LITERATURE CITED

1. B. D. Brondz, Byull. Éksp. Biol. Med., No. 5, 64 (1964).
2. B. D. Brondz and I. F. Kotomina, Byull. Éksp. Biol. Med., No. 9, 69 (1973).
3. B. D. Brondz, E. Ya. Khachikyan, G. I. Drizlikh, et al., Byull. Éksp. Biol. Med., No. 6, 723 (1977).
4. B. D. Brondz, S. G. Egorova, and I. F. Kotomina, Eur. J. Immunol., 5, 733 (1975).
5. N. A. Kraskina, in: Modern Methods of Experimental Immunology and Various Aspects of their Use [in Russian], Moscow (1976), pp. 5-12.
6. E. Ya. Khachikyan, B. D. Brondz, G. I. Drizlikh, et al., Byull. Éksp. Biol. Med., No. 2, 189 (1978).
7. B. F. Argyris and F. DeLustro, Cell Immunol., 28, 390 (1977).
8. R. Elie and W. S. Lapp, Cell Immunol., 34, 38 (1977).
9. W. H. Fridman, D. Fradelizi, A. Guimezanes, et al., Eur. J. Immunol., 7, 549 (1977).
10. J. L. Glick, C. Lockwood, J. Williams, et al., Transplantation, 18, 86 (1974).
11. P. A. Goger et al., Transplant. Bull., 3, 142 (1956).
12. R. J. Hodes, L. M. Nadler, and K. S. Hathcock, J. Immunol., 119, 961 (1977).
13. R. R. Rich and S. S. Rich, J. Exp. Med., 140, 158 (1974).
14. M. Taniguchi and J. F. A. P. Miller, J. Exp. Med., 146, 1450 (1977).
15. S. L. Tonkonogy and H. J. Winn, J. Immunol., 116, 835 (1976).
16. G. A. Truitt, R. R. Rich, and S. S. Rich, J. Immunol., 119, 31 (1976).

FORMATION OF HEMATOPOIETIC COLONIES OF DONOR'S TYPE IN THE BONE MARROW OF IRRADIATED CHICKS AFTER TRANSPLANTATION OF QUAIL YOLK SAC AND LIMB BUD CELLS INTO THEIR BONE MARROW

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Ability to form hematopoietic cell colonies from the yolk sac and limb bud of a quail embryo at the 60 h incubation stage (i.e., before establishment of a closed circulation) was studied in the bone marrow of sublethally irradiated 3-week-old chicks. The experimental results are based on the ability to distinguish between quail and chick cells by means of a natural marker (Feulgen-positive nucleolus). After transplantation of limb bud cells roughly 3 times more hematopoietic colonies were found to be formed than after transplantation of yolk sac cells of the quail embryo. With the dose of irradiation used, about 75% of exogenous (quail) and 25% of endogenous (chick) hematopoietic colonies were identified in the bone marrow.

KEY WORDS: bone marrow; hematopoietic colonies; quail embryonic yolk sac and limb bud.

The method of obtaining hematopoietic colonies in the bone marrow of lethally irradiated chicks after injection of chick embryonic bone marrow and yolk sac cells was first developed by Samarut and Nigon [3]. In their investigations the recipients were 3-week-old chicks, irradiated twice in doses of 750 and 970 R, and into which hematopoietic cells were injected intravenously 4 h after the second irradiation. On the 6th-10th day after injection of the cells, benzidine-positive macrocolonies were detected in the bone marrow. The clonal origin of these colonies was later established [4]. This method has not found widespread application, for the survival rate of the birds after this dose of irradiation is very low.

However, when modified, this method offers considerable opportunities for the study of the number of colony-forming units in presumptive anlagen of the hematopoietic organs in avian embryos.

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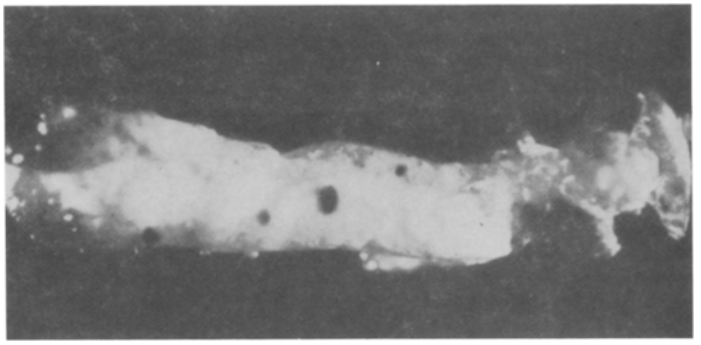

Fig. 1. Benzidine-positive macrocolonies (black spots) in chick bone marrow.

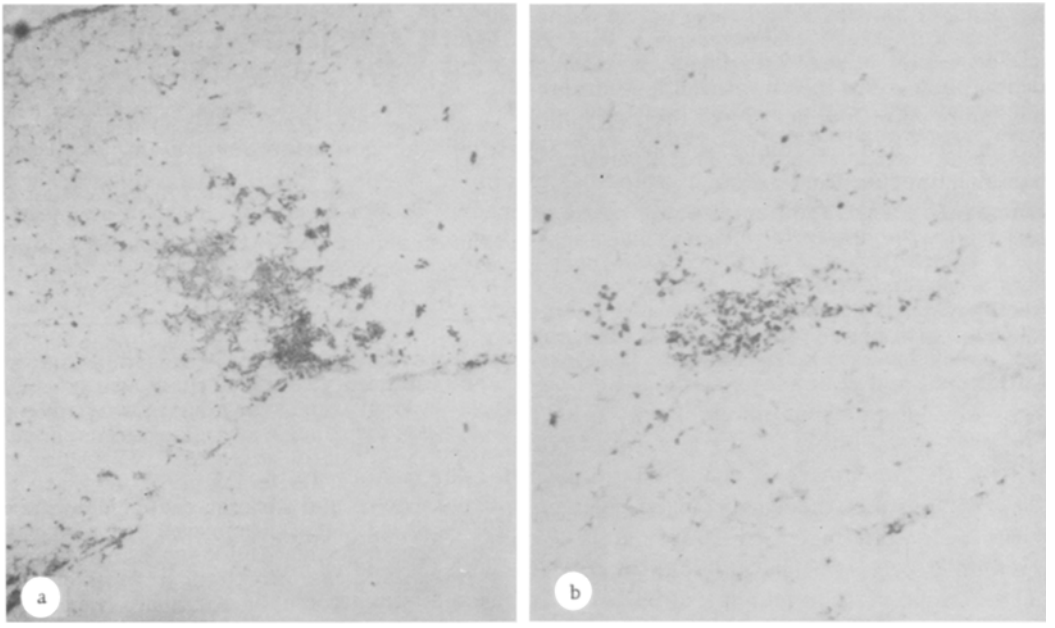

Fig. 2. Hematopoietic colonies in bone marrow section: a) differentiated colony; b) younger colony. Benzidine reaction followed by methyl green–pyronine; 160×.

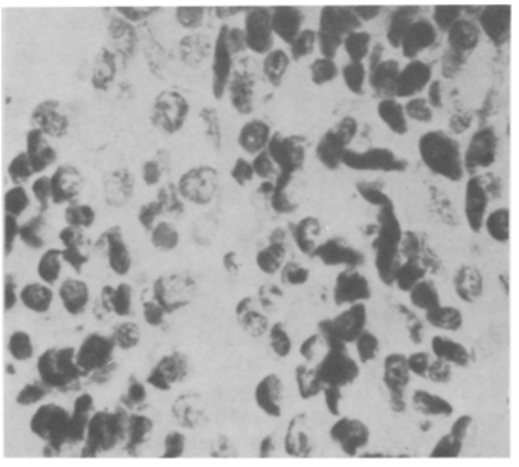

Fig. 3. Hematopoietic colony in chick bone marrow consisting of quail cells. Benzidine reaction followed by Feulgen's reaction; 180×.

In the last decade the method of natural cell markers, based on distinguishing Japanese quail cells from chicken cells by the presence of a Feulgen-positive nucleolus in the quail [2], has achieved widespread popularity.

If the two methods are combined and if cells from presumptive anlagen of the hematopoietic organs of the quail embryo are injected into sublethally irradiated chicks, not only the number of macrocolonies, but also the number of exogenous and endogenous colonies can be determined.

The ability of yolk sac and limb bud cells of quail embryos at the 60 h incubation stage to form colonies was investigated. Hind limb buds were taken when it was impossible to colonize them with hematopoietic stem cells from the yolk sac through the blood stream.

EXPERIMENTAL METHOD

Hens of the "Russian White" breed and embryos of the Japanese quail Coturnix coturnix japonica L. of the "Pharoah" breed were used. The 3-week chicks were irradiated sublethally: A dose of 750 R with a dose rate of 50 R/min on the RUT-250-15-3 (RUM-13) apparatus, with a tube voltage of 180 kV, tube current 15 mA, and filters: Al 1 mm and Cu 0.5 mm. One day after irradiation 4×10^8 quail yolk sac cells and 1.5×10^8 hind limb bud cells from a quail embryo at the 60 h incubation stage were injected into a wing vein. The volume of suspension injected was 0.6 ml. On the 6th-10th day after injection of the cells the chicks were decapitated, the limb bones were carefully opened, and the bone marrow was extracted and fixed in methanol. The benzidine reaction was carried out and the number of benzidine-positive macrocolonies counted in eight bones of each chick (femur, tibia, humerus, ulna). For histological treatment the bone marrow was taken through cedar wood oil, embedded in paraffin wax, and serial sections were cut to a thickness of 5-6 μm. The Feulgen reaction was carried out (cold hydrolysis for 3 min) on the sections and they were stained with methyl green and pyronine by Brachet's method. The number of microcolonies was counted in every fifth section of the series and the numbers of exogenous (quail) and endogenous (chick) colonies distinguished. Irradiated chicks not receiving injections of donor's cells served as the control for survival.

The hind limb bud and yolk sac of the quail embryo were isolated at the 60 h incubation stage and placed in buffered Ringer's medium for avian embryos [1] at 37°C. The tissue was then transferred to 3 ml buffered Ringer's medium with the addition of 40 units/ml of collagenase and 50 units/ml hyaluronidase, and incubated for 30 min at 37°C with constant rotation. During incubation the cells were partly dispersed by careful pipeting 3 times after 10 and 20 min of incubation. The cell suspensions were then filtered through two layers of Kapron filter. The filtrate was centrifuged for 10 min at 1700 rpm. The supernatant was then poured off and the cells suspended in medium No. 199 in Hanks's solution, pH 7.2, with the addition of 10,000 units/100 ml penicillin and 0.01 g/100 ml streptomycin. The number of cells in 1 ml suspension was counted.

EXPERIMENTAL RESULTS AND DISCUSSION

All the irradiated chicks which did not receive injections of donor's cells died 4 days after irradiation. About 50% of the chicks injected with donor's cells survived until the 10th day after injection of the quail cells. The first benzidine-positive colonies were found on the 6th-7th day after injection of the donor's cells. Colonies were most clearly defined on the 8th-10th days after injection of the cells (Fig. 1).

On the 9th day after injection of yolk sac cells 30.8 ± 4.3 colonies per chick were formed for every 10^8 donor's cells, and after injection of the limb bud cells 107.9 ± 22.2 colonies per chick were formed for every 10^8 donor's cells. It thus follows that the limb bud contains about 3 times as many colony-forming units as the yolk sac at the 60 h incubation stage.

In the bone marrow sections, against the background of general aplasia of the organ hematopoietic colonies could be seen (Fig. 2). Erythroid colonies as a rule were located at the periphery of the bone marrow, and a few myeloid colonies could be seen nearer the central part of the organ. In the region of the epiphyses, colonies were more numerous than in the diaphysis. Exogenous (quail) colonies accounted for $76.4 \pm 6.24\%$ of the total number of colonies in the sections.

After the benzidine reaction and further staining of the section by Brachet's method the degree of differentiation of the colonies could be distinguished on the basis of the number of benzidine-positive cells they contained. By the 9th-10th day after transplantation of the donor's cells the more differentiated colonies were more numerous. An exogenous colony with many benzidine-positive cells is shown in Fig. 3.

The method of obtaining hematopoietic colonies in chick bone marrow by the use of transplantation of quail cells enables the number of the host's stem cells still remaining in the blood stream and capable of repopulating the bone marrow to be counted and the dynamics of the pool of the donor's stem cells to be studied.

LITERATURE CITED

1. L. J. Hale, Biological Laboratory Data, London (1965).
2. N. Le Douarin, Exp. Cell Res., 77, 459 (1973).
3. J. Samarut and V. Nigon, J. Embryol. Exp. Morph., 33, 259 (1975).
4. J. Samarut and V. Nigon, J. Embryol. Exp. Morph., 36, 247 (1976).

EFFECT OF THYMECTOMY ON FORMATION OF IMMUNOLOGIC TOLERANCE IN DELAYED-TYPE HYPERSENSITIVITY EFFECTORS

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The effect of thymectomy on the formation of tolerance of delayed-type hypersensitivity (DTH) to sheep's red blood cells was investigated. If tolerance was induced by combined injection of a massive dose of antigen and cyclophosphamide, thymectomy did not prevent this process and prolonged the state of tolerance. If area activity was induced by a massive dose of antigen alone, thymectomy restored the ability to form DTH and prevented the formation of suppressor cells. Thymectomy weakened DTH formation somewhat in intact animals, but not in animals receiving cyclophosphamide. The results confirm views regarding the diversity of the mechanism of tolerance (clonal-deficiency and suppressor). It is also suggested that among DTH effectors and their precursors there are two subpopulations which differ in their sensitivity to cyclophosphamide and thymectomy.

KEY WORDS: immunologic tolerance; delayed-type hypersensitivity; cyclophosphamide; thymectomy; suppressor cells.

The use of thymectomy in adult animals enabled the life span of individual subpopulations of thymus-dependent lymphocytes to be determined, and if combined with other procedures, it can provide information on the precise mechanism of individual immunologic phenomena [9], including the mechanism of different forms of immunologic tolerance [6, 7, 10, 13, 14]. The object of the present investigation was to study the effect of thymectomy on the formation of tolerance to sheep's red blood cells (SRBC) in effectors of delayed-type hypersensitivity (DTH) in mice.

EXPERIMENTAL METHOD

(CBA × C57BL/6)F_1 hybrid mice weighing 20-22 g were used. Areactivity of the DTH effectors was obtained either by injection of 6×10^9 SRBC along or by successive intraperitoneal injections of 6×10^9 SRBC, followed after 42-45 h by cyclophosphamide (CP) in a dose of 200 mg/kg [3].

On the 14th day after this treatment the mice were sensitized by intravenous injection of 10^5 SRBC in physiological saline [11]. The DTH level was determined by skin tests [11]. For this purpose, on the 4th day after sensitization, 10^8 SRBC in 40 μl physiological saline was injected into a footpad of the mice. The reaction was read 24 h after the reacting injection of antigen. The difference between the thickness of the footpad

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