COLONY-FORMING HEMATOPOIETIC CELLS IN THE MOUSE EMBRYONIC LUNG

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In the course of embryonic development of the mammalian lungs several stages are distinguished: pseudoglandular, canalicular, and alveolar. It has been shown that as early as during the pseudoglandular stage stem cells, serving as the source for the macrophage population, are present in the rat lung [8]. Meanwhile it has been shown that hematopoietic stem cells, capable of colony formation, penetrate into several organs (liver, thymus) in the early stages of embryogenesis [1-3].

The lungs of adult mice are the source of factors of colony stimulation, which support proliferation of macrophages [6, 7], whereas lungs of infants aged 4-10 days (postnatal development) contain substances facilitating growth of colonies of bone marrow cells [4]. It can be tentatively suggested that the microenvironment of the embryonic lung is favorable for the colonization of this organ by polypotent hematopoietic stem cells, whose pathway of differentiation depends on the microenvironment.

The aim of the present investigation was to discover the presence of hematopoietic stem cells in the embryonic mouse lung.

EXPERIMENTAL METHOD

The colony-forming activity of the embryonic lung cells was revealed by the method in [9]. As irradiated recipients, 26 adult CBA mice weighing 25-30 g were used. The donors of the cells were mouse embryos of the same line, at 14 and 15 days of intrauterine life. The recipient mice received an intravenous injection of a suspension of embryonic lung or liver cells in a concentration of 10^6 cells/ml 2-6 h after irradiation with a sublethal dose of 8.5 Gy on a γ-ray apparatus (189 R/min). The control recipient mice received 1 ml of medium 199 intravenously. A lung cell suspension was prepared by using 4-8 embryos simultaneously at each time of development.

There were two repeated series of experiments: I) in March and II) in October, 1988 (Table 1). In cases when the recipient received the embryonic lung cell suspension, visual counting of discrete colonies in the recipients' spleen on the 8th day after irradiation did not reflect their true number sufficiently completely, because of the small sizes of the colonies. Accordingly, quantitative estimation of colony formation was carried out on serial sections 7 μ thick, stained with hematoxylin and eosin, and also with azure II and eosin.

In each case the number of macro- and microcolonies in each section of the spleen was determined under a magnification of 150, after which the total number of colonies in all sections was counted. In parallel tests, the contours of each section were traced on a "Mikrofot" apparatus (magnification 11) and the total area of all sections in which the colonies were analyzed was calculated. In this way it was possible to determine the number of colonies per square millimeter of section through the spleen (Table 2). The numerical data were subjected to statistical and lysis by Student's test.

EXPERIMENTAL RESULTS

On the 8th day after irradiation 37% of the control mice survived, whereas among recipients receiving embryonic liver cells, the survival rate 100%. Recipient mice, receiving an embryonic lung cell suspension, occupied an intermediate position:

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TABLE 1. Number of CBA Mice Used for Transplantation of Embryonic Lung and Liver Cells and Their Survival Rate on the 8th Day after Irradiation

Series of experiments	Group of animals and age of embryos	Source and number of transplanted cells	Number of animals used	Number of animals which survived	Presence of visible colonies in spleen (number of cases)
I	Control	Medium 199 (1 ml)	3	1	—
	Exptl. (14 days)	Lung (10^6 cells/ml)	3	3	3
		Liver (10^6 cells/ml)	3	3	3
II	Control	Medium 100 (1 ml)	5	2	—
	Exptl. (15 days)	Lung (10^6 cells/ml)	8	4	4
		Liver (10^6 cells/ml)	4	3	3

TABLE 2. Number of Colonies in Spleen of Irradiated CBA Recipient Mice on 8th Day after Intravenous Transplantation of Embryonic Lung Cells

Source and number of transplanted cells	Age of embryo, days	Total number of colonies per square millimeter section through spleen	Number of	
			macrocolonies	microcolonies
Lung (10^6 cells/ml)	14 (n=3)	2,32±0,39	1,21±0,25	1,11±0,55
	15 (n=4)	2,34±0,57	0,60±0,26	1,74±0,57
Medium 199, 1 ml (control)	(n=3)	0,52±0,04	—	0,52±0,04

63% of the irradiated animals survived (Table 1). The weight of the spleen of recipient mice receiving embryonic lung cells was 14% higher than the weight of the spleen of the surviving control mice. After transplantation of embryonic lung cells, one or two small colonies could be seen visually on the surface of the spleen of the experimental mice. In spleens of the reconstituted mice, receiving an injection of embryonic lung cells, both macro- and microcolonies were found (Fig. 1a, b). The total number of colonies, counted per square millimeter of section through the spleen, averaged 2.31 ± 0.39 in the case of transplantation of lung cells from 14-day embryos, and 2.34 ± 0.57 in the case of 15-day embryos. Macrocolonies in the first case accounted for 52.5%, and in the second case only 2.5% of the total number of colonies (Table 2). Among endogenous colonies in the spleen of the control mice there were no microcolonies. The criteria of macrocolonies which was adopted wars the number of cells in them (over 60), and also their size (they must be not less than 1 mm in diameter).

As a rule the microcolonies were arranged beneath the capsule of the spleen or along its trabeculae. Many cells of microcolonies had the appearance of blast forms, and it was therefore difficult to establish the type of colony. However, cells of the myeloid series predominated in them. Evidently the source of the microcolonies in this case was committed stem cells, unable to reproduce a large cell population of the different branches of hematopoiesis.

Macrocolonies were formd by myeloid cells, and mixers colonies also were found. Cells of the granulocytic series in the various stages of differentiation predominated in the myeloid colonies. Blast forms were present in the center of the colony: single cells were found in a state of mitosis (Fig. 1c). Mixed colonies contained cells of the myeloid, erythroid, and megakaryocytic series (Fig. 1d).

Incidentally, the character of the colonies formed in the spleen of the recipient mice from lunar cells at the 14th day of intrauterine development differed from that of colonies formed by cells from the 15-day embryonic lung. When lung cells from 15-day embryos were injected, microcolonies predominated (97.5% of the total number of colonies, see Table 1).

The colony-forming activity of the embryonic liver at the 14th and 15th days of embryonic development was high: the recipients' spleen contained large, confluent macrocolonies, most of which were mixed or erythroid. Colonies formed by cells of the embryonic lung at 14 and 15 days of development differed from colonies formed by the liver cells at the same times of development, in the number, smaller size, and relative proportions of the different types of colonies.

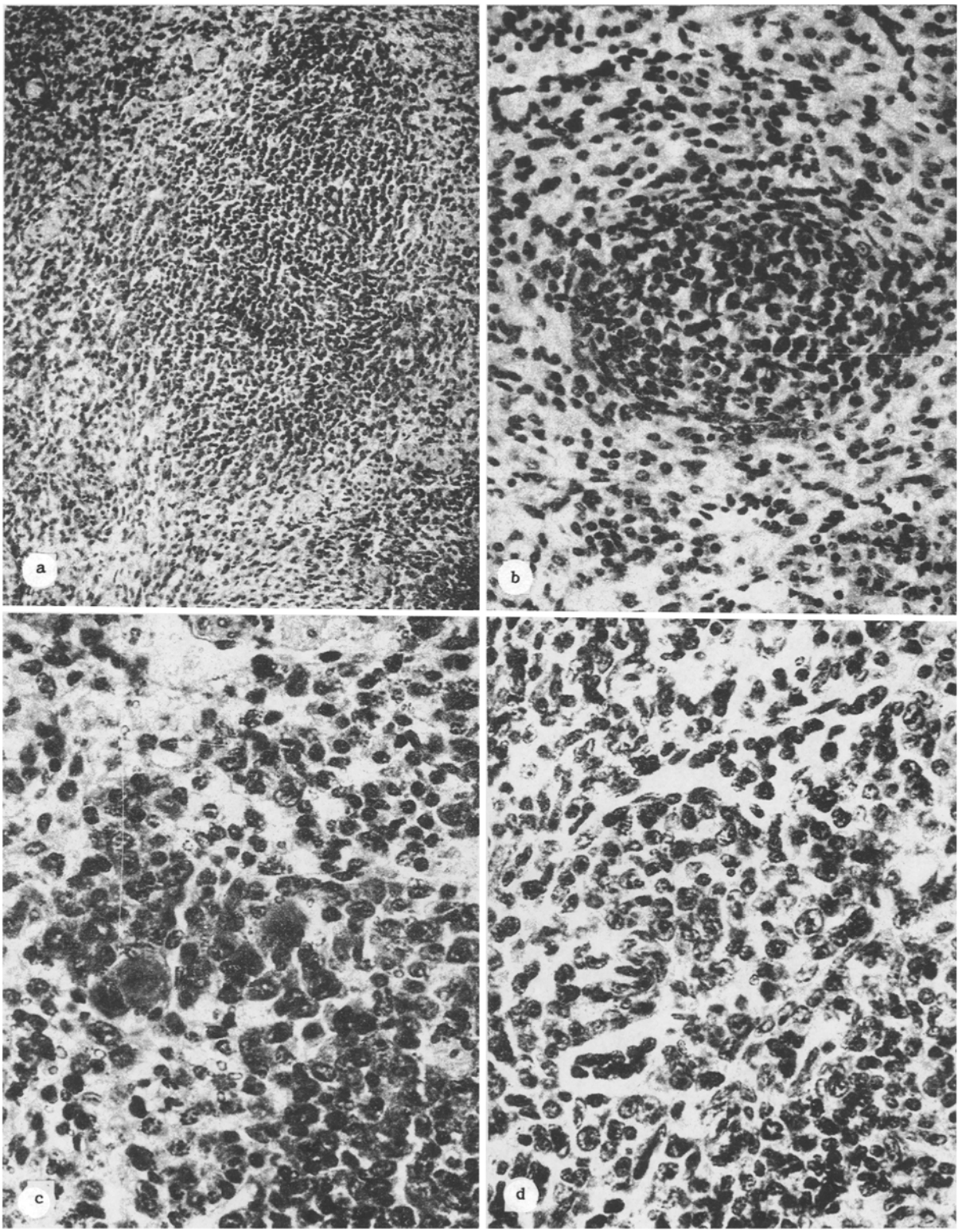

Fig. 1. Spleen of irradiated CBA recipient mouse after intravenous injection of embryonic lung cells at 14-15 days of development. a) Macrocolony, 170×; b) microcolony, 340×. Stained with hematoxylin and eosin. c, d) Fragments of mixed colonies containing cells of megakaryocytic, granulocytic, and erythroid series. Mitosis in megakaryocyte (arrow). 540×. Stained with hematoxylin and eosin.

Our data showing that the properties of hematopoietic stem cells can change in different periods of embryonic development are in agreement with the results of investigations by or her workers [1, 3, 4].

The formation of colonies by blood cells, settling in the embryonic lung, can be ruled out by data showing that cell suspensions of the embryonic heart of CBA mice at the same times of development, if injected into irradiated recipients, virtually never form colonies [1].

The following conclusions can be drawn from the results: 1) hematopoietic stem cells migrate in vivo into the embryonic lung, just as also into the liver and thymus; 2) the colony-forming ability of these cells diminishes with an increase in the period of embryogenesis of the lung; 3) stromal elements of the spleen of the irradiated recipient facilitate realization of the colony-forming potential of hematopoietic stem cells in the lungs and determine the pathway of their differentiation. All these finding's suggest that the lung in vivo is colonized by hematopoietic stem cells in the early stage of embryogenesis of the mouse (evidently before the 14th day of development).

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ISLET-CELL AGGREGATES: STRUCTURE AND INSULIN-PRODUCING ACTIVITY DURING IN VITRO CULTURE

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With rapid developments in the subject of free transplantation of the pancreatic islet-cells (PIC) in diabetes, particular importance is attached to research aimed at obtaining cultures with high insulin-producing activity, and purified from cells carrying class II histocompatibility antigens — so-called passenger leukocytes. Different approaches have been used to solve this problem: isolation of islets followed by their culture in vitro at 24-26°C or in contact with the gaseous phase, containing 95% of oxygen, the preparation of monolayer cultures with a high PIC content and of floating cultures of organotypical character from fetal pancreas [1-4]. There have also been isolated reports of the possibility of using "neoislets," formed by aggregation of PIC during roller-tube culture [5].

The aim of this investigation was to study the possibility of obtaining neoislets during culture of rat PIC in an ordinary stationary system, and to the investigation of their structure and insulin-producing activity.

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