Differentiation and grafting of haemopoietic stem cells from early postimplantation mouse embryos

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Summary

Haemopoietic stem cells evidently arise in early post-implantation mouse embryos at day 6 of gestation, a day earlier than previously thought (Moore & Metcalf, 1970). Disaggregated embryonic cells were injected into mice given a lethal dose of X-irradiation. The presence of donor haemoglobin (Whitney, 1978) and donor lymphocytic glucose phosphate isomerase (GPI) (Siciliano & Shaw, 1976) to detect donor erythrocytes and lymphocytes, respectively, were monitored by starch gel electrophoresis. The presence of donor cells was also assessed by using donor embryos carrying the T6 marker chromosomes. Decidual cells dissected free of embryos did not colonize any recipients. Disaggregated cells from early mouse embryos first colonized the liver and then repopulated the haemopoietic systems of recipients, producing adult donor haemoglobin within 2–3 days and donor GPI within 3–5 days. 80 % of grafted X-irradiated recipients survived and donor markers were found in each of them. All nongrafted controls died within 14 days of X-irradiation and none of them showed donor markers. Disaggregated embryonic cells could be grafted across major histocompatibility barriers unlike adult bone marrow. Haemopoietic stem cells could not be identified in disaggregated cells from embryos aged less than 6 days gestation.

Key words: grafting, haemopoietic, mouse, X-irradiation.

Introduction

It has long been thought that grafts of haemopoietic stem cells differentiating in the early embryo might be capable of repopulating the bone marrow of adults with a deficiency in haemopoiesis (Edwards, 1982). Moore & Metcalf (1970) showed that this could be achieved in mice using tissue from embryos aged between 7 and 15 days, but they failed to produce evidence of biochemical donor cell markers in the recipients. The tissue used in most work on grafting fetal tissue is fetal liver which carries a risk of inducing graft-*versus*-host disease (GVHD) in the recipient unless inbred animals are used. Fleischman & Mintz (1979) showed that microinjection of fetal liver *via* the placenta can reverse genetic anaemia in fetal mice. Fetal liver has also been used clinically in an attempt to treat various human diseases (Touraine, 1983).

The use of donor cells from embryos very early in growth should eliminate the problem of GVHD, since the donor thymus has not differentiated and embryonic haemopoietic cells have not gained their immune 'competence'. The present work describes the use of tissue taken from embryos soon after implantation for grafting into recipients given a lethal dose of X-irradiation.

Materials and methods

Animals and X-irradiation

Donor embryonic cells were obtained from strain CBA, CBA-T6T6, MF1 and C57B1/10 (Olac Ltd). All donor strains were typed for their electrophoretic variants of haemoglobin (Hb) and glucose phosphate isomerase (GPI). No polymorphism of GPI or Hb was found in MF1 mice. Recipient mice came from strain Balb/c and 129 (Olac Ltd) and were also typed for Hb and GPI.

X-irradiated recipients were obtained by exposing Balb/c or 129 mice to a dose of 8·5 Gy of X-irradiation, from a 250 kV Marconi Orthovoltage X-ray machine, set at 15 mA. Dose–response assays showed this dose to be lethal to all the irradiated mice. Balb/c mice carry the histocompatibility antigen $H2^d$ and were used as recipients for CBA donors, which carry $H2^k$, in studies on grafting across major histocompatibility barriers (Klein, 1975). C57B1/10 and

129 mice carry the histocompatibility antigen H2^b. MF1 mice are outbred.

Preparation of donor embryonic cells

Donor female CBA, CBA-T6T6, MF1 and C57B1/10 mice were superovulated with 2 i.u. pregnant mare serum (PMS) (Folligon, Intervet) to stimulate follicle growth and 2 i.u. human chorionic gonadotropin (HCG), (Chorulon, Intervet) to induce ovulation (Fowler & Edwards, 1957). The day of finding the vaginal plug was taken as day 0 of gestation.

Cells from day 5 embryos were obtained by removing the uterine horns from mice of day 5 gestation and carefully locating the implantation site of the embryos by using a dissecting microscope. Once located, the embryos were scraped free of uterine tissue. Cells from embryos aged day 6 or 7 of gestation were obtained by removing the uterine horns, which were placed in Hank's medium (Flow Laboratories). The embryos were freed of uterine tissue by careful dissection and disaggregated by initial aspiration into the barrel of a 1 ml syringe followed by aspiration through needles of decreasing size, starting at 19 gauge then 21 gauge, 25 gauge and finally 27 gauge, which was the needle used for intravenous injection. The tissue was passed through each needle up to a maximum of ten times; persistent pieces of tissue were removed from the needle using a sterile swab. The cell viability after disaggregation as assessed by trypan blue exclusion test was 90 %, with occasional small clumps of cells in the final preparation. All disaggregation steps were performed under a laminar flow hood with sterile techniques. Cell disaggregates were kept on ice during preparation. In most experiments maternal decidual cells were included in these disaggregates.

The disaggregates were centrifuged at 500 g for 5 min and resuspended in 0·1 ml of Hank's medium.

The cells were then ready for grafting. Three embryos from a single female donor were usually used to make each single graft inoculum and in no instance were embryos from different females mixed.

In a second experiment, day 7 embryos were dissected completely free of maternal decidual cells under the dissecting microscope. Decidual and embryonic cells were then separately disaggregated as before and prepared for grafting into X-irradiated recipients.

Adult bone marrow cells

Bone marrow cells were obtained from the mothers that produced donor embryos. These cells were used for grafting in order to compare their properties with those of embryonic cells. Bone marrow was obtained by flushing the femoral shafts with Hank's medium. The tissue was disaggregated by passage through needles, and the resulting cells were centrifuged at 500 g for 5 min and resuspended in 0·2 ml of Hank's medium prior to their intravenous injection into X-irradiated mice. Each recipient mouse received between 3·3×10^6 and 5·6×10^6 bone marrow cells.

Grafting of donor embryonic cells

All grafts were given intravenously without anaesthetic via the tail vein in a volume of 0·1–0·2 ml. The cells remaining in the barrel of the syringe were used for cell counts to estimate the number of cells injected, using standard techniques (Dacie & Lewis, 1975). The number of cells, including decidual cells, injected per mouse ranged from 1·5×10^6 to 6·5×10^6 nucleated cells. All X-irradiated mice received their graft within 3 h of irradiation. A total of 272 Balb/c recipients received grafts of embryonic cells from CBA, CBA-T6T6 or C57B1/10 mice and a further 40 129 recipients received grafts from MF1 donors.

Some grafts were carried out across a known major histocompatibility barrier (H2^k→H2^d). Six X-irradiated Balb/c mice were given intravenous injections of embryonic cells of day 6 embryos taken from six CBA mothers. A further six X-irradiated Balb/c mice were given intravenous injections of femoral bone marrow from the same six mothers. The numbers of cells injected were counted as above. This experiment was repeated using day 7 embryonic cells as the donor tissue. In each of these experiments, control animals were injected with either physiological saline or Hank's medium.

Analysis of grafted recipients

Blood sampling

Approximately 90 μl of tail vein blood were collected daily from newly grafted recipients and at weekly or longer intervals from longterm survivors into a heparinized capillary tube, and transferred immediately to 1 ml of phosphate-buffered saline. The cells were then layered onto Ficoll-Paque (Pharmacia Fine Chemicals) and centrifuged for 15 min at 1000 g to separate lymphocytes from erythrocytes. Each fraction was assessed morphologically to check for unwanted cellular contamination.

The erythrocytes and lymphocytes were washed and then lysed by the addition of 1 ml of distilled water and exposure to freeze–thaw cycles. The lysates were then centrifuged at 1500 g for 10 min and the resulting supernatants were then ready for analysis of haemoglobin (erythrocyte lysates) or GPI (erythrocyte or lymphocyte lysates). Some lysates were stored at −20 °C until required.

Electrophoresis

Starch gel electrophoresis (LKB Instruments) of haemoglobin (Hb) and glucose phosphate isomerase (GPI) was performed as described previously (Whitney, 1978; Siciliano & Shaw, 1976). 10 μl of each lysate was applied to the gel and gels were run at 25 mA for 15 h, and stained with Ponceau S to show the Hb bands. Staining of GPI was performed as previously described (Siciliano & Shaw, 1976). Photographs were taken of all gels immediately after fixation.

Once fixed and dried the electrophoresis gels were scanned on a transmission densitometer (Chromoscan, Joyce-Loebl & Co. Ltd) at 495 nm for haemoglobin gels and 585 nm for glucose phosphate isomerase, to assess the relative intensities of donor and recipient bands. The accuracy of the densitometer was assessed using known mixtures of donor and recipient Hb and GPI. The lower limit of detection of embryonic haemoglobin was also assessed using known dilutions of embryonic haemoglobin.

Table 1. *Summary of intravenous grafting of MF1, C57B1/10 or CBA embryonic or bone marrow cells into X-irradiated 129 or Balb/c mice*

Series	No. receiving embryonic cells	No. surviving for >350 days(%)	No. receiving bone marrow	No. surviving for >350 days(%)
1(MF1→ 129)	20	16(80)	10	2(20)
2(MF1→ 129)	10	8(80)	10	3(30)
3(MF1→129)	10	8(80)	10	3(30)
4(C57→ Balb)	30	25(83)	10	3(30)
5(C57→ Balb)	20	15(75)	10	2(20)
6(C57→ Balb)	10	7(70)	10	0(0)
7(C57→ Balb)	20	15(75)	10	2(20)
8(CBA→ Balb)	12	10(83)	12	2(16)
9(C57→ Balb)	20	16(80)	10	2(20)
10(C57→ Balb)	20	16(80)	10	3(30)
11(C57→ Balb)*	10	10(100)	5	5(100)
Total	172	136(80)	102	22(21)

* Series 11 is not included in any calculation or in the total. Series 1, 2 and 3 are now all dead.

Migration of CBA-T6T6 donor cells in X-irradiated Balb/c recipients

140 Balb/c recipients were autopsied at different times after grafting in order to trace the destination of the donor cells. The donor cells were taken from CBA-T6T6 embryos at day 7 of gestation. Recipients were Balb/c females given 8·5 Gy X-irradiation just prior to grafting. Chromosome preparations were made from the bone marrow, spleen and liver of mice receiving CBA-T6T6 embryonic cells following the protocol of Ford, Hamerton, Barnes & Loutit (1956). In the case of liver preparations the whole of the slide was scanned for donor mitoses; in bone marrow and spleen 20 mitoses were scored for each recipient and the result expressed as percentage donor.

Haematological assays

The survival of all grafted animals was checked by routine haematological assays on blood samples taken weekly or at longer intervals in longterm survivors. Haemoglobin levels, erythrocyte and leucocyte counts were performed using standard techniques (Dacie & Lewis, 1975).

Results

Cells from day 5 embryos grafted into X-irradiated recipients

Day 5 embryonic cells of strain MF1 were injected intravenously into a group of ten X-irradiated 129 mice, each recipient receiving between 2×10^6 and 4×10^6 nucleated cells. All of the recipients died within 12–14 days of X-irradiation. Ten nongrafted X-irradiated controls injected with physiological saline or Hank's medium died at the same time.

Cells from day 6 and day 7 embryos grafted into X-irradiated recipients

Eleven different series of studies were carried out (Table 1). In all except one series (no. 11), the saline-injected control X-irradiated mice died at approximately 12 days postirradiation, as expected. All the X-irradiated mice survived in series 11, and this series has been excluded from analyses of the data since the X-ray dose was apparently too low. The remaining series gave consistent findings. All mice in series 1, 2 and 3 are now dead, presumably from old age since those surviving radiation lived for a mean of 600 ± 30 days. All successfully grafted mice in series 4–10 are alive and well at >450 days postgraft.

The fast-migrating variant of haemoglobin (Hb single) and the GPI marker typical of the MFI and C57B1/10 donors (GPI 1B) were absent from the erythrocytes and lymphocytes of X-irradiated 129 and Balb/c recipients before grafting. Recipient 129 mice carry the markers Hb diffuse and GPI 1A. Donor haemoglobin and donor GPI were identified in 80 % of the grafted recipients given day 6 and day 7 embryonic cells (Table 1; Figs 1, 2).

Donor haemoglobin appeared in some recipients within 24 h postgraft and their own haemoglobin disappeared within 30 days postgraft. Densitometry readings on samples from mice in series 1 showed that the donor band contributed a maximum of approximately 20 % of the total haemoglobin on day 1 in the most heavily colonized recipients. The donor band steadily increased in intensity with time until day 30 postgraft when it was the only one detected on scanning (Table 2). Adult donor haemoglobin was identified electrophoretically and the three characteristic bands of mouse embryonic haemoglobin were not detected in any recipients. The lower limit of detection of embryonic haemoglobin was approximately 5 %. Donor erythrocytic GPI appeared at the same time as donor haemoglobin.

Lymphocytic GPI was first detected a little later, within 3–4 days postgraft at the earliest, the recipient
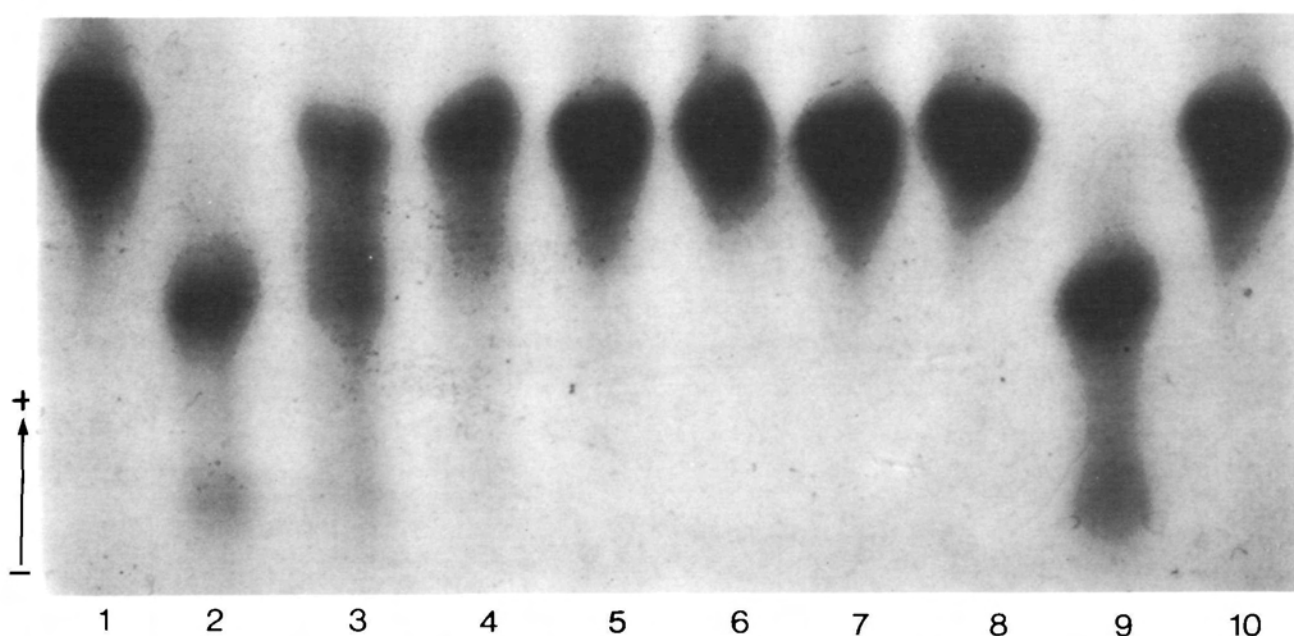
Fig. 1. Haemoglobin electrophoresis of MF1, 129 and X-irradiated 129 mice grafted with day 6 MF1 embryonic cells (series 1).

Well number	Sample	Time postgraft (days)
1 and 10	Normal donor MF1	N/A
2 and 9	Normal recipient 129	N/A
3	Grafted 129	1
4	Grafted 129	14
5	Grafted 129	30
6	Grafted 129	60
7	Grafted 129	120
8	Grafted 129	240

N/A, not applicable.
Grafts of day 7 embryonic cells gave similar results.
The time course was on a single typical recipient.

GPI persisting for up to 30 days postgraft. Densitometry readings on samples from mice in series 10 showed that donor GPI formed approximately 20% of the total at day 3 postgraft in the most heavily colonized recipients, increasing to >95% by day 30 postgraft (Table 2).

No clear correlation could be found between the rate of appearance of donor Hb and lymphocytic GPI in the recipients (Table 3).

By day 30 postgraft, donor haemoglobin and GPI were the dominant markers present in grafted mice, except series 11, all recipient markers having declined. 80% of grafted animals given day 6 or day 7 embryonic cells showed donor markers (Table 2). None of the remaining 20% of recipients that subsequently died showed donor markers.

Blood and ear clips from each of the surviving grafted recipients were assessed for donor and recipient GPI. These samples came from mice in series 4–10; mice were 300 days or older postgraft when the samples were taken. Each recipient displayed donor GPI in blood and recipient GPI in ear clips. There was no evidence of any reversion to their own GPI in the blood of any recipient.

None of the control mice given injections of saline or Hank's medium displayed donor haemoglobin or GPI. Each of them except one group (series 11) died within 12–14 days postinjection of X-irradiation damage of the haemopoietic system. Two groups of ten X-irradiated recipient mice were given lysates of homogenized day 6 and day 7 embryonic cells. None of them produced any donor markers and they all died within 12–14 days postgraft.

Decidual and embryonic cells grafted separately into X-irradiated recipients

None of the 20 mice receiving decidual cells showed any signs of colonization and died at the same time as saline-injected X-irradiated controls. Each mouse received the decidual cells from three embryos.
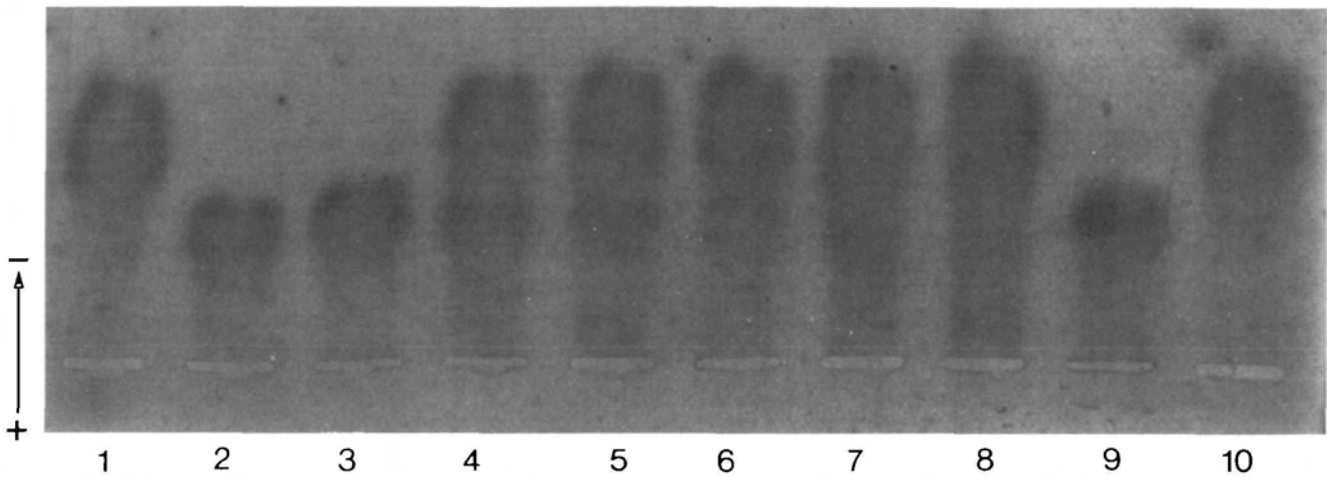
Fig. 2. Lymphocytic glucose phosphate isomerase (GPI) electrophoresis of MF1, 129 and X-irradiated 129 mice grafted with day 6 MF1 embryonic cells (series 1).

Well number	Sample	Time postgraft (days)
1 and 10	Normal donor MF1	N/A
2 and 9	Normal recipient 129	N/A
3	Grafted 129	1
4	Grafted 129	3
5	Grafted 129	15
6	Grafted 129	30
7	Grafted 129	120
8	Grafted 129	240

N/A, not applicable.
Grafts of day 7 embryonic cells gave similar results.
The time course was on a single typical recipient.

16 of the 20 mice (series 9), each receiving cells from three disaggregated embryos at day 7 of gestation, showed donor markers and survived in the same way as those animals given embryonic and decidual cells in the earlier experiment.

Bone marrow from adult donors grafted into X-irradiated recipient mice

Bone marrow from each adult MF1 or C57B1/10 embryo donor was injected intravenously into X-irradiated 129 or Balb/c recipient mice. Altogether bone marrow was taken from 102 donors and injected into X-irradiated recipients. These recipients all developed donor markers (except series 11) but approximately 80 % died within 35–40 days postgraft, evidently of graft-*versus*-host disease (Table 1). One group of ten bone marrow recipients (series 6) died at 12 days postgraft probably because of an infection in the colony at that time.

Chromosomal analysis of X-irradiated Balb/c recipients grafted with CBA-T6T6 embryonic cells

Mitoses carrying donor T6 chromosomes were found in the liver of many recipients of CBA-T6T6 embryonic cells by 2 days postgraft. At this stage, the bone marrow and spleen of recipients did not contain any marker chromosomes. By day 4 postgraft, cells carrying the donor T6 chromosomes appeared in the recipient bone marrow, alongside recipient mitoses. From day 4 onwards, mitoses with donor chromosomes proportionally increased in recipient bone marrow as the number in the liver decreased. Donor T6 chromosomes have been detected in the spleens of recipient animals by day 64 postgraft (Table 4).

Cells from day 6 and day 7 embryos traversing a major histocompatibility barrier

Two group, each of twelve female X-irradiated Balb/c mice, were used as recipients for CBA embryonic cells of day 6 and day 7 embryos, respectively. In each group, six mice were given CBA embryonic cells of the appropriate age and six were given femoral bone marrow from the same embryonic cell donors (series 8).

83 % of the mice given day 6 or day 7 embryonic cells survived the X-irradiation with no apparent ill effects. In contrast 83 % of bone marrow recipients died within 35–40 days postgraft, presumably from graft-*versus*-host disease.

Table 2. *Percentage of donor MF1 haemoglobin and glucose phosphate isomerase in X-irradiated 129 recipients grafted with day 6 or 7 embryonic cells as assessed by densitometry (series 1)*

Time postgraft (days)	Donor MF1 haemoglobin (%)					
	0	1–10	11–25	26–50	51–75	76–100
(a) *Number of recipients with donor haemoglobin*						
1	4	12	4			
2	4	11	5			
4	4	9	7			
8	4	7	7	2		
16	4	4	5	4	3	
32						16
450						16

Time postgraft (days)	Donor lymphocytic GPI (%)					
	0	1–10	11–25	26–50	51–75	76–100
(b) *Number of recipients with GPI*						
1	20					
2	20					
4	4	14	2			
8	4	12	4			
16	4	8	6	1	1	
32						16
450						16

Table 3. *Correlation between time of first appearance of donor haemoglobin and lymphocytic GPI (series 1–10)*

		Time of appearance of erythrocytic Hb (days postgraft)			
		1	2	3	4
Time of appearance of lymphocytic GPI (days postgraft)	3	14	41	12	2
	4	36	15	8	
	5	2	4	2	

Haemoglobin markers could not be used to establish the origin of the haemopoietic cells in the recipients, because both donors and recipients carry the same diffuse haemoglobin markers. The two strains carry different allozymes for glucose phosphate isomerase (Balb/c: GPI 1A, CBA: GPI 1B) which were used as electrophoretic markers. Donor lymphocytic GPI appeared in grafted animals on day 3–4 postgraft as in previous experiments.

Haematological parameters of grafted recipients

Haematological parameters in the surviving recipients were also used to assess the success of the graft. Haemoglobin levels were 13·0–15·2 g/100 ml, erythrocyte counts were $7·2–8·6 \times 10^9$ ml^{-1} and leucocyte

Table 4. *Chromosomal analysis of X-irradiated Balb/c mice grafted with CBA-T6T6 embryonic cells*

Time postgraft (days)	Number of donor mitoses/slide (%)					
	0	1–10	11–25	26–50	51–75	76–100
(a) *Number of recipients with liver colonization*						
1	16	4				
2	9	7	4			
4	2	4	10	4		
8	0	9	8	3		
16	20					
32	20					
64	20					
(b) *Number of recipients with bone marrow colonization*						
1	20					
2	20					
4	14	6				
8	3	9	8			
16	0	4	6	6	4	
32	0	2	9	5	4	
64	0	0	0	3	5	12

Donor T6 chromosomes were seen in recipient spleens on day 64 postgraft at a level of 10 %, no later assays have been done.

counts were $3·9–4·5 \times 10^6$ ml^{-1} during the postgraft period. These figures are similar to values for normal control mice.

Discussion

Embryonic cells from entire mouse embryos aged 6 or 7 days can repopulate the haemopoietic system of lethally X-irradiated recipients (Hollands, 1986). Four parameters supported this conclusion: the use of electrophoretic markers for erythrocytes and lymphocytes, survival from a lethal dose of X-rays, the detection of cells carrying donor chromosomes in grafted animals and the re-establishment of normal haematological parameters in the recipients. Haemopoiesis is actually occurring by day 7 in the mouse fetus, when erythrocytes first appear in the yolk sac blood islands (Russell & Bernstein, 1966).

Embryonic cells obtained from day 5 embryos did not colonize any recipients. A simple dilution effect seems unlikely because the total number of cells injected was similar to that used in experiments with day 6 and day 7 embryos. The failure of these grafts may be attributed to a lack of differentiation of haemopoietic progenitors at this stage in gestation.

Embryonic cells from day 6 and day 7 mouse embryos include precursor haemopoietic cells in sufficient numbers to repopulate the haemopoietic system of lethally X-irradiated recipients with remarkable efficiency. These cells arise a day earlier than previously thought (Moore & Metcalf, 1970).

Donor haemoglobin was detected in some recipients within 24 h postgraft, and was not derived from contaminating maternal haemoglobin since embryonic homogenates do not contain a significant number of maternal erythrocytes. It must have arisen from the differentiating erythroid progenitor cells responding to adult hormones, e.g. erythropoietin. Donor glucose phosphate isomerase (GPI) was detected in the lymphocytes of some grafted recipients within 3–4 days postgraft. Lysates of day 6 or day 7 embryonic cells did not repopulate X-irradiated animals, excluding the possibility of chemical factors within the graft stimulating endogenous stem cells in the recipient.

Donor haemoglobin appeared in successful recipients up to 48 h before lymphocytic GPI. This result possibly reflects the relatively rapid production of erythrocytes compared to that of lymphocytes by the embryonic stem cells. It is possible that the maturational stages of erythrocyte precursors are shorter than those of lymphocytes in this situation. The rate of production of donor haemoglobin and donor lymphocytic GPI in the same recipient occurred independently, suggesting that variable numbers of each progenitor were present in each embryonic cell disaggregate, or there are different differentiation times for the erythroid and lymphoid cell lines.

Recipient haemoglobin and GPI persisted for up to 30 days postgraft in the X-irradiated recipients, because of peripheral blood cells resistant to X-irradiation, sustaining lethally X-irradiated mice without any treatment for 12–14 days. In grafted recipients the residual peripheral cells die by 30 days postgraft, as the embryonic cells become fully active and presumably colonize the marrow spaces.

Decidual cells of the embryo evidently play no part in colonization, for recipients given only decidual cells died at the same time as saline-injected controls. Recipients given disaggregated cells from isolated embryos survived normally, as cells with donor chromosomes colonized their liver and bone marrow. Cells from day 3 blastocysts grown *in vitro* for 72 h also colonize recipients (P. Hollands, unpublished data), showing that decidual cells are unnecessary for colonization.

The rapid appearance of donor haemoglobin when compared to grafts of fetal liver or bone marrow was surprising, and it could be due to the brief cell cycles of 2·5 h postulated to occur in mouse embryos (Snow, 1977). Densitometer scans of the gels revealed that approximately 20 % of the haemoglobin in the most rapidly colonized recipients came from the donor at 24 h postgraft.

Each recipient received three embryos, which is the equivalent of 45 000 embryonic cells (Snow, 1977). If it is assumed that 20 % of these cells are haemopoietic and that there were no cells in advanced differen-

tiation, then approximately 10^4 haemopoietic cells were grafted. A normal mouse has a total of approximately 7×10^9 erythrocytes. The donor haemoglobin at 24 h therefore represents 20 % of 7×10^9 cells, i.e. $1·4 \times 10^9$. Assuming that a donated embryonic cell contains the same amount of haemoglobin as an adult erythrocyte the donor cells must have a remarkable cell cycle of 1·5 h, which must exclude this explanation of simple colonization.

Embryonic erythropoietic progenitor cells committed to the production of adult haemoglobin are present at day 8 of gestation (Wong, Chung, Reicheld & Chui, 1986; Wong, Chung, Chui & Eaves, 1986). These progenitors could be contributing significantly to the observed donor adult haemoglobin at 24 h postgraft. Some of the donor haemoglobin might have been produced by cells already advanced in differentiation when they were grafted into the recipients.

The method of electrophoresis used in the present work gives estimates of donor glucose phosphate isomerase that are slightly higher than those obtained by another method of electrophoresis (W. Reik, personal communication), although both methods identified the same chimaeras. The exact degree of colonization is currently being assessed by measuring erythrocytic GPI in samples obtained at various intervals between 12 and 96 h after grafting and by comparing results from different methods of electrophoresis.

Embryonic cells with chromosome markers colonized grafted recipients very rapidly. They appeared in liver within 24 h and in bone marrow two days later. Haemopoietic stem cells in the yolk sac of the developing embryo normally migrate from the yolk sac to the fetal liver and then to the bone marrow around the time of birth. The grafted embryonic cells appear to follow the same route and environments in the adult recipients. Nevertheless, it is possible that the cells found in the liver and the bone marrow are totally separate cell populations and not a result of migration.

The environment within the haemopoietic systems of X-irradiated recipients appeared to stimulate the donor haemopoietic stem cells to synthesize adult instead of embryonic haemoglobin within 24 h postgraft. This rapid transition from embryonic to adult haemoglobin might involve a switch by cells already producing haemoglobin or the stimulation of a different set of donor stem cells (Wood, 1982; Wong *et al.* 1986). The liver was the first site to be colonized in grafted recipients and this organ might stimulate all embryonic stem cells to produce adult haemoglobin.

The haemopoietic stem cells in embryonic cell homogenates from day 6 and 7 mouse embryos also repopulated the haemopoietic systems of lethally

X-irradiated recipients across a major histocompatibility barrier for periods much longer than 30 days. This is in contrast to adult bone marrow which does not survive in incompatible recipients unless mature adult lymphocytes are removed using monoclonal antibodies (Korngold & Sprent, 1978; Slavin *et al.* 1985).

Current work has shown that the lowest number of cells sustaining a successful graft was $8 \cdot 0 \times 10^5$ nucleated cells or, perhaps more significantly, the cells from at least one complete embryo (Hollands, 1986). Grafts using less than one embryo were unsuccessful in X-irradiated recipients. The number of precursor cells might not have been sufficient or some necessary interactions might not occur when low numbers of cells are grafted. Work is in progress to estimate the number of haemopoietic stem cells in the embryonic homogenates using the methods of Dexter, Allen & Lajtha (1977) and to purify them using density gradient centrifugation.

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