Hemopoietic Stem Cells in Embryogenesis of the Mouse

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Introduction

Erythropoietic islands located in the yolk sac (YS) mesoderm are known to be the first sites of hemopoiesis in the ontogeny of mice. Committed precursors colony-forming unit–granulocyte-macrophage (CFU-GM) and burst-forming unit–erythroid (BFU-E), as well as pluripotent progenitors (CFU_{mix}) were found in murine YS as early as 8 days of gestation; in embryos proper (bodies) and in the circulation, committed precursors appeared 1 day later than in YS [1–3]. As for CFU–spleen (CFU-S), there are few communications and they are contradictory. Moore and Metcalf [1] reported that CFU-S initially appeared in murine YS on day 8 of gestation, and they were evident in the embryonic circulation by day 10 of development. However, other investigators failed to find macroscopic spleen colonies after the injection of day-9 YS cells [4, 5]. After the cultivation of a day-9 YS in organ culture for 24–96 h, CFU-S were readily found [4]. In the present study we tested the accurate stages – in somite pair (SP) numbers – of embryonic development when CFU-S and CFU-GM appeared for the first time in the YS, circulation, embryos proper, and liver rudiments (LR). We have used the organ culture system to examine whether pre-CFU-S, capable of differentiating into CFU-S in vitro, exist in the 9-days YS and embryo proper.

Materials and Methods

C57Bl/6 females were mated with CBA males. The day on which the vaginal plugs were observed was designated as day 0 of gestation. SP were counted in 8 to 10-day embryos. Embryonic blood was collected after the separation of the YS from the bodies. Beginning with the 9th day of gestation, LR were removed from the bodies and investigated separately. For routine CFU-GM testing the tissues were minced and treated with 0.1% trypsin. For CFU-S testing tissues were minced and pipetted gently. Cell suspensions were injected i.v. to lethally irradiated (^{137}Cs, 12.5 Gy) CBF_1 mice; 7th-day macroscopic colonies were counted. The filter organ culture method [6] was used with some modifications. Briefly, whole 9-day embryos or separated embryos and YS were cultured on Millipore filters (HA, pore size 0.45 μm), supported by a stainless steel grid (37 °C, 5% CO_2 in air). After cultivation explants were tested for CFU-S content.

Results

CFU-GM were observed first in the 8-day YS (0 to 5-SP stage), and they were practically absent from the body at that time. Reliable numbers of CFU-GM in the embryo body appeared from day 9. The appearance of CFU-GM in the embryo proper generally coincided with the beginning of their detection in the circulation (13 to 16-SP stage). The maximum content of CFU-GM was observed on day 10 of development both in the YS and

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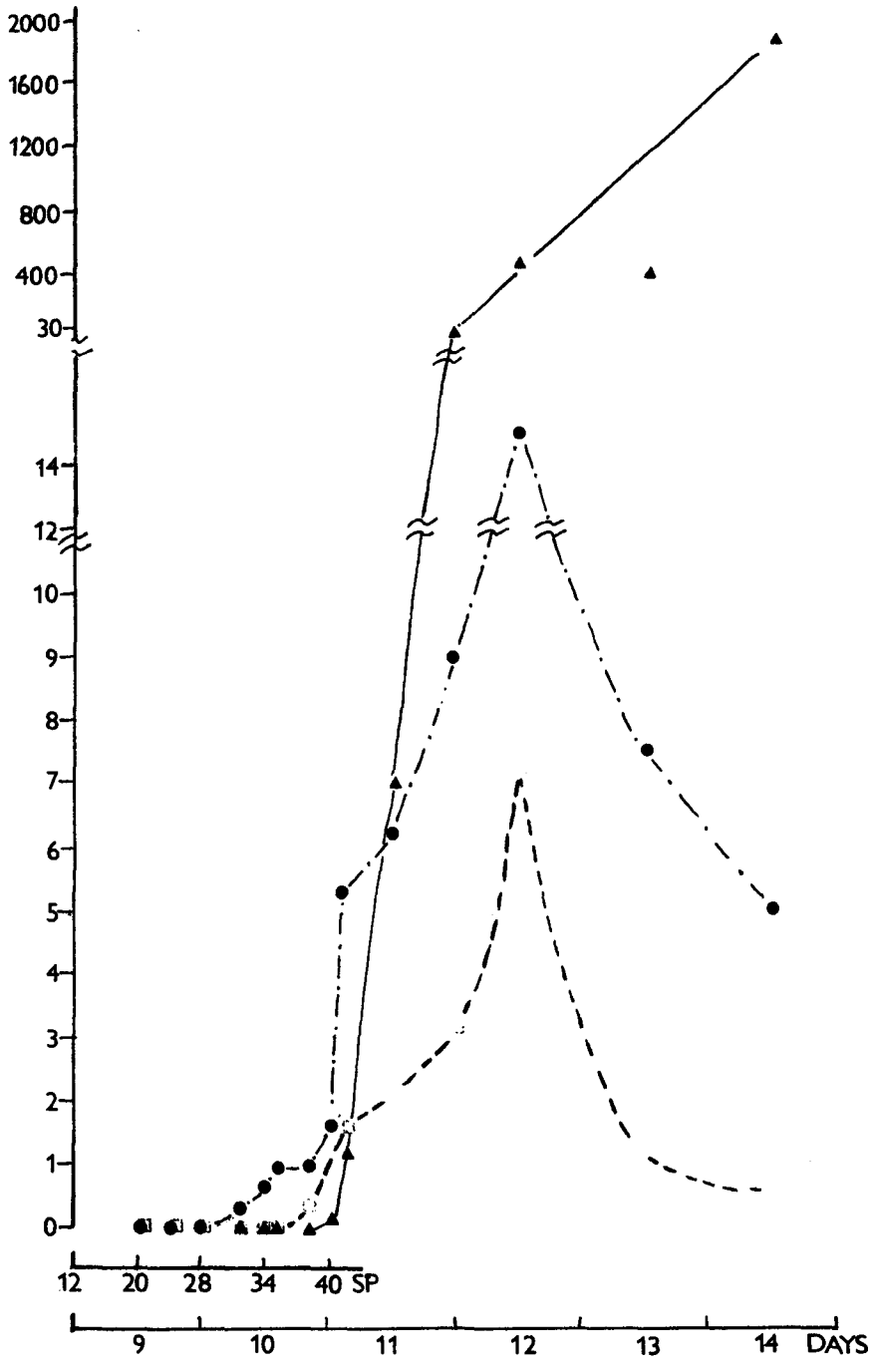

Fig. 1. CFU-S in embryonic tissues (per embryo) during 9th–14th days of development. *Abscissa*, Days of embryonic development and numbers of somite pairs (SP); *ordinate*, the number of CFU-S in the YS (□–□), the body (●—●), and the liver (▲—▲)

the body. It must be emphasized that the first CFU-GM which were found in the body of the embryo were not localized in the LR, where they appeared from approximately 25 to 26-SP stage (9th day). From the 11th day of development the liver became the main source of GM progenitors in embryos (data not shown).

CFU-S first appeared in the embryo proper, out of the LR, at the 30 to 33-SP stage on day 10 (Fig. 1). In the YS, CFU-S could be found beginning at the stage of 37–38 SP. In the circulation, CFU-S were observed from about the 37-SP stage. From the initial level of 0.24 CFU-S per 10^6 cells, their concentration increased to 1.69 per 10^6 cells by day 11, and reached a maximum on day 13 (3.5 per 10^6 cells). The detectable number of CFU-S in LR was found only at the end of the 10th day

Table 1. CFU-S in 9-say-old embryos after in vitro cultivation

Culture	Age of donor (SP)	Number of embryos	Colonies/spleens
Whole embryo	14–23	105	1/30
	24	43	3/13
	25–28	41	7/16[a]
	29–30	5	3
Yolk sac	23–24	9	0/1
	25–28	92	9/19[a]
	29–30	28	6/7
Body with liver rudiment	23–24	9	0/1
	25–28	56	5/22[a]
	29–30	7	2/5
Body without liver rudiment	25–28	62	13/18
	29–30	18	1/9
Liver rudiment	26–30	61	0/8
Irradiation only			1/96

[a] One large colony was selected for chromosome analysis. Donor origin (T6 chromosome) of CFU-S was confirmed.

of gestation ($\approx$ 40 SP). CFU-S number in the YS as well as in the embryo body attained a peak on day 12 and then decreased. From the 11th day, the CFU-S content in embryonic liver increased suddenly.

After 4 days in organ culture CFU-S were not detected in any tissues explanted earlier than the 25 to 28-SP stage, with the exception of the 24-SP whole embryo group in which quite a few colonies were found (Table 1). In the YS and the embryo body explanted at the stage of 25–28 SP or later, significant numbers of colonies were observed. Embryo bodies without LR were cultured in order to test whether CFU-S production in the embryo could be ascribed to the developing liver. The results indicate that CFU-S production in cultures of "the body without LR" was no less than in cultures of "the body with LR." No CFU-S were produced in isolated LR.

Discussion

We have failed, as others [4, 5], to support the data of Moore and Metcalf [1] that CFU-S first appear on the 8th day of murine development. We could not demonstrate any CFU-S in the YS or in the embryonic body till the 10th day of gestation. Moreover, CFU-S did not appear earlier in the YS than in the body. So we encountered the fact that CFU-S were absent from the hierarchy of hemopoietic progenitors in the 8 to 9-day YS, though committed precursors (CFU-GM) were present. Whether these two kinds of embryonic hemopoietic precursors have a common origin or *take* arise independendently is not clear now. One cannot rule out the possibility of a transitory hemopoiesis in the YS of an early (7–8 days) murine embryo such as takes place during development in birds [7]. It has been shown earlier [4] that the YS of a 9-day embryo contains pre-CFU-S which can differentiate into CFU-S after 1–4 days in organ culture. We have not only confirmed this observation but have also found that pre-CFU-S appeared both in the YS and embryos proper simultaneously in the late 9-day (25–29 SP) embryos.

In conclusion, the early (8–9 days) embryonic (YS) period of hemopoiesis

differs from adult and fetal hemopoiesis in that CFU-S are absent from the stem cell compartment. Before fetal liver hemopoiesis starts (the 10th day of development), there are two sites, the YS and the embryo body, from which CFU-S could migrate into the developing liver. According to the data obtained, one cannot prefer the YS or the body as the source of CFU-S origin since CFU-S as well as pre-CFU-S are detected in both sources simultaneously.

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