Effects of Transplantation on the Primitive Immunohematopoietic Stem Cell

By David E. Harrison,* Mervyn Stone,‡ and Clinton M. Astle*

*From *The Jackson Laboratory, Bar Harbor, Maine 04609; and the ‡Department of Statistical Science, University College, London, WC1E 6BT, United Kingdom*

Summary

Transplantation has strong deleterious effects on the primitive immunohematopoietic stem cells (PSC) from which circulating lymphocytes and erythrocytes are descended. We studied these effects over 300–400 d, testing whether PSC numbers, repopulating abilities, or both, were reduced. Equivalent PSC numbers were estimated in recipients of mixtures of genetically different cells, using the binomial model with covariance. Percentages of lymphocyte and erythrocyte types were closely correlated, as were percentages of either type sampled at intervals of several months. This suggests that the same PSC produced lymphoid and myeloid cells, and that most circulating cells were descended from the same PSC over hundreds of days. Equivalent PSC concentrations were $\sim 1/10^5$ fresh marrow cells, and were about twofold lower using previously transplanted marrow. However, such marrow repopulated only one-seventh to one-eighth as well as fresh marrow. Apparently, transplantation not only reduces PSC concentrations, but also reduces the repopulating ability per PSC. This may result from excessive stimuli to differentiate that overbalance the stimuli for PSC to replenish themselves.

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The most primitive stem cell (PSC)[1] functioning in adults is the immunohematopoietic PSC. It continuously replaces all renewable lymphoid cells and myeloid cells (1–3), as well as a variety of other cell types including phagocytic and mast cells (4). The PSC is characterized by maximum self-renewal and maximum differentiating ability (1–3).

However, transplantation reduces marrow repopulating abilities dramatically (5–7). Such transplantation effects have theoretical importance, not only for the hematology of stem cells, but also for one of the classic questions in biology: the maximal proliferative capacity of somatic cells. Studies by Carrel (8) and Ebeling (9) convinced people for half a century that such cells had potentially unlimited proliferative capacities. However, modern studies of fibroblasts reversed that opinion (10–12). It is still unknown whether the loss of PSC function with transplantation results from exhaustion of limited proliferative capacity or whether untransplanted PSC may proliferate without limit.

Transplantation effects also have clinical importance, because PSC are essential in successful marrow transplantation. Furthermore, their huge differentiating and repopulating abilities have the potential to make them important vehicles for gene transplantation therapy, especially since they can be conveniently removed and transplanted in marrow cell suspensions.

There are two general mechanisms that may cause transplantation damage: reductions in PSC concentrations, and diminished repopulating ability per PSC. In the present report we find damage from both of these mechanisms, since transplanted marrow cells had a twofold reduction in PSC concentration, but a seven- to eightfold reduction in repopulating ability, compared with fresh marrow. We derive our estimate of equivalent PSC concentration from the covariance of joint measurements on lymphocytes and erythrocytes, a new technique that avoids a serious problem in variance calculations, the subtraction of an estimate of variance resulting from experimental error (1, 2). Functional abilities were estimated from competitive repopulation studies (5–7).

Materials and Methods

Mice. Different mouse strains may have genetic disparities affecting histocompatibility and hybrid or allogeneic resistance. Since these may influence PSC development, we used C57BL/6J (B6) and congenic B6-*Hbb^d/Hbb^d*, *Gpi-1^a/Gpi-1^a* (B6-*Hbb^d Gpi-1^a*) male donors (1, 2) with B6 recipients. All were 2–4-mo-old male mice in both experiments, except that donors of previously transplanted cells had been lethally irradiated and given 100×10^5 marrow cells 5–6 mo before the experiment, and therefore were 8–10 mo old.

Mice were produced and maintained at The Jackson Laboratory, which is fully accredited by the American Association for the Accreditation of Laboratory Animal Care. They were maintained in isolated, environmentally controlled rooms with filtered air under positive pressure, temperatures at 22°C, and lighting from 07:00 to 19:00 hours. Mice were fed a pelleted, pasteurized diet (96WA; Emory Morse, Guilford, CT), and were not exposed to known

[1] *Abbreviations used in this paper:* Gpi, glucosephosphate isomerase; PSC, primitive stem cell.

pathogens; randomly sampled mice from the colony showed no antiviral titers when tested for 10 standard mouse viruses. Details of the animal husbandry are published in the Handbook on Genetically Standardized Jax Mice, and quarterly updates are published in the Animal Health and Genetic Quality Control Report (The Jackson Laboratory, Bar Harbor, ME).

Marrow Transplantation. Recipients were lethally irradiated with 1,100 rad in a ^{137}Cs gamma irradiator (Shepherd Mark I; J.L. Shepherd & Associates, Glendale, CA) at a dose rate of 220 rad/min 15–18 h before the marrow was transplanted. Marrow cell suspensions were prepared from femurs and tibias of each donor. The bones were cleaned, the ends were opened, and the marrow was washed out by repeated flushing with IMDM through a 23-gauge needle. Clumps were dissociated by gently forcing them through a 1.00-ml syringe against the bottom of a test tube. Marrow suspensions were filtered with 100-mesh nylon cloth, and marrow cells were stored on ice. Nucleated cells were counted using a Coulter model ZBI Electronic Cell Counter (Coulter Electronics, Inc., Hialeah, FL), after erythrocytes were lysed using standard lysing agents for white cell counting. Cells were injected intravenously through 26-gauge needles into the lateral tail veins of warmed recipients. In each of the experiments, lethally irradiated B6 recipients were given the marrow doses detailed in the table footnotes in 0.50 ml, and all recipients of a given dose were intravenously injected with equal portions from the same mixture. Marrow cells of each type were taken from a single donor cell pool in each experiment.

Recipient Cell Recovery. We estimated the contribution to the total amounts of B6-type cells due to recovery of recipient cells in groups of seven lethally irradiated B6 recipients in each experiment that were given 10 or 20 × 10^5 previously transplanted B6-*Hbb^d Gpi-1^a* competitor cells alone. Averaging the means for tests at each time point, and pooling Hb and GPI results, since they were similar, percentages of host (B6) cells were very low, 4.1 ± 2.4(7) or 2.1 ± 1.9(7), given as mean ± SD (number of individual tests). Percentages of host cells may have had a slight tendency to decline with time, as shown in Table 6. Thus, host recovery did not contribute significant numbers of differentiated cells, even in recipients of the lowest number of previously transplanted cells.

Percentages of B6-type Lymphocytes (P_l) and Erythrocytes (P_e). Lymphocytes from the blood were separated by density gradient centrifugation as detailed previously (2), and the percentages of B6 type glucosephosphate isomerase (Gpi) were determined by densitometry after electrophoresis. Donor B6 mice have the *Gpi-1^b* allele at the *Gpi-1* locus; in electrophoresis this type forms a more rapidly migrating band from positive to negative than does the *Gpi-1^a* allele. Erythrocytes were taken from the same blood samples and percentages of B6 type hemoglobin (Hb) were determined by densitometry after electrophoresis. B6 mice have *Hbb^s* (s, single) hemoglobin, which forms an electrophoretic band migrating farthest from negative to positive on the standard cellulose acetate gels used, while *Hbb^d* (d, diffuse) hemoglobin has the *Hbb-b1^d* and *Hbb-b2^d* bands that are intermediate and slowest migrating, respectively. Details of the Gpi and hemoglobin electrophoretic techniques have been published previously (2). Blood was sampled at intervals of >100 d, and each blood sample was tested for both lymphocyte and erythrocyte percentages of B6 type. Results are given as sample means estimating P_l and P_e.

Estimation of PSC Numbers and Concentrations. For each individual in a group numbering n recipients getting identical aliquots from the same marrow cell pool, we measure the B6 percentages for lymphocytes and for erythrocytes, P_l and P_e, at the same sampling time after transplantation. We calculate: Mean = the average of all $2n$ percentages which is the average of the 2 sample means estimating P_l and P_e, the percentages of B6 type lymphocytes and erythrocytes; SD_L, SD_E = the standard deviations of the two sets of n measurements; r = lymphocyte/erythrocyte Pearson correlation coefficient (L/E r); high correlations, suggest that the sampled lymphocytes and erythrocytes are descended from the same precursors. Then covariance = $r \times SD_L \times SD_E$.

Estimates of PSC number and concentration are calculated from the binomial formula as in Eqs. 1 and 2 below. If in each individual there were just N such PSC that contributed equal fractions 1/N of lymphocytes and of erythrocytes, then equivalent PSC number would be an estimate of N and equivalent PSC concentration an estimate of the concentration of such PSC.

$$\text{Equivalent PSC number} = [(\text{mean})(100\text{-mean})]/\text{covariance} \quad \text{(Eq. 1)}$$

$$\text{Equivalent PSC concentration} = \text{equivalent PSC number/number of injected cells} \quad \text{(Eq. 2)}$$

The reciprocal of equivalent PSC number is an estimate of the parameter introduced by Stone (13) and labeled π_c. If the genetic marking (B6 or not) does not affect clonal development, as should be the case using congenic lines, π_c is the probability that a randomly picked lymphocyte and a randomly picked erythrocyte are descended from the same transplanted PSC. Equivalent PSC number and concentration clearly have useful interpretations in terms of the fundamental probability parameter π_c, even when the assumption that each PSC contributes equally is unrealistic. They should then be regarded as experimentally ascertainable, inverse measures of the common PSC clonality of the lymphocyte and erythrocyte compartments. Furthermore, if each precursor does not contribute equally, estimates of the equivalent PSC number and concentration reflect those precursors contributing the most, and these are especially interesting.

Estimation of Numbers and Concentrations for Continuously Functioning Precursors. The methods described in the preceding paragraphs are also applicable if the two measurements are B6 percentages of either lymphocytes or erythrocytes at different sampling times. As long as these times are far enough apart so that circulating cells have been renewed during that interval, estimations of common continuously functioning precursors will still be labelled as PSC. In these cases, r is the correlation coefficient relating the measures on the same recipients at the two times; $SD1$ and $SD2$, the standard deviations of measurements at those times, are substituted for SD_L and SD_E. High correlations suggest that the differentiated cells are descended from the same precursors over the time interval used.

Results

PSC Concentrations. In each of two experiments, percentages of B6 type lymphocytes and erythrocytes, P_l and P_e, were measured repeatedly at intervals of ∼100 d to follow long-term changes in these populations. The basic feature of the simple binomial neutral-marker model (1–3, 14–18) associating increasing variance with decreasing PSC numbers was supported. Using previously transplanted cells, standard deviations were highest in recipients of 20 × 10^5 cells, reducing to about one-third of their value as the dose was increased to 200 × 10^5 cells. Standard deviations were about the same in recipients of 8 × 10^5 fresh marrow cells as in recipients of 20 × 10^5 previously transplanted cells, suggesting that PSC concentrations are higher in fresh marrow. PSC concentrations were estimated in two ways: as common

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Table 1. *Frequencies of Common Lymphoid/Erythroid Precursor Cells (Exp. 1)*

Sampling times	Calculated statistics	Total number of marrow cells injected (× 10^5)				
		8-F	20	40	80	200
d						
107	Means P_l,P_e	49,45	59,52	49,45	55,53	56,51
	SD_L,SD_E	12,16	16,15	7,8	6,8	5,5
	L/E *r*	0.73	0.93	0.82	0.64	0.33
	n	16	15	16	16	16
	Concentration	2.1	0.6	1.3	1.0	1.6
243	Means P_l,P_e	54,47	57,63	54,54	59,61	60,60
	SD_L,SD_E	14,21	16,19	12,17	9,14	5,5
	L/E *r*	0.94	0.77	0.80	0.88	0.85
	n	16	15	16	15	16
	Concentration	1.1	0.5	0.4	0.3	0.6
362	Means P_l,P_e	59,50	60,66	59,60	60,66	64,64
	SD_L,SD_E	19,23	20,18	16,20	14,14	8,8
	L/E *r*	0.83	0.68	0.91	0.84	0.79
	n	16	14	14	14	15
	Concentration	0.8	0.5	0.2	0.2	0.2
463	Means P_l,P_e	60,50	54,66	61,73	67,67	63,67
	SD_L,SD_E	21,23	29,21	19,23	11,15	9,7
	L/E *r*	0.85	0.72	0.81	0.65	0.74
	n	9	9	11	9	10
	Concentration	0.8	0.3	0.2	0.3	0.2

Marrow doses totaled 8 × 10^5 (8-F), half fresh B6 cells, and half fresh B6-*Hbb^d Gpi-1^a* cells, or 20, 40, 80, and 200 × 10^5 cells, half from carriers transplanted with B6 cells, and half from carriers transplanted with B6-*Hbb^d Gpi-1^a* marrow. All but the first dose (given as cell number "-F" for fresh marrow of each type) came from lethally irradiated B6 carriers given 100 × 10^5 marrow cells 5–6 mo previously. Recipients were bled and tested 107, 243, 362, and 463 d after they were given the marrow cell mixtures. P_l and P_e are the percentages of B6-type lymphocytes and erythrocytes, respectively, and SD_L,SD_E are their sample standard deviations. The *r* values (L/E *r*) are Pearson's product moment correlation coefficients of P_l and P_e. The equivalent PSC number was calculated as P(100 − P)/covariance, where *P* is overall mean percent B6 type, and covariance = *r* × (SD_L × SD_E). "Concentration" is the equivalent PSC concentration = equivalent PSC number/number of injected cells, and *n* is the number of recipients for which both P_l and P_e were measured. Estimations of concentrations become more meaningful as *r* approaches 1.00 and the SDs approach the same value.

precursors of lymphocytes and erythrocytes in one blood sample, or as precursors producing differentiated cells during two samplings 101–145 d apart.

Common Precursors of Lymphocytes and Erythrocytes. Lymphoid/erythroid correlation coefficients (L/E *r*) in the same recipients were very high in most groups. For example, at 243 d in Table 1, L/E *r* = 0.94 for groups receiving 8 × 10^5 fresh cells (8-F); and 0.77, 0.80, 0.88, and 0.85, for groups receiving 20, 40, 80, and 200 × 10^5 previously transplanted marrow cells, respectively. These high *r* values suggest that most differentiated lymphoid and erythroid cells were descended from a small number of transplanted precursors, as expected of true PSC. Equivalent PSC concentrations were ~1/10^5 for fresh marrow, and less than half of that for transplanted marrow. In the example mentioned above, concentrations per 10^5 marrow cells were 1.1 for groups receiving 8 × 10^5 fresh cells; and 0.5, 0.4, 0.3, and 0.6, for groups receiving 20, 40, 80, and 200 × 10^5 previously transplanted marrow cells, respectively. Equivalent PSC numbers for retransplanted cells increase roughly proportionally with the number of marrow cells injected, so that taking Tables 1 and 2 together, there is no evidence of any systematic dependence of equivalent PSC concentration on dose.

Precursors of Differentiated Cells Over Hundreds of Days. Correlation coefficients between P_l (or P_e) in the same recipients at different times were also very high in most groups after the first time point (Tables 3 and 4). Thus between times 2 and 3 (243–362 d) in Exp. 1, lymphoid cell correlation coefficients (L2/L3 *r*) were 0.91 for groups receiving 8 × 10^5 fresh cells; and 0.78, 0.94, 0.80, and 0.73 for groups

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Table 2. *Frequencies of Common Lymphoid/Erythroid Precursor Cells (Exp. 2)*

Sampling times	Calculated statistics	Total number of marrow cells injected ($\times 10^5$)				
		8-F	20	40	80	200
d						
152	Means P_l,P_e	53,53	59,59	59,58	64,63	66,62
	SD_L,SD_E	18,26	16,25	10,12	6,7	5,6
	L/E *r*	0.92	0.96	0.95	0.70	0.69
	n	13	13	15	16	16
	Concentration	0.7	0.3	0.6	0.9	0.5
297	Means P_l,P_e	58,57	62,64	65,62	68,67	67,68
	SD_L,SD_E	19,26	22,27	14,15	6,7	8,9
	L/E *r*	0.76	0.91	0.92	0.50	0.90
	n	13	12	15	16	16
	Concentration	0.8	0.2	0.3	1.4	0.2
398	Means P_l,P_e	53,68	59,58	66,58	75,67	73,66
	SD_L,SD_E	33,31	32,30	16,19	5,8	7,9
	L/E *r*	0.73	0.98	0.91	0.11	0.21
	n	8	9	11	13	12
	Concentration	0.4	0.1	0.2	6.4	0.8

Footnotes are the same as in Table 1, except that this is an independent Exp. 2, and recipients were tested 152, 297, and 398 d after they were given marrow mixtures.

Table 3. *Frequency of Continuously Functioning Precursor Cells (Exp. 1)*

Sampling times	Calculated statistics	Total number of marrow cells injected ($\times 10^5$)				
		8-F	20	40	80	200
d	Lymphoid cells					
107–243	L1/L2 *r*	0.61	0.70	0.78	0.66	0.49
	Concentration	2.9	0.6	1.0	0.9	1.1
243–362	L2/L3 *r*	0.91	0.78	0.94	0.80	0.73
	Concentration	1.3	0.5	0.4	0.4	0.4
362–463	L3/L4 *r*	0.97	0.97	0.92	0.70	0.59
	Concentration	0.8	0.2	0.2	0.3	0.2
	Erythroid cells					
107–243	E1/E2 *r*	0.86	0.93	0.75	0.86	0.62
	Concentration	1.0	0.5	0.6	0.3	0.7
243–362	E2/E3 *r*	0.91	0.93	0.94	0.94	0.94
	Concentration	0.7	0.4	0.2	0.2	0.3
362–463	E3/E4 *r*	0.93	0.98	0.97	0.98	0.88
	Concentration	0.6	0.3	0.1	0.1	0.2

Footnotes are the same as in Table 1, except that instead of P_l and P_e, calculations are based on P_l and P_l (or P_e and P_e) for sampling times 1-2, 2-3, and 3-4.

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Table 4. *Frequency of Continuously Functioning Precursor Cells (Exp. 2)*

Sampling times	Calculated statistics	Total number of marrow cells injected ($\times 10^5$)				
		8-F	20	40	80	200
d	Lymphoid cells					
152–297	L1/L2 *r*	0.79	0.98	0.92	0.48	0.73
	Concentration	1.2	0.3	0.5	1.7	0.4
297–398	L2/L3 *r*	0.91	0.98	0.90	0.58	0.33
	Concentration	0.5	0.2	0.3	1.7	0.6
	Erythroid cells					
152–297	E1/E2 *r*	0.88	0.96	0.94	0.65	0.73
	Concentration	0.5	0.2	0.4	0.8	0.3
297–398	E2/E3 *r*	0.81	0.98	0.87	0.82	0.87
	Concentration	0.4	0.2	0.3	0.6	0.2

Footnotes are the same as in Table 3.

receiving 20, 40, 80, and 200 $\times$ 10^5 previously transplanted marrow cells, respectively (Table 3). These high correlations suggest that most differentiated cells were descended from the same precursors over hundreds of days, as expected if PSC and their descendants function continuously. As above, equivalent PSC concentrations were $\sim 1/10^5$ for fresh marrow, and about one-third that for transplanted marrow. In the example mentioned above, equivalent PSC concentrations per 10^5 marrow cells were 1.3 for groups receiving 8 $\times$ 10^5 fresh cells; and 0.5, 0.4, 0.4, and 0.4, for groups receiving 20, 40, 80, and 200 $\times$ 10^5 previously transplanted marrow cells, respectively. Just as for the previous tables, Tables 3 and 4 together show that the equivalent PSC concentration for retransplanted cells is roughly the same at all dose levels.

Equivalent PSC concentrations are summarized in Table 5 as "concentration from L,E" (Tables 1 and 2), "concentration over time L" (Tables 3 and 4, top half) and "concentration over time E" (Tables 3 and 4, bottom half). Equivalent PSC concentrations tend to decline with time proportionally in fresh and in previously transplanted cells; ratios of

Table 5. *Summaries of PSC Concentration Estimates*

		Concentrations from L, E			Concentrations over time L			Concentrations over time E		
Exp.	Days	Fresh	Retrans	Ratio	Fresh	Retrans	Ratio	Fresh	Retrans	Ratio
1	107	2.1	1.15	0.55	–	–	–	–	–	–
1	243	1.1	0.45	0.41	2.9	0.95	0.33	1.0	0.55	0.55
1	362	0.8	0.2	0.25	1.3	0.4	0.31	0.7	0.25	0.36
1	463	0.8	0.25	0.31	0.8	0.2	0.25	0.6	0.15	0.25
2	152	0.7	0.55	0.79	–	–	–	–	–	–
2	297	0.8	0.25	0.31	1.2	0.45	0.38	0.5	0.35	0.7
2	398	0.4	0.5	1.25	0.5	0.45	0.9	0.4	0.25	0.63

In this summary of results in Tables 1–4, values for fresh marrow were taken from the group receiving 8 $\times$ 10^5 fresh marrow cells, while medians of values for previously transplanted marrow (Retrans) were taken from groups receiving 20, 40, 80, and 200 $\times$ 10^5 retransplanted marrow cells. "Concentrations from L, E" are summarized from Table 1 and 2. "Concentrations over time L or E" are summarized from Tables 3 and 4. The median of the concentration values for any two sampling times is listed at the later time. Fresh and retrans concentration values were campared by the Wilcoxon signed-rank test; for Exp. 1 (n = 10), $Z = -2.8$ with $P = 0.0049$ and for Exp. 2 (n = 7), $Z = -2.0$ with $P = 0.041$. Ratios calculated as concentration Retrans/concentration Fresh have means of 0.36 for Exp. 1 and 0.71 for Exp. 2, with medians 0.32 and 0.70, respectively.

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equivalent PSC concentrations in retransplanted marrow/fresh marrow did not change consistently with time. They averaged 0.36 in Exp. 1, and 0.71 in Exp. 2.

Effects of Transplantation on Functional Abilities. Relative repopulating abilities of fresh and retransplanted cells may be estimated from the percentages of B6 type in the following experiment (Table 6): 10 × 10^5 fresh (10-F), or 33, 100, or 300 × 10^5 previously transplanted B6 cells, were each mixed with 10 × 10^5 cell portions of fresh competitor cells. In parentheses are calculated relative repopulating abilities of B6 compared with competitor cells, where 100% means equal repopulating abilities. With the same doses of fresh B6 marrow these averaged 91% in Exp. 1 and 139% in Exp. 2. However, transplanted cells had average relative repopulating abilities of 11.7% in Exp. 1 and 19.6% in Exp. 2 (Table 6). Adjusted to a cell for cell basis, fresh marrow repopulated eight times as well as previously transplanted marrow in Exp. 1, and seven times as well in Exp. 2.

Table 6. *Repopulating Abilities of Retransplanted Cells*

Exp.	Days	B6 percent in groups receiving the following cell numbers (× 10^5): 10-F	33	100	300	10-C	20-C
1	107	46 (85)	40 (20)	55 (12)	73 (9)	3	3
1	243	48 (92)	29 (12)	54 (12)	72 (9)	7	3
1	362	49 (96)	32 (14)	49 (10)	73 (9)	4	0
1	463	–	25 (10)	–	–	3	1
2	152	55 (122)	52 (33)	61 (16)	76 (11)	8	3
2	297	61 (156)	46 (26)	60 (15)	82 (15)	2	5
2	398	–	45 (25)	61 (16)	85 (19)	2	0

The same cell pools used in Exps. 1 and 2 were used, with the following recipient groups: 10 × 10^5 fresh B6 cells (results under 10-F), 33, 100, or 300 × 10^5 previously transplanted B6 cells (results given under 33, 100, and 200) were mixed with 10 × 10^5 fresh B6-*Hbb^d^ Gpi-1^a^* competitor cells, and given to groups of three to four lethally irradiated B6 recipients. Host cell recovery was tested in groups of seven lethally irradiated B6 recipients that were given 10 or 20 × 10^5 previously transplanted B6-*Hbb^d^ Gpi-1^a^* competitor cells alone (results given under 10-C and 20-C). Percentages of B6 type hemoglobin and GPI were measured with those given in Tables 1–4. Both Hb and GPI data gave similar results so their average values are given here. In the columns where B6 type cells were mixed with competitors, relative repopulating percentages of the B6 cells, given in parentheses, were calculated as follows: M = the number of B6 cells (× 10^5) that would be needed to produce the observed percent B6 (called "P") assuming both types had the same repopulating ability, as is likely with congenic cells. The competitor cells were given at a dosage of 10 × 10^5, so $P = 100 \times [M/(10 + M)]$. By simple algebra, $M = 10P/(100\text{-}P)$. Relative repopulating percentages are then $100M$/(actual number B6 cells). These averaged 11.7% for retransplanted and 91% for fresh cells in Exp. 1; they were 19.6% for retransplanted and 139% for fresh cells in Exp. 2. Thus the transplanted cells repopulated 12.9% (∼1/8) and 14.1% (∼1/7) as well as fresh, in the two experiments.

Discussion

Understanding how transplantation alters PSC, precursor cells with the most repopulating ability over the longest period of time, is important for three reasons: (*a*) These cells are of basic interest as they have many properties of undifferentiated embryonal cells but are found in adults; (*b*) they are vital for long-term success in marrow transplantation, a treatment of increasing clinical importance; and (*c*) they will be vital in gene therapy in adults, when the genetically altered cells will have to successfully repopulate over long time periods in competition with the host's untreated cells.

The competitive repopulation techniques used in this study directly assay the most important properties of PSC, their long-term repopulating and differentiating functions over hundreds of days. Estimates of PSC concentrations are not exact because each precursor may not contribute equally to the differentiated cell populations. However our estimates of equivalent PSC concentrations represent the most interesting cells, the precursors from which most of the differentiated cells are descended. In addition they have a precise interpretation in terms of the probability parameter π_c (13, 18). It is limited numbers of these precursors that cause most of the covariance in Eq. 1. Numbers of precursors contributing less to the differentiated cell populations will have little effect on the covariance and will be underestimated (13).

The drop in estimated equivalent PSC concentrations after 107 d in Exp. 1 probably occurred because exhaustible precursors, less primitive than true PSC, were present in the original marrow grafts, but gradually were exhausted after 107 d (1, 2, 19). Since each PSC probably contributed much more to the differentiated populations than did any of the differentiated precursors, the observed covariances mostly reflect limited PSC numbers, not numbers of more differentiated cells.

An advantage in using covariance-based rather than variance-based estimates is that bias arising from random experimental error or any other source of error that is uncorrelated in the two compartments is not present. Had variances been used in the binomial formula as is usual (3, 13–17), estimates of measurement error would have been needed for comparison with experimental standard deviations. Such estimates are usually obtained from repeatedly measuring a standard sample and do not include all random experimental error, for example that due to effects of irradiation on individual recipients, or that due to different conditions at different sampling times when recipients are followed over time.

Transplantation reduced equivalent PSC concentrations about twofold (Table 5). However, this reduction was not sufficient to explain the seven- to eightfold reductions in repopulating ability caused by transplantation in the same experiments (Table 6). Thus, transplantation reduces the functional ability per PSC as well as reducing PSC numbers.

There are two potential causes of transplantation damage: (*a*) There may be excessive stimuli for PSC to differentiate in the lethally irradiated recipients relative to the stimuli for

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PSC to proliferate and maintain their numbers. This would result in dilution of the slowly proliferating PSC by more rapidly proliferating differentiated precursors, as suggested by Jones et al. (20). (*b*) Removal of PSC from their microenvironments and transplantation into irradiated recipients may cause damage.

We tested whether PSC were damaged due to irradiation injury of the recipient stromal tissue by comparing transplanted marrow from genetically anemic W/W^v recipients that were repopulated without irradiation, and marrow from lethally irradiated recipients of identical cells. No beneficial effects of transplantation into W/W^v recipients were found, even when marrow was transplanted by parabiosis to avoid handling (21). Hybrid resistance or graft-vs.-host effects may have damaged the transplanted B6 marrow in the WBB6F1 W/W^v recipients used, so the experiment should be repeated using congenic recipients.

Transplantation damage should be preventable if it occurs because transplanted PSC are suddenly exposed to high levels of stimuli to differentiate, and these overbalance the stimuli to replicate. Stimuli for PSC to differentiate may be reduced, stimuli for them to replicate may be increased, or both.

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Address correspondence to Dr. David E. Harrison, The Jackson Laboratory, Bar Harbor, ME 04609.

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