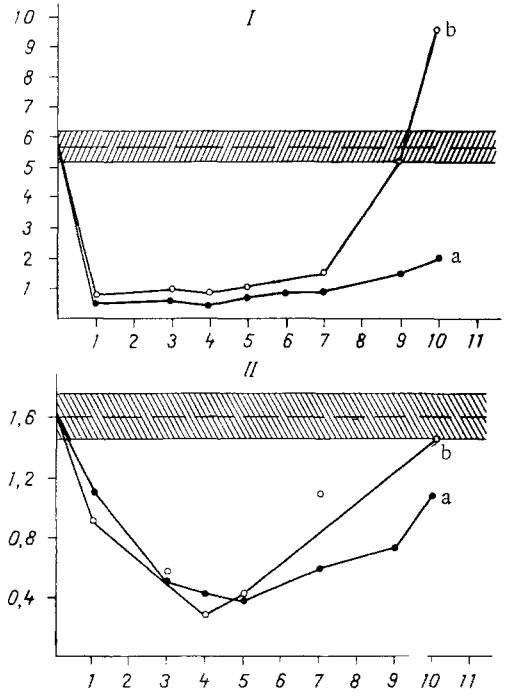

Fig. 1. Time course of incorporation of ^{59}Fe into erythroid cells after sublethal irradiation (6.2 Gy). Abscissa, time after irradiation (in days); ordinate, incorporation of ^{59}Fe (in %). I) Spleen; II) bone marrow. a) Normal; b) whooping cough vaccine.

spleen was stable: Regeneration began with the 8th day after irradiation, and by the 10th day it had reached 40% of the normal level. An increase in incorporation of ^{59}Fe into the spleen of mice vaccinated with *B. pertussis* was observed 24-48 h earlier than in the control, and on the 10th day after irradiation an "overshoot" was observed: Consumption of the isotope was almost twice as high as the radioactivity of the spleen of intact mice.

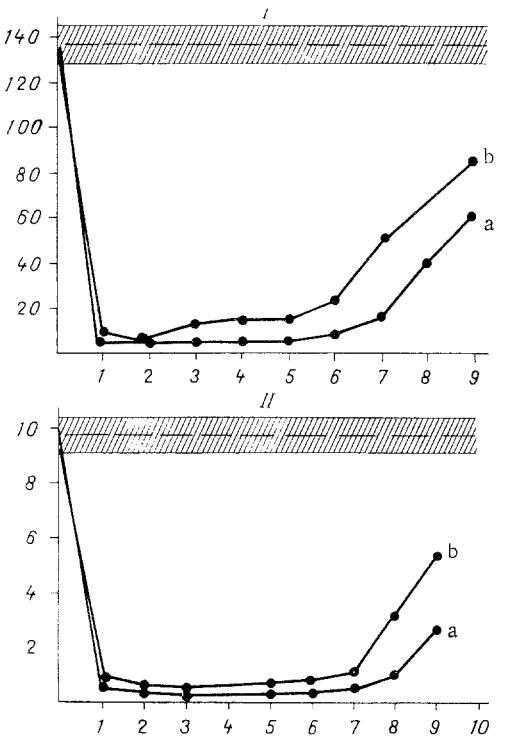

Fig. 2. Regeneration of cell composition of bone marrow and spleen after sublethal irradiation (6.0 Gy). Abscissa, time after irradiation (in days); ordinate: I) number of cells in spleen ($\times 10^6$), II) number of cells in one femur ($\times 10^6$). I) Spleen; II) bone marrow. a) Normal, b) whooping cough vaccine.

Regeneration of erythropoiesis in the bone marrow of the control mice began with the 7th day after irradiation (Fig. 1, II) and reached 70% on the 10th day. Injection of whooping cough vaccine accelerated regeneration of erythropoiesis in the bone marrow, although not to the same degree as in the spleen. On the 10th day after irradiation incorporation of ^{59}Fe was almost the same as in the bone marrow of intact, nonirradiated mice.

A similar time course was observed in recovery of the cell populations of the spleen and bone marrow after irradiation (Fig. 2).

Injection of killed whooping cough vaccine 24 h before irradiation of mice in a sublethal dose thus causes acceleration of regeneration of erythropoiesis and of the cell population of the hematopoietic organs; these effects, moreover, are more marked in the spleen than in the bone marrow. It was shown previously [1] that vaccination with whooping cough vaccine 1-3 days before irradiation of mice is accompanied by marked stimulation of endogenous colony formation. In the next stage of the present investigation an attempt was made to discover the stimulating activity of *B. pertussis* on postradiation regeneration of erythropoiesis which acts as a differential stimulus for HSS proliferating after sublethal radiation damage. For this purpose CBA mice were irradiated in a dose of 6.5 Gy, and *B. pertussis* was injected into them at different times after irradiation in a dose of 10^7 or 10^{10} microbial cells. It will be clear from Table 1 that vaccination of the mice on the 4th-5th day after irradiation led to a significant increase in endogenous colony formation, which was recordable on the 9th day after irradiation. On the one hand, this could be evidence of the action of the vaccine on the cells less differentiated than erythropoietin-sensitive cells, for erythropoiesis in the spleen in the first 4-5 days after sublethal irradiation, damaging more than 90% of HSS, is insensitive to specific stimuli, such as to injection of exogenous erythropoietin [6, 10]. On the other hand, the increase in the number of endogenous colonies may reflect increased release of HSS from the bone marrow and an increase in their migration into the spleen; killed whooping cough vaccine has been shown to have a similar action on HSS of unirradiated mice [12]. The absence of a stimulating effect 5 days after injection into irradiated mice can be explained on the grounds that HSS subsequently commencing differentiation were simply unable to get through the necessary number of mitoses, in the time which remained, to form macroscopically visible colonies.

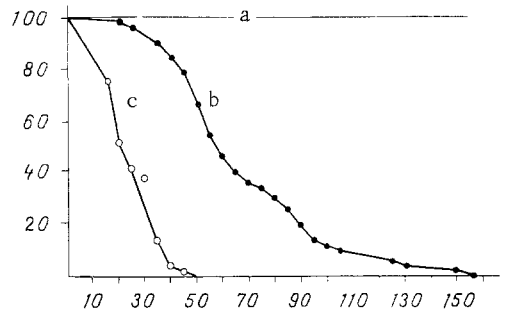

Fig. 3. Time course of survival of mice after injection of RLV. Abscissa, time after injection of RLV (in days); ordinate, number of surviving mice (in %). a) *B. pertussis*; b) RLV; c) *B. petussis* 1 day before injection of RLV.

If true stimulation of production of erythroid precursor cells from HSS takes place under the influence of whooping cough vaccine, this may be of great importance in the genesis of virus leukemia. It was shown previously [2] that during mixed infection of $B6_1$ mice (resistant to RLV) with *M. arthritidis* stimulates the formation of erythroid target cells for RLV, and this leads to the induction of leukemia [3-5]. *M. arthritidis* had the maximal stimulating effect on hematopoietic cell proliferation when injected 24 h before irradiation [3], just as with *B. pertussis* [1]. Consequently, 24 h before infection with RLV, BALB/c mice (sensitive to RLV) were injected with 10^{10} bacterial cells of whooping cough vaccine and the survival rate of the mice and times of development of leukemia were determined. The results showed (Fig. 3) that preliminiary injection of whooping cough vaccine led to the much earlier development of leukemia and death of the mice: Only 50 days after inoculation 100% of the mice had died, whereas in the case of infection with RLV alone, the last mouse died after 156 days. Injection of whooping cough vaccine alone did not cause death of the animals. This fact suggests that the mechanism of stimulation of development of Rauscher leukemia by *B. pertussis* may involve an activating action of the vaccine on erythroid target cells for RLV. Moreover, the possibility cannot yet be ruled out that vaccination may be accompanied by depression of synthesis of interferon, which participates in protection against virus infection. This last hypothesis requires experimental investigation.

A combination of the available facts thus indicates that killed whooping cough vaccine has a stimulating action on erythropoiesis in normal mice, and also in mice irradiated in a sublethal dose. In the latter case, *B. pertussis* has a stronger action on regeneration of erythropoiesis in the spleen. Bacterial endotoxin has a similar effect on postradiation regeneration of hematopoiesis [13]. We know, however, that intensive regeneration of hematopoiesis in the spleen is not always accompanied by adequate regeneration in the bone marrow [8]. Since the latter is much more important for postradiation survival of mice [13], it is possible that this was why in preliminary experiments an increase in survival of mice vaccinated with *B. pertussis* 24 h before irradiation in a lethal dose could not be demonstrated. This distinguishes the action of whooping cough vaccine from *M. arthritidis*, injection of which into mice 24 h before irradiation in a lethal dose caused an almost tenfold increase in survival recorded 30 days after irradiation [3]. The mycoplasma also stimulated endogenous colony formation in mice even if injected 24 h after irradiation [3], whereas, for example, endotoxin of *S. typhosa* was active only if injected immediately after irradiation [7]. Otherwise the time course of regeneration of erythropoiesis after irradiation of mice vaccinated with whooping cough vaccine resembles that of regeneration of erythropoiesis by endotoxin [7]. Since there is little in common in the regulation of erythropoiesis and immunopoiesis, and considering the adjuvant properties of *B. pertussis* vaccine, it can be postulated that the results of the present investigation will contribute to a better understanding of the mechanisms of regulation of anti-infectious and antitumor immunity.

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ROLE OF DIFFERENT MOUSE ESOPHAGEAL EPITHELIAL CELL POPULATIONS IN FORMATION OF THE CIRCADIAN RHYTHM OF PROLIFERATION

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KEY WORDS: circadiam rhythm; cell populations; heterogeneity.

The degree of participation of the cell population passing synchronously through the mitotic cycle (MC) in the formation of the circadin rhythm of mitotic activity was studied previously [1, 2] over a period of several days. However, the role of the asynchronous population in the formation of the circadian rhythm of cell proliferation has not yet been elucidated [3], and the investigation described below was undertaken to study this problem.

EXPERIMENTAL METHOD

Noninbred male albino mice weighing 25 g were kept in the animal house under conditions of 12 h daylight and 12 h darkness (daylight from 8 a.m. to 8 p.m.). There were two series of experiments. The animals in both series were given a single injection of [^{3}H]thymidine in a dose of 1 μCi/g body weight (specific radioactivity 8.8 Ci/mole). The mice of series I (n = 235) were given the isotope at 1 a.m., the mice of series II (n = 205) at 1 p.m., at times of maximal and minimal DNA-synthetic activity of cells of the stratum basale of the esophageal epithelium; the index of labeled nuclei (ILN) was 155 and 72°/₀₀ respectively. The animals were killed 1, 2, 3, 4, 5, 7, and 9 h later, and thereafter every 2 h for 90 h (series I) and 78 h (series II) after injection of the isotope. Paraffin sections of the esophageal epithelium were coated with type M emulsion (Moscow Technical Photographic Plate Factor) and exposed for 45 days. On the basis of analysis of 5000-10,000 cells of the stratum basale in each case the mitotic index (MI) and the index of labeled mitoses (ILM — the number of labeled mitoses divided by the total number of cells of the stratum basale) were determined and expressed in pro mille. The intensity of labeling of dividing cells (ILDC) also was determined. The numerical results were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

A circadian rhythm of MI was found for the animals in both series, with maxima at the end of the dark and beginning of the light periods of the day (Figs. 1a and 2a).

Investigation of ILM in the animals of series I showed (Fig. 1b) that it changed in the course of the experiment, with four peaks corresponding in time with periods of maxima of MI in the circadian rhythm. The first peak of ILM practically repeated the MI curve (about 76%

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