DIFFERENTIATION OF THYMOCYTES IN MOUSE EMBRYOGENESIS

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Data in the literature and the writer's own observations demonstrate the existence of a regular sequence of change in the fine structure of thymus lymphocytes and the appearance of differentiation antigens on their surface during embryogenesis of the chick [10] and man [1, 3]. It has not yet been explained to what extent this principle is characteristic of other species of animals, notably mice, as regards the degree of differentiation of the thymocytes, which can be judged not only on the basis of their fine structure [9] and the presence of surface receptors [2, 7], but also by their colony-forming ability [5].

In this investigation the fine structure, surface properties, and colony-forming ability of thymus lymphocytes were compared in mice at different stages of embryonic development.

EXPERIMENTAL METHOD

The investigation was conducted on 250 CBA mouse embryos at the 13th, 15th, and 17th days of development and on 300 mature male mice of the same line. To analyze the ultrastructure of the thymocytes, thymus glands from 60 embryos of each age were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4), and then postfixed with osmic acid. After embedding in Araldite semithin and ultrathin sections were cut. The semithin sections were stained with 1% methylene blue solution and examined under the light microscope. Ultrathin sections were stained with lead citrate by Reynolds' method and examined in the JEM—100B electron microscope. The remaining embryos at different times of development were used to prepare suspensions of lymphocytes from the thymus and liver and heart cells in a concentration of 10^6 cells/ml, which were then used in the cytotoxic test with anti-θ-serum, and also for an experiment to verify their colony-forming ability. The presence of θ-antigen on the surface of the lymphocytes was determined by a cytotoxic test, using the method in [8]. The antiserum against mouse T lymphocytes used in the reaction was provided by Professor B. B. Fuks, to whom the writer is grateful.

The reaction was set up as follows: 0.2 ml of lymphocyte suspension containing 10^6 cells/ml was mixed with 0.2 ml of serum taken in dilutions of 1:2, 1:4, 1:8, 1:16, and 1:32, and 0.1 ml of guinea pig serum was added as complement. The mixture was incubated at 37°C for 45 min. The lysed cells were identified by staining with 0.1% trypan blue (the stain was made up immediately before use). For the control, cell suspensions of organs were incubated with buffered physiological saline instead of antiserum. The percentage of lysed cells was calculated by the formula $[(a - b)/a]$, where a stands for the number of living cells in suspensions without antiserum, and b the number of living cells in suspensions with antiserum, expressed per 100 cells counted altogether. To study the colony-forming ability of the cells the method in [11] was used. After lethal irradiation in a dose of 850 R on a gamma-ray source (189 R/min) the recipient mice received an intravenous injection of a suspension of thymus cells in a concentration of 10^6 cells/ml in a dose of 1 ml per animal. The animals of three groups constituted the control. Mice of group 1 were injected with 1 ml of medium 199, those of group 2 with 1 ml (10^6 cells) of a suspension of liver cells, and

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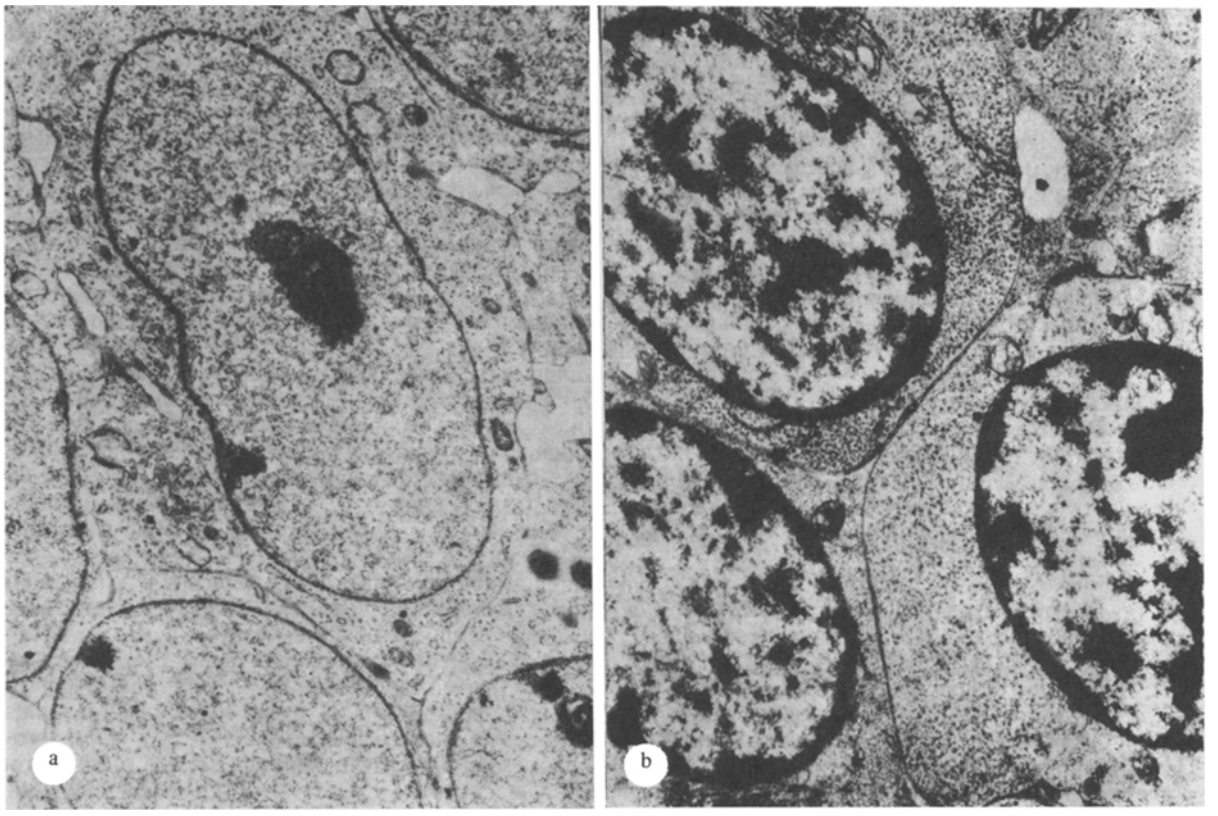

Fig. 1. Ultrastructure of mouse embryonic thymocytes: a) mouse embryo at 13th day of development: elongated lymphocytes with large nuclei containing diffuse chromatin and one or two large nucleoli, 8000 ×; b) 15-day mouse embryo: thin layer of juxtamural chromatin visible in lymphocyte nuclei, cytoplasm is electron-dense, 9600 ×; c) 17-day embryo: thymocytes have compact chromatin near nuclear membrane and throughout area of nucleus, 9000 ×[(c) missing in Russian original — Publisher].

those of group 3 with 1 ml (10^6 cells) of a suspension of heart cells. The number of discrete colonies in the recipients' spleen was counted visually on the 8th day after injection of the cells. For microscopic study the spleens were fixed in Carnoy's fluid and serial sections were stained with azure II-eosin and methyl green with pyronine. The following types of colonies were counted in sections: erythroid, myeloid, megakaryocytic, mixed, and undifferentiated. The numberical data were subjected to statistical analysis by Student's formula.

EXPERIMENTAL RESULTS

Thymus lymphocytes of 13-day mouse embryos were often elongated in shape, with large oval or round nuclei containing diffusely distributed chromatin, and with one or two large nucleoli (Fig. 1a). The cytoplasm contained ribosomes, mitochondria, and sometimes a Golgi complex; other organelles most frequently were absent. In all dilutions studied the antiserum had hardly any cytotoxic action on thymus lymphocytes of 13-day mouse embryos. The percentage of stained cells did not exceed 4. These same lymphocytes possessed colony-forming activity. The number of colonies found in the spleens of the reconstituted mice was 11.6 ± 2.7, of which 2.3 ± 0.3 were macrocolonies (Table 1). Thymus lymphocytes of embryos of this period of development formed colonies of all types, but most frequently colonies of the myeloid branch of hematopoiesis, consisting of cells which rarely survived to the stage of mature forms. Most frequently they were arrested at the promyelocyte stage.

Condensation of chromatin began in lymphocyte nuclei of the thymus of 15-day mouse embryos, a thin layer of juxtamural chromatin appeared, and the cytoplasm became electron-dense (Fig. 1b). Thymus lymphocytes of 15-day mouse embryos became more circular in shape. More than half (57%) of the lymphocytes were lysed by antiserum in a dilution of 1:16. The colony-forming activity of these lymphocytes was depressed. The number of colonies found in

TABLE 1. Colony Formation by Mouse Thymus, Liver, and Heart Cells during Embryogenesis

Source of transplanted cells and their number	Age of embryo, days	Total No. of colonies	No. of macrocolonies (> 1 mm)	Types of hematopoietic colonies				
				erythroid	myeloid	megakaryocytic	mixed	undifferentiated
Thymus (10^6)	13 (n=5)	11,6±2,7	2,3±0,3	0,8±0,5	8,2±3,4	0,39±0,3	1,4±0,7	0,8±0,4
	15 (n=4)	4±1,2	1,3±0,3	1,3±0,4	2,3±1,3	—	0,5±0,2	—
	17 (n=5)	4,2±0,6	0,8±0,4	1,4±0,3	2,8±0,7	—	—	—
Liver (10^6)	13 (n=4)	27,5±2,9	17,8±1,1	8±1,2	7±2,3	1,8±0,6	10,5±1,2	0,3±0,2
	15 (n=3)	26,3±3,2	17,5±1,54	6±2,3	7±2,3	1,8±0,6	10,5±1,2	0,3±0,2
	17 (n=3)	23±0,5	17,3±1,4	6,3±1,8	3,6±2,3	2±0,58	11±2,5	—
Heart (10^6)	13 (n=3)	0,3±0,03	—	0,25±0,03	—	—	—	—
	15 (n=3)	0,4±0,01	—	0,4±0,01	—	—	—	
	17 (n=3)	—	—	—	—	—	—	
	1 ml of med. 199 (n=3)	—	—	—	—	—	—	

Legend. n) Number of spleens studied microscopically.

the recipients' spleens was 4 ± 1.2, of which 1.3 ± 0.3 were macrocolonies (Table 1). The colonies were formed by erythroid and myeloid branches, and mixed colonies also were present. As before, the commonest colonies were myeloid in type.

The zone of condensed chromatin in nuclei of thymus lymphocytes from 17-day mouse embryos was considered enlarged (Fig. 1c). The lymphocytes contained electron-dense cytoplasm, and 88% of the cells were lysed by antiserum in a dilution of 1:16. The colony-forming activity of the lymphocytes was low. Only 0.8 ± 0.4 macrocolony was found in the recipients' spleens.

Colony formation of blood cells present in the thymus could be excluded by the fact that the heart cell suspensions, if injected into irradiated recipients, formed virtually no colonies (Table 1).

The colony-forming activity of the liver in embryos at all times of development remained at a uniform high level. In this case mixed and erythroid colonies predominated. Colonies formed by thymus lymphocytes of embryos at the 13th, 15th, and 17th days of development, it will be noted, differed from colonies formed by liver cells at the same stages of development in their smaller size, and also the ratio between the numbers of different types of colonies (Table 1). These observations do not disagree with the generally known fact that the properties of hematopoietic stem cells change in the course of individual development [4].

The results are in agreement with observations according to which thymus lymphocytes of mouse embryos at different times of development may be the source for repopulating the spleen of irradiated recipients [5], and they suggest that lymphoid stem cells, more advanced in the series of differentiation than the stem cells of the liver, migrate into the embryonic thymus.

Condensation of chromatin in thymus lymphocyte nuclei of 15-day mouse embryos takes place simultaneously with the appearance of θ-antigen on their surface. This is in agreement with the generally accepted view that a characteristic feature of conversion of thymocyte precursors into T cells is the appearance of a large quantity of θ-antigen on their surface [2]. Restriction of the colony-forming activity of thymus lymphocytes of 15-day embryos and its disappearance in 17-day embryos are probably connected with committal and limitation of their potential.

It can be concluded from these results that differentiation of mouse thymocytes during embryonic development is characterized by condensation of chromatin in their nuclei, accumulation of θ-antigen on their surface, and loss by the cells of their ability to form colonies in the spleens of irradiated recipients.

The sequence of events during differentiation of thymus lymphocytes in embryogenesis of mice thus completely repeats the changes which arise during thymocyte differentiation in embryogenesis of the chick and man [1, 3, 10].

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EFFECT OF SIZE-FRACTIONATED THYMOCYTES ON NUMBER OF HEMATOPOIETIC STEM CELLS IN BONE MARROW OF SUBLETHALLY IRRADIATED MICE

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T lymphocytes have an influence on the functional properties of hematopoietic stem cells (CFU-S) [1, 3, 4]. In particular, thymocytes have been shown to stimulate CFU-S proliferation [8, 9]. The stimulating effect of thymus cells has been noted mainly in a nonsyngeneic system, whereas the results of studies of interaction between thymocytes and CFU-S under conditions of a syngeneic donor–recipient combination are contradictory [2, 10, 11]. Lymphocyte subpopulations may exhibit various functional properties, and methods of their fractionation have now been developed, whereby the cells can be separated without disturbance of their function [7, 12]. Repeated injections of small doses of glucocorticoids increase the number of CFU-S in the bone marrow of intact mice [5], possibly on account of changes in the cell composition of the thymus as a result of the effect of the hormone on the cortisone-sensitive subpopulation of T cells.

The object of this investigation was to study the effect of thymus cells, fractionated according to size, and obtained from intact mice and mice treated with dexamethasone, on the number of CFU-S in the bone marrow after sublethal irradiation.

EXPERIMENTAL METHOD

Experiments were carried out on male (CBA × C57BL)F_1 hybrid mice weighing 20-24 g. A thymocyte suspension was prepared in medium No. 199 from the thymus glands of intact mice or of mice receiving an intraperitoneal injection of dexamethasone-^{21}Na-phosphate (DM) in a dose of 0.001 mg per mouse daily for 7 days. The thymocytes were fractionated by size by

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