DEVELOPMENT OF ADULT BONE MARROW STEM CELLS IN *H-2*-COMPATIBLE AND -INCOMPATIBLE MOUSE FETUSES

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Hematopoietic stem cells undergo regulated proliferation and differentiation in a series of tissue environments, chiefly, the liver prenatally and the bone marrow postnatally. The relationships between the stem cell and each of these environments appear to be specific and critical but are poorly understood. We have described (1) a means of characterizing the biological potential of mouse hematopoietic stem cells during development through this series of environments in vivo by introducing the cells via the placental circulation into early fetuses near the inception of liver hematopoiesis. The use of *W*-locus mutant recipients, with a macrocytic anemia and reduced stem cell proliferation (2), confers a selective advantage on normal donor cells without irradiation of the recipient. The host's immunological immaturity (3) circumvents rejection problems.

Fully allogeneic cells taken from livers of fetuses close in age to the recipients are able to seed the host's liver and progress to the bone marrow. In the best cases, the donor stem cells undergo lifelong self-renewal and coordinate differentiation into myeloid as well as lymphoid lineages. Thus, fetal liver contains a population defined as developmentally totipotent hematopoietic stem cells (THSC)[1] (4).

The present study was undertaken to learn whether cells that are the counterpart of fetal liver THSC are still present in adult bone marrow. The results of transplacental inoculation of genetically marked marrow cells into early *W*-mutant fetuses show that, despite maturation in the bone marrow, THSC capable of seeding the fetal liver and undergoing sustained self-renewal and differentiation into myeloid and lymphoid cells are indeed present. However, their self-renewal capacity is reduced and, along with other irreversible changes, histocompatibility restriction has become a partially limiting factor, especially at the *H-2* locus.

Materials and Methods

Mice. The recipient fetuses in all transplacental injections were from matings involving *W*-locus mutant alleles. The macrocytic anemia of these genotypes is most severe (and

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[1] *Abbreviations used in this paper:* THSC, totipotent hematopoietic stem cells.

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lethal shortly after birth) in *W/W* and is progressively less so, and compatible with viability, in W/W^v, W^v/W^v, W/W^f, W^f/W^f, and $W^v/+$. *W/+* is nonanemic (5, 6). In all segregating matings, coat color differences also due to *W* alleles enabled the genotypes to be recognized. W^f/W^f mice, unlike *W/W* and W^v/W^v, are fertile. Injected W/W^v fetuses were from matings between *W/+* and $W^v/+$ heterozygotes congenic with the C57BL/6 strain. In a few experiments, *W/+*-C57BL/6 animals used in matings had the Hbb^d (*diffuse*) hemoglobin electrophoretic variant substituted for the Hbb^s (*single*) allele characteristic of the congenic C57BL/6 strain. W^f/W^f homozygotes were coisogenic with the C3H/He inbred strain as a result of a mutation in that strain and were originally obtained from the Institut Pasteur, Paris, France (6). Injected W/W^f fetuses were from matings of W^f/W^f-C3H homozygotes with *W/+* heterozygotes of either the C57BL/6 or WH strains. *W/W* fetuses used for histological examination were from matings between *W/+*-C57BL/6 females and *W/+*-WH males.

Adult mice used as bone marrow donors were all of nonanemic genotypes and were from Icr sublines of inbred strains C3H, DBA/2, C57BL/6, and C3H.SW ($H\text{-}2^b/H\text{-}2^b$, congenic with C3H), or their F_1 hybrids. Some $H\text{-}2^k/H\text{-}2^k$ mice congenic with C57BL/6 were also used; these were derived from animals kindly sent by Edward A. Boyse of the Sloan-Kettering Institute, New York.

Microinjection of Cells from Adult Bone Marrow. The microinjection procedure was the same as originally described by Fleischman and Mintz (1). Instead of fetal liver donor cells, suspensions of bone marrow cells from mice 2–4 mo old were prepared, and 5×10^5 cells were injected into the placental circulation of fetal recipients on day 11 of gestation, as described by Blanchet et al. (7).

Blood Analyses. Blood was collected from the incised tail tip or the retroorbital sinus, and erythrocyte lysates were analyzed for the relative proportions of strain-specific hemoglobin variants due to alleles at the *Hbb* locus (8). The transparentized gels were densitometrically scanned as previously described (4). Strain-specific variants of immunoglobulin allotypes, indicative of the B lymphocyte strains of origin, were analyzed by Ouchterlony double-diffusion immunoprecipitation in agar, using allotype-specific antisera.

Histology. Liver tissue was fixed and stained as previously described (4).

Results

Development of Adult Cells in Fetal Livers

At ~11 d of gestation, the liver of a normal mouse fetus begins to increase rapidly in size and to acquire a uniform dark red color due to the onset of hematopoiesis and hemoglobin production (9). By comparison, livers of *W/W* fetuses, characterized by a severe hypoplastic anemia and a defect in the hematopoietic stem cells (2), are reduced in size and pale in color throughout fetal life (10).

To learn whether normal adult bone marrow, like normal fetal liver (4), contains hematopoietic stem cells able to competitively seed the fetal liver microenvironment, four *W/W* experimental fetuses, identified by their relative pallor and residual anemia, were autopsied on day 18 of gestation, 1 wk after the injection of bone marrow cells via the placenta. Two proved to be hematopoietic mosaics, as seen by the presence of 25 and 40% donor-type hemoglobin, respectively, in the peripheral blood (data not shown). The two unsuccessfully treated nonmosaics, which were seen in sections to be uniformly hypocellular (Fig. 1*A* and *C*), were indistinguishable from untreated *W/W* fetuses and, along with the latter, served as controls. The livers of both mosaic animals had several discrete, nonraised, dark red areas easily visible on the background of otherwise pale liver tissue (Fig. 1*B*). Such regions were distinct individual clusters of rapidly

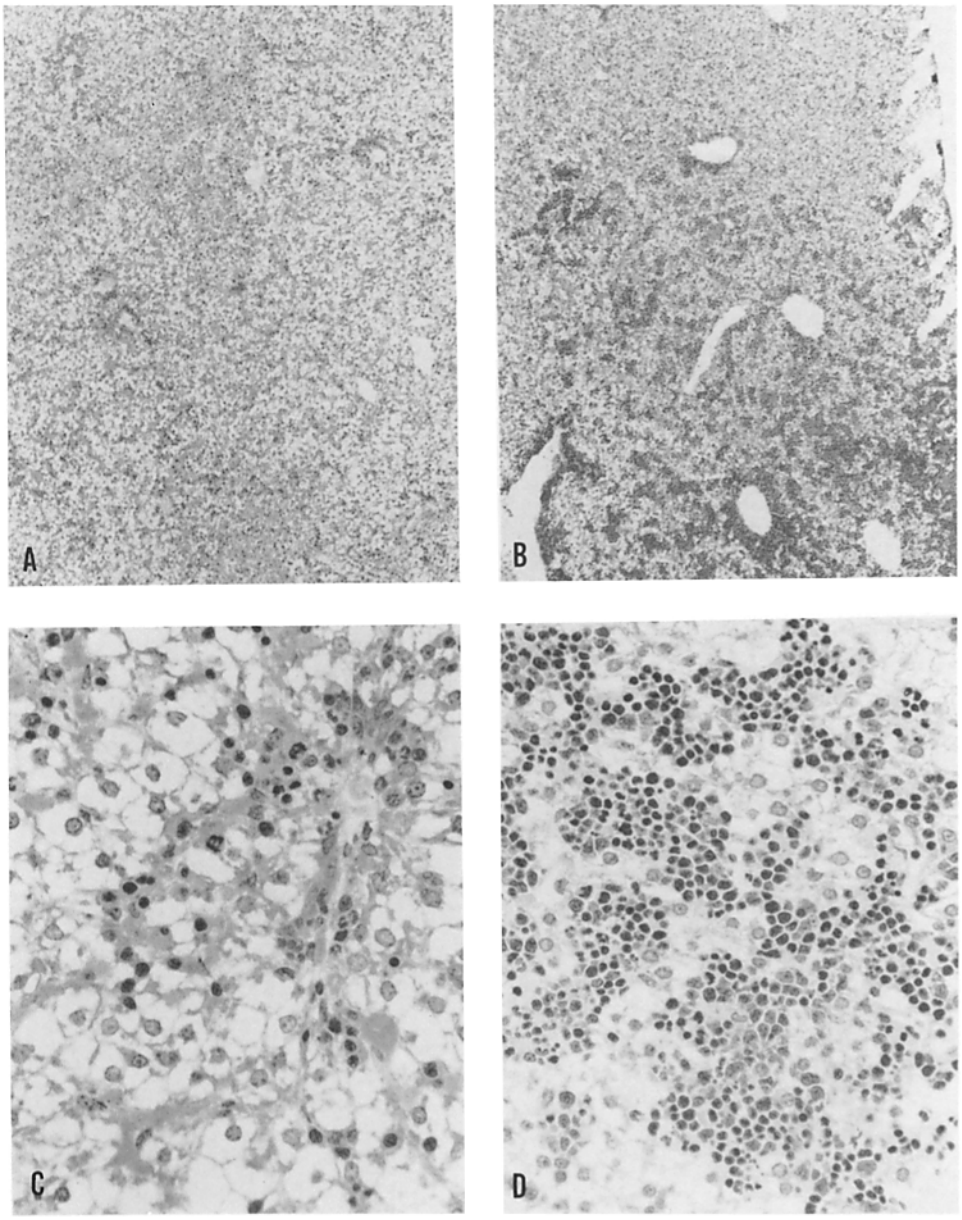

FIGURE 1. Histological sections of fetal livers at 18 d of gestation. (*A*) *W/W*-(C57BL/6 × WH) mutant anemic control. (*B*) *W/W*-(C57BL/6 × WH) mutant fetus with 40% donor-strain hemoglobin in the peripheral blood, after receiving normal (+/+-C57BL/6) adult bone marrow cells on day 11 of gestation. (*C*) Higher magnification of (*A*) showing the uniform hypocellularity and the rarity of hematopoietic cells. (*D*) Higher magnification of (*B*) showing the focal region of hypercellularity evident in (*B*), with numerous hematopoietic cells. Stained with hematoxylin-eosin (63× in *A*, *B*; 400× in *C*, *D*).

proliferating hematopoietic cells clearly derived from the donor source (Fig. 1*D*). Each of these hypercellular foci contained predominantly erythroid cells, with smaller numbers of identifiable granulocytic and megakaryocytic cells.

Survival to Birth

The largest experimental group consisted of fetuses from $W/+ \times W^v/+$ matings, in which the W/W^v segregants, with a relatively severe genetic defect, offer the greatest possibility of competitive engraftment by normal donor stem cells (4). All were on the C57BL/6 ($H\text{-}2^b/H\text{-}2^b$) strain background. To learn whether histocompatibility and strain differences affected the outcome, we used donor bone marrow cells from a variety of inbred strains (Table I). Three groups of donors shared at least a haplotype at the *H-2* locus with the recipients; these were C57BL/6, (C3H × C57BL/6)F_1, and C3H.SW ($H\text{-}2^b/H\text{-}2^b$). In three other groups, the donors were *H-2*-incompatible with the recipients; these were congenic $H\text{-}2^k/H\text{-}2^k$-C57BL/6, DBA/2 ($H\text{-}2^d/H\text{-}2^d$), and C3H ($H\text{-}2^k/H\text{-}2^k$).

Of a total of 1,458 injected fetuses in these groups, there were 1,064 live births, or an overall survival rate of 73%. Losses of entire litters accounted for a major proportion of the missing animals. Incompatibility for *H-2* alleles clearly did not affect survival to birth (Table I). There was slightly superior survival with C57BL/6 donors, whether *H-2*-compatible or -incompatible.

TABLE I

Survival of W/W^v-C57BL/6 Fetuses to Birth after Injection with H-2-Compatible vs. -Incompatible Cells from Adult Bone Marrow of Various Strains

Donor genotype	Number injected	Number born (%)	W/W^v neonates	
			Number	Percent of expected*
H-2 compatible				
C57BL/6‡	151	129 (85)	17	46
(C3H × C57BL/6)F_1	86	57 (66)	11	73
C3H.SW	284	191 (67)	19	33
Totals	521	377 (72)	47	43
H-2 incompatible				
$H\text{-}2^k/H\text{-}2^k$-C57BL/6‡	230	187 (81)	23	42
DBA/2	262	185 (71)	32	63
C3H	445	315 (71)	34	36
Totals	937	687 (73)	89	42

* In matings of $W/+ \times W^v/+$, each segregating genotype would be expected to constitute 25% of the animals born, assuming equal survival in utero. Because the number of W/W^v neonates actually seen was significantly below 25%, for purposes of this calculation the number of W/W^v births expected theoretically was derived by dividing the total of all other segregants (+/+, $W/+$, and $W^v/+$) born by three, thus representing an equal (25%) share of all births.

‡ In these experiments, the C57BL/6 donors and the W/W^v recipients from $H\text{-}2^k/H\text{-}2^k$-C57BL/6 ($Hbb^s/Hbb^s$) donors were Hbb^d/Hbb^d or Hbb^d/Hbb^s, thereby permitting donor erythroid cells to be distinguished from those of the hosts by means of their hemoglobin differences; all other donors were Hbb^d/Hbb^d or Hbb^d/Hbb^s and all other recipients were Hbb^s/Hbb^s.

More than half of the expected W/W^v segregants appeared to have died in utero, or else were destroyed by mothers soon after birth. The known poor viability of W/W^v on the C57BL/6 strain background (11), in contrast to an F_1 hybrid background, undoubtedly accounts for the low survival of W/W^v. Nearly identical rates of survival were observed in comparisons between congenic donors with *H-2* differences but in which one member had the same *H-2* type as the recipient, i.e., C57BL/6 and $H\text{-}2^k/H\text{-}2^k$-C57BL/6 or C3H.SW and C3H (Table I). Thus, graft-vs.-host disease directed against *H-2* antigens of the host is apparently ruled out as a cause of prenatal losses in allogeneic combinations.

Self-renewal of Donor Stem Cells in Severely Defective Recipients

In the first week after birth, 71 W/W^v-C57BL/6 recipients among the 136 animals born were identified, by means of the hemoglobin electrophoretic marker in peripheral blood, as having erythrocytes of the donor strain. The calculated frequency with which each of the donor genotypes contributed any cells was strikingly similar (approximately half for each genotype) (Table II). Of the 71 mosaic W/W^v neonates, 19 did not survive to weaning and are therefore designated in the table as "unclassified" with respect to the kinetics of postnatal replacement of host by donor cells. The 52 survivors were analyzed at regular intervals with the hemoglobin marker previously found to reflect not only erythrocyte replacement but also, over time, the relative success of engraftment by self-renewing stem cells (4). As in our observations when the donor cells were

TABLE II

Frequency of Successful Injection of Bone Marrow Cells and of Stem Cell Engraftment in Relation to Histocompatibility and Strain Differences Between Donors and C57BL/6 Hosts*

Donor genotype	Percent W/W^v neonates positive for donor cells‡	Number unclassified	Number with transient replacement	Stem cell engraftment	
				Number	Percent success
H-2 compatible					
C57BL/6§	59	2	2	6	60
(C3H × C57BL/6)F_1	45	0	1	4	80
C3H.SW	58	4	1	6	55
Totals	54	6	4	16	62
H-2 incompatible					
$H\text{-}2^k/H\text{-}2^k$-C57BL/6§	52	2	4	6	50
DBA/2	41	4	6	3	23
C3H	59	7	11	2	10
Totals	51	13	21	11	27

* Successful engraftment was ascertained from the persistence of donor strain hemoglobin variants in repeated postnatal analyses.

‡ Calculated by dividing the number of W/W^v animals positive for donor cells (the sum of the unclassified, transient, and engraftment groups in this table) by the actual total number (including negatives) of W/W^v animals born and tested for presence of the donor-specific hemoglobin variant (Table I, third column of numbers). Animals that did not survive to weaning, and therefore yielded insufficient data on postnatal replacement by donor cells, are designated as unclassified.

§ See footnote ‡ in Table I.

from fetal livers, there were two biological classes of results: Some animals showed only a brief proliferation of donor strain erythroid cells, of <12 wk duration; these were described as having "transient replacement". In others, proliferation was sustained for a much longer period; these were classified as having either "complete replacement," or as "limited replacement" if donor strain cells were substantially represented but limited in duration or extent. True engraftment by stem cells has been shown (4) to be attributable only to those cases in the "complete" or "limited" group. The frequency of these outcomes differed in relation to some histocompatibility and strain differences between bone marrow donors and hosts, as will now be presented in detail.

Syngeneic and semisyngeneic combinations. With syngeneic (C57BL/6-*Hbb*^{d}/*Hbb*^{d} or -*Hbb*^{d}/*Hbb*^{s}) or F_1 semisyngeneic donors (C3H × C57BL/6), 10 of the 15 *W*/*W*^{v}-C57BL/6 recipients (67% combined) that were initially positive for donor strain erythroid cells achieved and maintained complete replacement in the erythroid lineage (Table II; Fig. 2*A* and *B*) and were therefore successfully engrafted with ongoing stem cells. Hematocrit measurements confirmed the associated correction of the recipients' genetic anemia (data not shown). Similar results were obtained in two recipients with sustained replacement by semisyngeneic bone marrow cells from (C57BL/6 × DBA/2)F_1 hybrid donors (data not shown).

Nearly half of these cases died of unknown causes between 8 and 12 mo of age, despite histocompatibility. This surprising result cannot be attributed to loss of tolerance or to graft-vs.-host disease, especially in the purely syngeneic cases (Fig. 2*A*). Recurrent anemia was seen in one case (Fig. 2*A*, open circles) after the dramatic decline and disappearance of donor-derived erythrocytes and may have contributed to the deaths of other animals in which accurate hematologic data could not be obtained postmortem.

Allogeneic H-2-compatible combinations. With *H-2*-compatible but otherwise allogeneic C3H.SW donors, a high frequency (55%) of stem cell engraftment in *W*/*W*^{v}-C57BL/6 hosts was also obtained (Table II, Fig. 2*C*). Except for one early death at 10 wk of age, those hosts achieving complete replacement have thus far survived for >1 yr. One case of limited replacement was also observed to have a recurrence of mild anemia before death at 50 wk of age (Fig. 2*C*, open inverted triangles). Additional cases of successful stem cell engraftment were found at a high frequency in F_1 *W*/*W*^{v} and *W*/*W* (WH × C57BL/6) hybrid recipients (*H-2*^{d}/*H-2*^{b}) after injection of bone marrow cells from allogeneic but *H-2*-haplotype-compatible DBA/2 donors (*H-2*^{d}/*H-2*^{d}) (data not shown).

Congenic H-2-incompatible combinations. With *H-2*^{k}/*H-2*^{k}-C57BL/6 donors in *W*/*W*^{v}-C57BL/6 (*H-2*^{b}/*H-2*^{b}) recipients with at least one *Hbb*^{d} allele substituted for *Hbb*^{s} at the hemoglobin marker locus, the results for the first 12 wk appeared similar to those in the *H-2*-compatible combinations (Table II, Fig. 3*A*): 5 of the 11 animals were characterized by complete replacement and one additional case was tentatively considered to represent stem cell engraftment although it died shortly after weaning (see Fig. 5, open inverted triangles). Between 3 and 5 mo of age, however, all five of the remaining animals died abruptly. All had appeared healthy just before their deaths and did not exhibit the runting, weight loss, hunched posture, or skin lesions seen in mice with graft-vs.-host disease. None

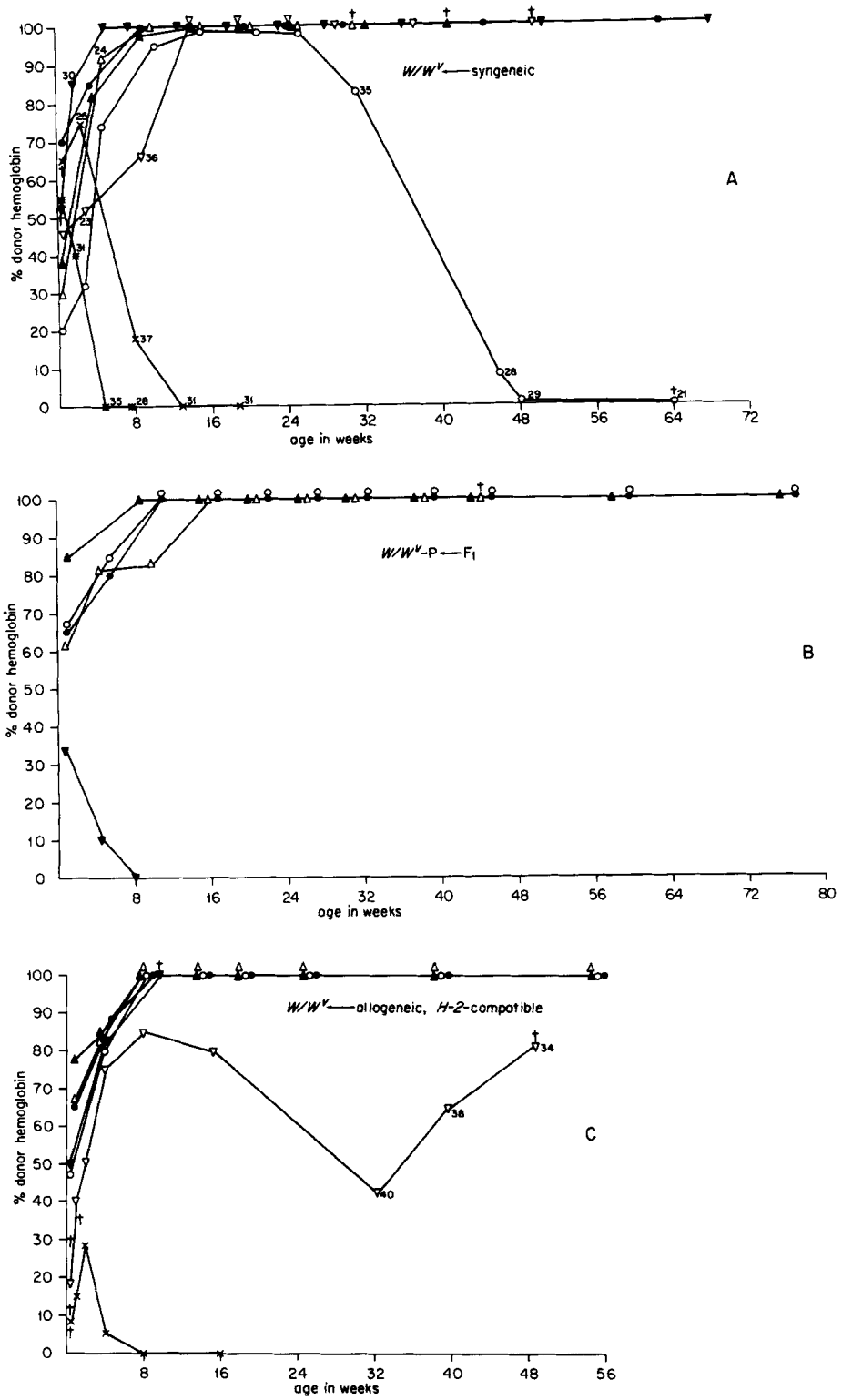

FIGURE 2. In vivo postnatal kinetics of erythroid replacement in severely anemic W/W^v-C57BL/6 hosts by *H-2*-compatible donor cells from adult bone marrow. (*A*) Syngeneic donors (C57BL/6) with Hbb^d substituted for one or both alleles at the hemoglobin marker locus. (*B*) Semisyngeneic F_1 donors (C3H × C57BL/6) into hosts of the C57BL/6 parental (P) strain. (*C*) Allogeneic, *H-2*-compatible donors (C3H.SW). Erythroid replacement was determined from electrophoresis of strain-type hemoglobin variants in erythrocyte lysates at progressive host ages. Numbers shown are those hematocrit measurements that fell below the normal range for nonanemic mice of these strains. The † (dagger) indicates the age at death of experimental animals.

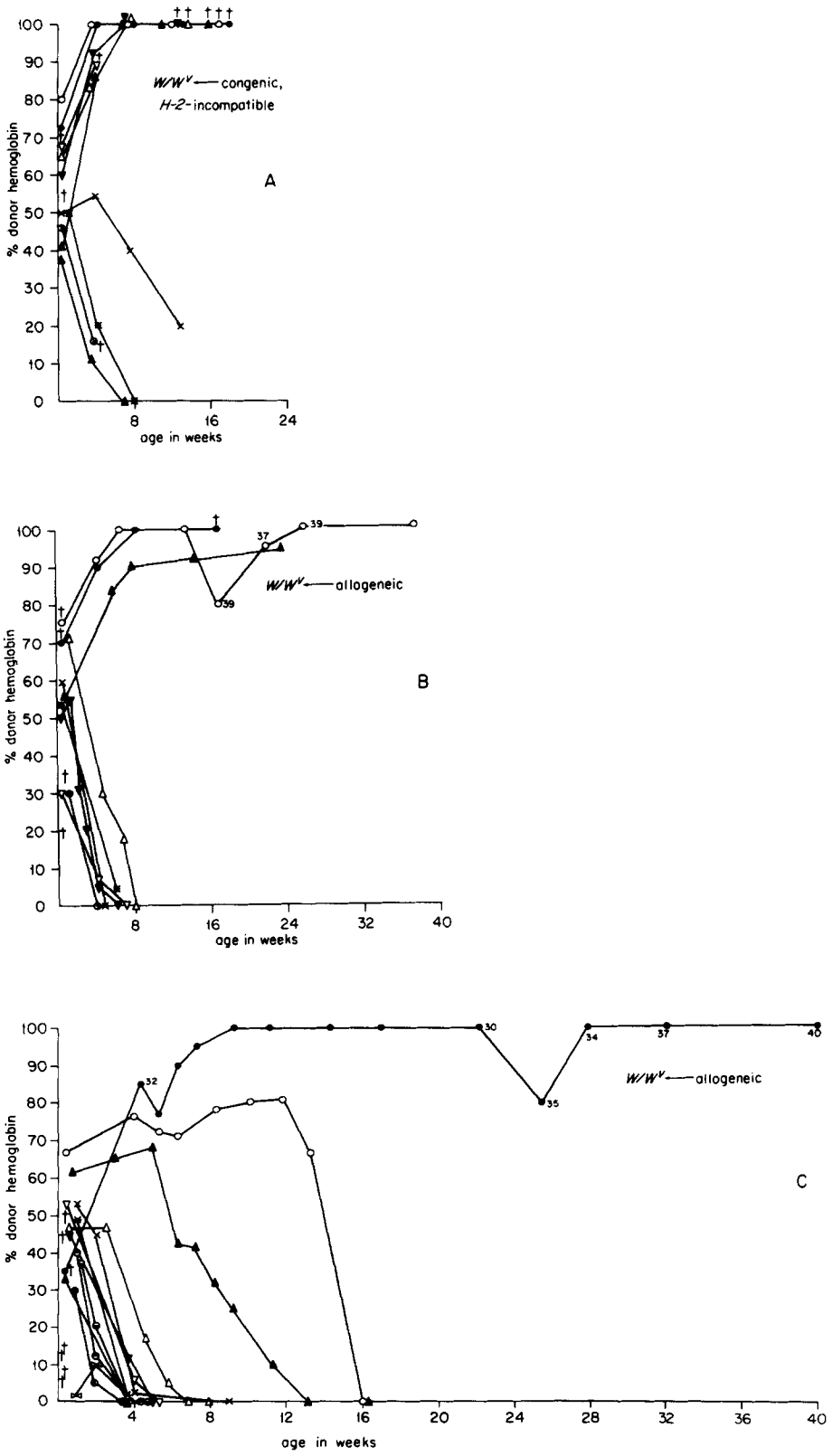

FIGURE 3. In vivo postnatal kinetics of erythroid replacement in severely anemic W/W^v-C57BL/6 hosts by *H-2*-incompatible donor cells from adult bone marrow. (*A*) Congenic donors ($H\text{-}2^k/H\text{-}2^k$-C57BL/6). (*B*) Allogeneic donors (DBA/2). (*C*) Allogeneic donors (C3H). The hosts used in *A* had an Hbb^d substitution at the diagnostic hemoglobin locus. Note the expanded time scale in (*C*). Numbers indicate abnormally low hematocrit readings.

were anemic. Postmortem changes prevented meaningful histological examination of the tissues in these cases.

Allogeneic H-2-incompatible combinations. With DBA/2 (Fig. 3*B*) or C3H (Fig. 3*C*) donors, engraftment of stem cells proved to be more difficult to achieve in W/W^v-C57BL/6 hosts. Nevertheless, at least one example of complete or limited replacement was obtained in each group. By far the most frequent result was either transient replacement for only 8–12 wk, or death of the recipient before weaning (unclassified). The very low initial levels of donor strain erythrocytes in many of these unclassified cases strongly suggests that stem cell engraftment was unlikely to have occurred, even if the animals had survived for a longer period.

A comparison of the congenic donor strains C3H.SW (Fig. 2*C*) and C3H (Fig. 3*C*) is particularly instructive. In both cases, the W/W^v-C57BL/6 recipients had nearly identical rates of survival in utero (Table I), incidence of donor strain erythroid cells neonatally, and survival to weaning (Table II). Yet the frequency of stem cell engraftment was dramatically different, in favor of the *H-2*-compatible C3H.SW donors. However, the $H\text{-}2^k/H\text{-}2^k$ constitution of C3H cannot alone explain this discrepancy, in view of the high frequency of stem cell engraftment from $H\text{-}2^k/H\text{-}2^k$-C57BL/6 donors in C57BL/6 recipients (Table II, Fig. 3*A*).

Comparison of Erythrocyte and B Lymphocyte Replacement

Serum samples from 15 W/W^v-C57BL/6 experimental mice were analyzed for donor-specific immunoglobulin allotype as a marker of B lymphocytes (Table III). The five cases with only transient erythroid substitution in the first 12 wk of life had no detectable donor strain immunoglobulin. Although four of these were tested a few wk after loss of donor-derived erythrocytes, the lymphoid cells, with a relatively longer half-life (12), would be expected to disappear more slowly. In contrast to the transient cases, all 10 with limited or complete erythroid replacement were positive for donor strain allotype.

In its limited erythroid replacement, for approximately 14 wk, the animal in Fig. 3*C* (open circles) was only marginally superior to transient cases such as the one (closed triangles) in the same figure. Nevertheless, the classification apparently represents a valid distinction signifying THSC engraftment in the former

TABLE III

Presence of Donor Strain Immunoglobulin Allotype in Relation to the Kinetics of Erythroid Replacement

Donor genotype*	Age (months)	Kinetics of erythroid replacement	
		Transient‡	Limited or complete‡
(C3H × C57BL/6)F_1	9–10	0/1	4/4
C3H.SW	6		4/4
C3H	3	0/4	2/2
Totals		0/5	10/10

* Donor strain allotype was distinguishable in all cases from the allotype of the W/W^v-C57BL/6 recipients by Ouchterlony double-diffusion tests.

‡ Expressed as the number of animals positive for immunoglobulin of the donor allotype over the number of animals tested.

and not in the latter. This is seen from the presence of donor strain allotype at 3 mo of age in the former; at 6 and 9 mo, there is a progressive decline and extinction of donor allotype (Fig. 4*A* and *B*). On the other hand, the serum of an animal characterized by sustained complete replacement with donor-derived erythroid cells (Fig. 3*C*, closed circles) was positive for immunoglobulin of the donor allotype at all ages tested up to 9 mo (Fig. 4*C*).

Self-Renewal of Donor Stem Cells in Mildly Defective Recipients

Unlike all the cases involving severely defective W/W^v recipients, discussed above, transplacental inoculation of normal adult bone marrow cells into fetuses of the mildly defective W^f/W^f genotype yielded no examples of successful engraftment in a series of 183 injected. The recipients (all of the C3H strain) received marrow cells from *H-2*-compatible (C57BL/6 × C3H)F_1 or *H-2*-incompatible (C57BL/6) donors. These results contrast sharply with those of our earlier study (4) in which fetal liver cell inoculations into 111 W^f/W^f fetuses yielded 6% with stem cell engraftment and at least 4% with transient donor-type erythroid cells.

Fetuses of a slightly more defective genotype, W/W^f, from matings of ($W/+$-C57BL/6 × W^f/W^f-C3H), proved to be more favorable hosts than W^f/W^f for competitive seeding by bone marrow cells from normal adult donors. Injection of 70 fetuses with cells from *H-2*-compatible $H\text{-}2^k/H\text{-}2^k$-C57BL/6 donors resulted in 57 live births of which 25 were W/W^f and 11 of these were initially positive for donor lineage erythrocytes (Fig. 5). Although four of the cases proved to be transient, six have thus far shown continued proliferation of donor stem cells for >9 mo. In an additional case (Fig. 5, closed squares), limited donor cell renewal occurred for several months. Further instances of stem cell engraftment were obtained with marrow from the *H-2*-compatible C3H or C57BL/6 strains (three cases, along with four transient ones) (data not shown). Sustained engraftment was also observed with an allogeneic *H-2*-incompatible combination (one case) with C57BL/6 donor cells and a W/W^f ($H\text{-}2^d/H\text{-}2^k$) recipient derived from ($W/+$-WH × W^f/W^f-C3H) (data not shown).

Discussion

The present results, in relation to our previous tests of fetal liver grafts into early fetuses (4), demonstrate that adult bone marrow, although many cell

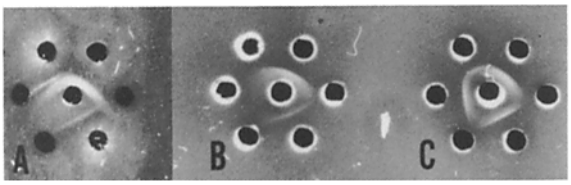

FIGURE 4. Serial tests for donor allotype immunoglobulin in two mice with prenatally engrafted bone marrow stem cells. Immunoprecipitation by antiserum (center wells) against the donor strain allotype (controls in upper right wells) shows in *A* the decreasing presence of the donor type in serum of a case of limited erythroid replacement (Fig. 3*C*, open circles) from 3 mo (upper left well) to 6 mo (lower right well) of age, and its loss in *B* from 6 mo (lower right) to 9 mo (leftmost well). In *C*, the serum of a case with complete erythroid replacement (Fig. 3*C*, closed circles) shows continued presence of donor allotype at 3 mo (lower right well), 6 mo (leftmost well) and 9 mo (upper left well) of age.

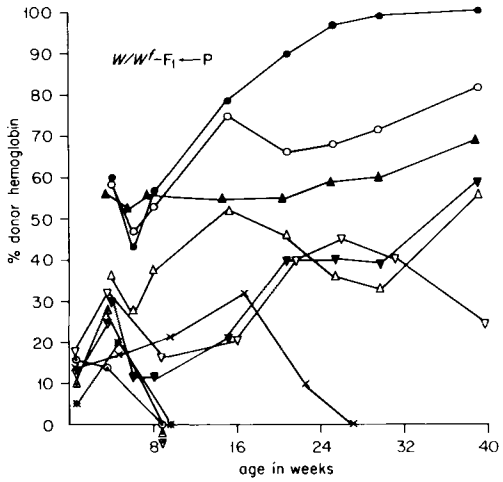

FIGURE 5. In vivo postnatal kinetics of erythroid replacement in mildly anemic W/W^f hosts. Donors of the parental (P) $H\text{-}2^k/H\text{-}2^k$-C57BL/6 strain were *H-2* compatible with the (C57BL/6 × C3H)F_1 background of the hosts.

generations removed from the precursor cells in the fetal liver, contains cells that are still able to proliferate and develop in the fetal liver environment (Fig. 1) and subsequently to colonize the bone marrow. The cells have also conserved the essential properties of totipotent hematopoietic cells, i.e., the capacity for long-term self-renewal and for coordinate differentiation into myeloid and lymphoid lineages (Figs. 2–4, Table III), similar to the THSC taken directly from fetal liver.

However, significant and irreversible maturational changes have apparently occurred during ontogeny, specifically among those bone marrow THSC still able to seed the fetal liver. One change is a decreased potential for self-renewal. This is evident from a comparison of the results with bone marrow and fetal liver inoculations into severely defective (W/W^v) vs. mildly defective (W^f/W^f) fetuses. With bone marrow, successful stem cell engraftment occurred at a high frequency in W/W^v (Table II) but failed to occur in W^f/W^f, whereas fetal liver stem cells yielded successful engraftment in these and all defective genotypes tested, albeit at a lower frequency in the less defective ones, such as W^f/W^f (4). Thus, bone marrow cells in the fetal environment were less successful in sustaining themselves than were fetal liver cells when the advantage to the donor cells was marginal. The more limited lifespan of some animals with complete replacement of their hematopoietic cells by bone marrow donor cells, even in purely syngeneic or in semisyngeneic combinations (Fig. 2*A* and *B*), could also be accounted for by exhaustion of donor stem cells after extensive proliferation in vivo, especially since seeding is likely to have occurred by only one or a few donor cells (4, and unpublished data). A decline in self-renewal of adult hematopoietic stem cells is likewise indicated by other kinds of experiments (13, 9). From the standpoint of self-renewal potential, fetal liver grafts might therefore be expected to be superior to bone marrow grafts in human clinical cases.

There is evidence that changes in expression of other genes, specifically

involved in differentiation, may become manifest during the stem cell phase, rather than in a single step from stem cell to differentiated progeny cell. Thus different kinds of stem cells would be generated that nevertheless share the basic features of THSC. As an example, we have previously noted (7) that the stem cells of adult bone marrow, after transplacental inoculation into W/W^v fetuses, generate erythrocytes which lack a fetal stage-specific erythrocyte antigen. Therefore, adult marrow THSC have an adult differentiation program that they continue to express even when maturation occurs in a fetal environment. In the present data, the few cases of stem cell engraftment by fully allogeneic DBA/2 or C3H bone marrow donors in W/W^v-C57BL/6 recipients (Table II, Fig. 3) may also partly signify seeding by a rare class of "adult" THSC of relatively less mature status with respect to its histocompatibility gene expression.

Histocompatibility relations between cells of adult donors and fetal hosts were explored in various combinations and yielded some surprising results. Successful engraftment occurred (in severely defective W/W^v-C57BL/6 recipients) at a high frequency with syngeneic and semisyngeneic combinations (Table II). Yet, as already noted, nearly half of these cases died at 8–12 mo. The importance of *H-2* matching was seen in the equally frequent success of engraftment with *H-2* compatible but otherwise allogeneic (C3H.SW) marrow cells. The somewhat better survival among these animals, in comparison with syngeneic C57BL/6 donors, is without obvious explanation. *H-2* incompatibility in otherwise syngeneic C57BL/6 donors led to high initial representation of donor-derived cells but was followed by relatively early deaths, at 3–5 mo. Fully allogeneic (DBA/2 or C3H) donors gave the poorest success, as expected, but included at least one successful case of engraftment in each group.

While the superiority of C3H.SW over C3H in achieving engraftment may seem expected in view of the *H-2* match between C3H.SW and the C57BL/6 recipient, it should be noted that $H\text{-}2^k/H\text{-}2^k$-C57BL/6 donors were mismatched with their recipients but gave a high initial frequency of stem cell engraftment. The fact that the $H\text{-}2^k$ region of $H\text{-}2^k/H\text{-}2^k$-C57BL/6 was derived from the AKR strain rather than from C3H (14) may account for the difference between the effect of $H\text{-}2^k$ in these C57BL/6 congenic donors and in C3H donors, if a gene closely linked to *H-2* plays a significant role in hematopoietic stem cell regulation. Some $H\text{-}2^k$ inbred strains, including C3H but not AKR, have indeed been found to yield relatively more limited proliferation of bone marrow cultures (15, 16).

The immunological relations between adult donors and fetal hosts have no counterpart at other ages although *H-2* compatibility in adult hematopoietic cells may be essential in many ways (17, 18), e.g., in stem cell regulation by stromal cells. Our hosts are not yet capable of resistance to or rejection of donor cells (3, 19). The donor cells are at first represented in marginal numbers, unlike the usual case with large numbers of bone marrow cells introduced into adult recipients that retain significant immunocompetence despite irradiation (20–22). Immunocompetent T lymphocytes capable of graft-vs.-host reactivity would be present in only small numbers in the bone marrow inoculum initially introduced into fetal recipients and an inoculum >10 times greater is required in adult mice to induce graft-vs.-host disease, which then occurs with delayed onset (23).

The deaths at 3–5 mo of successfully engrafted recipients of $H\text{-}2^k/H\text{-}2^k$-C57BL/

6 bone marrow may have resulted from gradual replacement of the host's own lymphocytes with lymphocytes of the donor strain, at a slower rate than erythroid cell replacement (e.g., Fig. 4). The ultimate complete or nearly complete presence of *H-2*-incompatible lymphocytes might produce an acquired immunodeficiency syndrome due to *H-2* restriction of lymphocyte function in a histoincompatible host (24) and result in sudden deaths from infection. If this is the case, the long-term survival of some cases with purely allogeneic donor cells might have involved less extensive lymphocyte replacement, but no data are available.

The proliferative potential of the THSC greatly exceeds that estimated for the committed erythroid stem cell, not only in our animals with the longest and most complete replacement but even with engraftment of a stem cell with limited self-renewal (25, 26). In all instances sufficiently examined for both myeloid and lymphoid derivatives in our transplants of bone marrow (15 cases) or of fetal liver (10 cases), both lineages have appeared in a coordinated fashion when engraftment was sustained. In addition, extinction of the erythroid lineage was accompanied by extinction of the lymphoid lineage in one case (Fig. 3*C*, open circles; Fig. 4*A* and *B*), at a rate consistent with the longer half-life of lymphoid cells (12). Therefore, the replacement in fetal recipients at the outset of hematopoiesis is attributable to a shared precursor, the THSC, and does not lend support to the hypothesis (27) of separate stem cells for the two lineages.

Other questions concerning the hierarchy of increasingly committed stem cells in hematopoiesis may be clarified by experimental entry into the hematopoietic system at the early fetal stage. These include control of progression through the developmental hierarchy, and the roles of mutant genes and tumorigenic viruses, in the stem cells or their tissue environments, in the stage-specific ontogeny of malignancies and other hematopoietic diseases.

Summary

Bone marrow of normal adult mice was found, after transplacental inoculation, to contain cells still able to seed the livers of early fetuses. The recipients' own hematopoietic stem cells, with a *W*-mutant defect, were at a selective disadvantage. Progression of donor strain cells to the bone marrow, long-term self-renewal, and differentiation into myeloid and lymphoid derivatives was consistent with the engraftment of totipotent hematopoietic stem cells (THSC) comparable to precursors previously identified (4) in normal fetal liver. More limited stem cells, specific for the myeloid or lymphoid cell lineages, were not detected in adult bone marrow. The bone marrow THSC, however, had a generally lower capacity for self-renewal than did fetal liver THSC. They had also embarked upon irreversible changes in gene expression, including partial histocompatibility restriction. While completely allogeneic fetal liver THSC were readily accepted by fetuses, *H-2* incompatibility only occasionally resulted in engraftment of adult bone marrow cells and, in these cases, was often associated with sudden death at 3–5 mo. On the other hand, *H-2* compatibility, even with histocompatibility differences at other loci, was sufficient to ensure long-term success as often as with fetal liver THSC.

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