# GENOTYPE-RESTRICTED GROWTH AND AGING PATTERNS IN HEMATOPOIETIC STEM CELL POPULATIONS OF ALLOPHENIC MICE

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Hematopoiesis is a life-long developmental process in which relatively short-lived, but abundant, mature cells must be continuously replaced by proliferation and differentiation of long-lived, but few, stem cells. Factors determining the allocation of stem cells between a proliferatively quiescent reserve pool on one hand and a proliferating, differentiating pool on the other hand, are at present poorly understood. Indeed, the number of proliferating and differentiating stem cell clones simultaneously supplying blood cell formation is controversial; different experimental approaches have yielded different answers to this question. Studies of radiation chimeras and stem cell-deficient mice engrafted with retrovirally "marked" stem cells have shown that hematopoiesis was oligoclonal (1, 2). Clones temporally waxed and waned and in some hosts pluripotent clones had productive lifespans of many months (3, 4). On the other hand, studies of allophenic mice and radiation chimeras, in which component genotypes contributing to hematopoiesis were analyzed using binomial statistics, determined from a lack of genotypic fluctuation that blood cell formation was polyclonal, with most, if not all, of the stem cells simultaneously productive (5, 6).

Aging in mouse and man is accompanied by a decline in the ability to resist disease and neoplasia, and it is reasonable to expect that genetic regulation of stem cell proliferation and function plays a role in this process. We previously uncovered a genetic difference in stem cell population kinetics among different inbred mouse strains that otherwise have similar numbers of mature blood cells, suggesting that similar production rates may be achieved through different developmental regulatory mechanisms (7). Two of these strains, a shorter lived strain with a normally high rate of stem cell division (DBA/2 [7, 8]) and a long-lived strain with a normally low fraction of dividing stem cells (C57BL/6 [7, 8]), were used in the present studies as sources of embryos to construct chimeric (allophenic) mice. One might expect that if the difference observed in stem cell population kinetics was extrinsically controlled, for example by a difference in cytokine titers in the inbred strains, the two stem cell populations would be expected to assume a similar kinetic phenotype in

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the common environment of the allophenic mouse. If, however, the kinetic control was intrinsic to the genetically different stem cells, for example via the sensitivity of stem cells to cytokine titer through inherent differences in receptor number or function, the two stem cell populations may be expected to maintain their phenotypic difference in a common environment and this difference may affect their participation in blood cell formation. Therefore, the purpose of these studies was to determine whether kinetic differences in genetically distinct stem cell populations affect their long-term blood cell outputs in two in vivo settings where the populations would be vying for representation in blood cell differentiation: allophenic mice and radiation chimeras engrafted with allophenic marrow. We report here that intrinsic genetic differences in stem cell populations of allophenic mice are reflected in the makeup of hematopoiesis over the lifetime of the animal. Stem cells from a strain with an inherently greater cycling rate and a shorter lifespan, have a competitive repopulation advantage, but become senescent both in allophenic mice and radiation chimeras, and are eclipsed by stem cells from a strain in which the population turns over at a slower rate and is long-lived.

## Materials and Methods

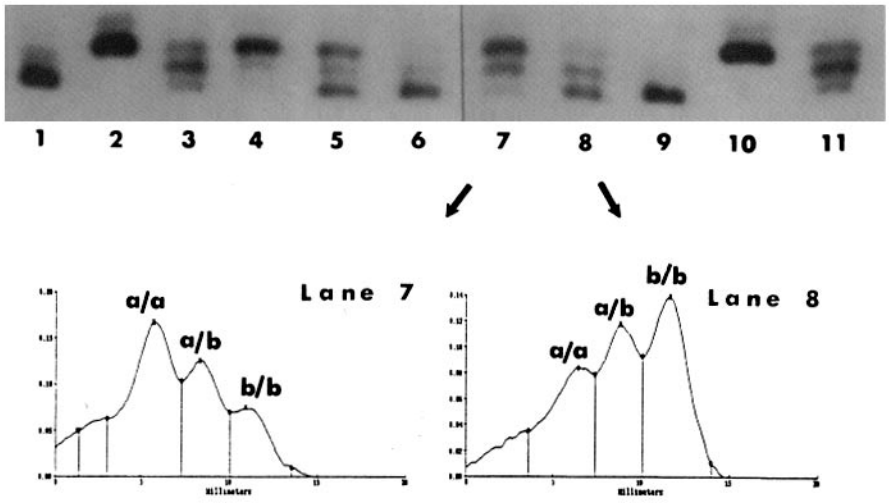
*Mice.* C57BL/6, DBA/2, and B6D2F1 mice were obtained through the Animal Resources Division of the National Institutes of Health. They were housed and maintained according to NIH guidelines. Allophenic mice were housed on enclosed cage racks supplied with filter-sterilized air; cages, bedding, food, and water were sterilized.

*Allophenic Mouse Construction.* Allophenic mice were produced by techniques fully described by Mintz (9). Briefly, eight-cell embryos were flushed from the uteri of DBA/2 and C57BL/6 mice on the morning of the second day of pregnancy. Pregnancy was determined by the presence of a vaginal plug in spontaneously mated females (day 0). The zona pellucida was removed from the embryos after a brief exposure to 0.5% pronase and they were manipulated together under a dissecting microscope and allowed to aggregate. The chimeric embryos were cultured overnight in the wells of Terasaki plates containing 0.1 ml of Brinster's medium (Gibco Laboratories, Grand Island, NY). In the afternoon of the next day the embryos, at the early blastocyst stage, were transferred to the uteri of pseudopregnant B6D2F1 females mated 2 d previously with vasectomized males. Chimeras were reliably born 17–20 d later.

*Tissue Preparation for Analysis of Genotypic Composition.* Blood samples, including isolation of red cells, platelets, granulocytes, and lymphocytes, were prepared using discontinuous Percoll (Pharmacia Fine Chemicals, Piscataway, NJ) gradients exactly as described (7). In studies where white cells were not sub-fractionated into granulocytes and lymphocytes, buffy coat cells were collected from the interface of a one-step Percoll (1.095 g/ml) cushion. Cell suspensions were prepared from lymph nodes, thymus, and spleen by squeezing them between frosted ends of microscope slides into culture medium. Care was taken not to include the stroma from these lymphoid organs. Cells from heart, liver, kidney, and brain were similarly prepared from minced tissues. Resulting cells were lysed by addition of the hypotonic sample buffer (1 mM EDTA, 50 mM Tris-HCl, 0.31 mg/ml dithiothreitol, pH 7.5) and repetitive cycles of freezing and thawing. Bone marrow cells were flushed from the tibiae and femora using 23 g needles. Individual spleen colonies were harvested 12 d after bone marrow transplant by carefully dissecting them from the organ surface under a dissecting microscope.

*Determination of Genotypic Composition.* Electrophoretic variants of glucose phosphate isomerase (GPI; D-glucose-6-phosphate ketol-isomerase, EC 5.3.1.9) were used to distinguish genotypic contributions to organs and tissues of chimeric mice. Electrophoresis of cell lysates on cellulose acetate membranes and subsequent staining for GPI activity has been previously described (7). Quantitation of chimerism in red cell samples from allophenic mice (Figs. 4 and 5) was done by eye with reference to electrophoretograms of artificial mixtures of DBA and B6 red cells. As before (7), genotypic composition could be estimated by eye with less than a 10% error and usually within 5% of the actual admixture.

Determination of chimerism reported in all other tables and figures was accomplished by analysis of electrophoretograms using a BioImage Visage 2000 image analyzer (BioImage, Ann Arbor MI). Chimerism in tissues that included $F_1$ cells presented special problems in analysis of electrophoretic bands. Hybrid cells contained three GPI dimers: the fastest migrating "b" homodimer, the intermediate "a/b" heterodimer and the slowest-migrating "a" homodimer; this in a theoretical ratio of 1:2:1, respectively (Fig. 1). The two homodimers were the only GPI species in B6 (b/b) and DBA (a/a) cells, thus the presence of the heterodimer was diagnostic of $F_1$ cells. However, quantitation of the inbred strain contributions was complicated by the $F_1$ contribution to the homodimeric bands. Therefore, we developed a computer-assisted method in which we were able to quantitate and subtract $F_1$-derived contributions to the optical densities of the homodimeric bands and thus accurately analyze tri-genotypic




c

| RBC Mix Composition (%) | | | Composition from BioImage (%) | | |
|---|---|---|---|---|---|
| DBA | F1 | B6 | DBA | F1 | B6 |
| 67 | 33 | 0 | 64 | 36 | 0 |
| 33 | 33 | 33 | 33 | 33 | 33 |
| 0 | 33 | 67 | 0 | 37 | 63 |
| 33 | 67 | 0 | 35 | 65 | 0 |
| 0 | 67 | 33 | 0 | 68 | 32 |

FIGURE 1. Image analysis provides accurate quantitation of three genotypes in a single blood sample. Red blood cells from B6, DBA, and $BDF_1$ mice were mixed, lysates were electrophoresed on cellulose acetate membranes, glucose phosphate isomerase was stained, and the membrane was scanned on an image analyzer. (*a*) Photograph of the electrophoretogram: (lane *1*) B6 ($Gpi\text{-}1^{b/b}$); (lane *2*) DBA ($Gpi\text{-}1^{a/a}$); (lane *3*) $BDF_1$ ($Gpi\text{-}1^{a/b}$); (lane *4*) 67% DBA red cells, 33% $BDF_1$ red cells; (lane *5*) 33% DBA, 33% $BDF_1$, 33% B6; (lane *6*) 33% $BDF_1$, 67% B6; (lane *7*) 33% DBA, 67% $BDF_1$; (lane *8*) 67% $BDF_1$, 33% B6; (lane *9*) B6; (lane *10*) DBA; (lane *11*) $BDF_1$. (*b*) Densitometric scans of lanes *7* and *8* and illustrate the relative optical densities of (from left to right) the *a* homodimeric band characteristic of B6, the *a/b* heterodimer characteristic of $BDF_1$ and the *b* homodimer characteristic of DBA. (*c*) A comparison of the known mixtures of B6, DBA, and $BDF_1$ with the composition arrived at by the computer-aided image analysis. See Materials and Methods for a description of the method used for computing tri-genotypic contributions to mixtures.

chimerism. An $F_1$ sample was included on each gel run and from it we determined the ratios of the optical densities of the homodimeric bands to the heterodimeric band, under the prevailing conditions for an individual gel. Using this information, the optical densities of the homodimeric contributions were calculated for each test lane on the strength of the unique heterodimeric band and those values were subtracted from the optical densities obtained for "a/a" and "b/b". The remaining optical density, if any, was then due to the DBA and B6 cells, respectively, contributing to chimerism. The efficacy of this technique is demonstrated in Fig. 1 *C* in which we mixed known numbers of DBA, B6, and $F_1$ cells, electrophoresed the lysates, stained for GPI activity, and analyzed the membrane on the image analyzer. The calculated chimerism in all samples was within 5% of the actual composition of the admixtures. Fig. 2 shows a representative electrophoretogram in which chimerism in blood cell types and hemato-lymphoid organs was analyzed. All tissues contained cells of different genotypes, although the proportions varied from tissue to tissue.

*Irradiation and Transplantation.* Mice were exposed to gamma irradiation from a $^{137}Cs$ source at a dose rate of 10.6 Gy/min. Typically, the irradiation (950–1,200 cGy) was delivered in two half-doses separated by 3 h. Marrow cell suspensions were injected intravenously into a lateral tail vein in a 0.5 ml volume, 3–6 h after the last irradiation.

*Treatment of Mice with Anti-asialo GM$_1$.* Mice were injected via a lateral tail vein with 50 μl of antibody (Waco Chemicals, Dallas, TX) mixed with 0.5 ml of medium. 2 d later mice were irradiated and injected with marrow cells.

*Cell Cultures.* Colonies derived from erythroid progenitors (CFU-E and BFU-E), myeloid progenitors (CFU-GM), and multipotential cells (CFU-GEMM) were grown in semi-solid medium in a humidified incubator gassed with 5% oxygen, 5% carbon dioxide, and the balance nitrogen. The medium was Iscove's Modified Dulbecco's Medium (IMDM; Gibco Laboratories) containing (final concentrations) 20% FCS (Defined/Supplemented grade; HyClone Laboratories, Logan, UT), 1% BSA (for cell culture no. 652 237; Boehringer-Mannheim, Indianapolis, IN), $10^{-4}$ M 2-ME, and 1% methylcellulose (4,000 cps; Sigma, St. Louis, MO). 1-ml cultures were grown in 35-mm culture dishes (Lux 5221-R; Miles Scientific, Naperville, IL). CFU-E growth was stimulated by 0.5 U/ml of erythropoietin (epo) (HyClone). BFU-E and CFU-GEMM growth was stimulated by 3 U/ml of epo and 30 U/ml of murine rIL-3 (Biogen, Geneva, Switzerland). CFU-GM colonies were grown with the addition of 25 μl/ml of serum collected from mice 4 h after intravenous injection of 10 μg of *Salmonella typhosa* LPS (Sigma Chemical Co., St. Louis, MO).

*GPI Analysis of Hematopoietic Colonies.* Individual BFU-E, CFU-GEMM, and CFU-GM colonies were picked from culture dishes under a dissecting microscope at 10–20× magnification using a pipettor equipped with Ultra Micro Tips (Eppendorf; Brinkmann Instruments, Westbury, NY). BFU-E colonies were harvested after 8 d of culture; CFU-GM colonies were harvested after 7–10 d; CFU-GEMM colonies were collected after 14–21 d of culture. CFU-E colonies (8–64 cells) were too small for GPI analysis on individual colonies; consequently colonies from culture dishes were pooled for analysis after 2 d of culture (10). We adopted the method of dispersing individual colonies into 200 μl of PBS (pH 7.2), 0.5% BSA to which 1 μl of human blood had been added in a 0.5 ml Eppendorf tube. The cells were spun down and the supernatant containing the methylcellulose was discarded; if necessary, the washing

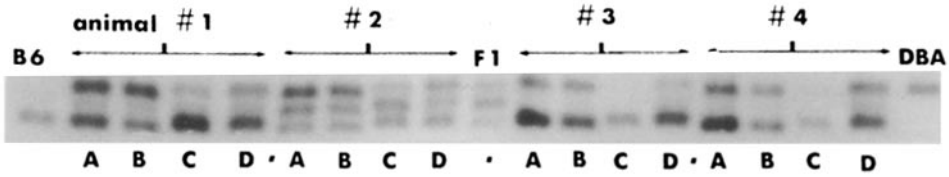



FIGURE 2. GPI electrophoretogram showing different B6, DBA, and $BDF_1$ representation in different tissues of the same radiation chimera engrafted with allophenic marrow. (*A*) Bone marrow; (*B*) spleen; (*C*) thymus; (*D*) lymph node.

procedure could be repeated. The resulting cell pellet was lysed in 3 μl of GPI sample buffer (1 mM EDTA, 50 mM Tris-HCl, 0.31 mg/ml dithiothreitol) and frozen and thawed several times before electrophoresing. Human red cells served as carriers enabling a high recovery of the mouse cells from the colony. Because human GPI had a higher electrophoretic mobility, it did not interfere with subsequent analysis of the murine GPI bands (Fig. 3). This method permitted the analysis of colonies consisting of 100 cells or more.

*Stromal Cell Cultures.* The method outlined by Dorschkind et al. (11) was followed. Briefly, marrow was grown in α medium (Gibco Laboratories) containing horse serum (20% Defined grade; HyClone Laboratories) and hydrocortisone sodium succinate ($10^{-6}$ M; Sigma Chemical Co.) in an incubator set at 33°C with a 5% carbon dioxide atmosphere. During the 26-d culture regimen the cells were subjected to two cycles of selection in mycophenolic acid (5 μg/ml; Sigma Chemical Co.) to deplete them of hematopoietic cells. The resulting stromal cell cultures, devoid of hematopoietic cells attached to the underlying adherent layer of macrophages, fibroblasts, endothelial cells, and fat cells, were trypsinized from the flask, washed, lysed, and electrophoresed.

*Statistical Analyses.* In Table I comparison between tissue types was performed by the rank sums test and the independence of age difference between tissues (2 × 2 contingency tables) was determined by Fisher's probability test (12). In both analyses a probability of $\leq 0.01$ was considered to be statistically significant. All other statistical comparisons were made with a paired *t*-test and a $p \leq 0.01$ was considered to be statistically significant.

## Results

*Long-Term Studies of Allophenic Mice.* A total of 64 allophenic mice were used in the various phases of these studies. 27 chimeras were bled every few months for 1.5–3 yr and thus represent the long-term study group from which selected examples are shown in Figs. 4 and 5. Genotypic contributions to blood cell lineages and tissues were determined by densitometric quantitation of electrophoretic variants of GPI (see Materials and Methods). Fig. 4 shows DBA and B6 contributions to the red cell pools of six allophenics over a period of ~2 yr. This group of six (and the group of three in Fig. 5) was selected for presentation because they best illustrate several points that emerged from the entire group of 27 mice studied long-term. First, the six in Fig. 4 spanned the entire range of red cell chimerism (from 90% DBA, 10% B6 to 20% DBA, 80% B6) at the time of their initial blood sampling. Second, during the first year or so the extent of chimerism was relatively stable, varying by <20% from the initial value obtained for each mouse. Chimerism, at least with respect

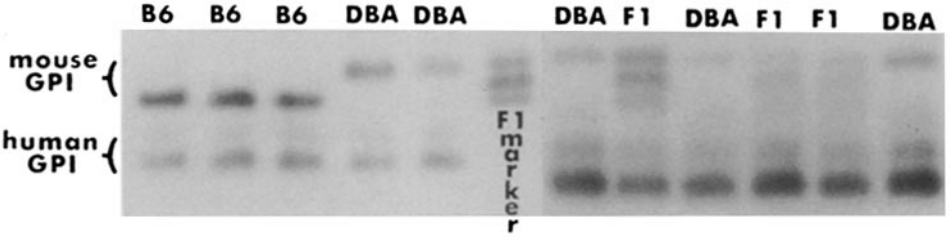



FIGURE 3. GPI electrophoretogram showing genotype of individual hematopoietic colonies grown in vitro. Human red cells were used as carriers in the preparation of the colonies for electrophoresis. The human GPI has a higher electrophoretic mobility than the murine isozymes and thus does not interfere with genotypic analysis of the murine colonies. The five colonies to the left of the $BDF_1$ marker are erythroid colonies derived from BFU-E; those six to the right are multilineage colonies derived from CFU-GEMM.

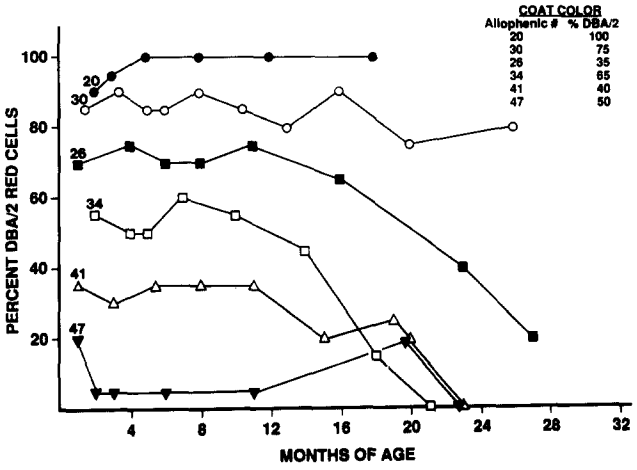



FIGURE 4. Genotypic composition of red cells in a group of "stable" allophenic mice. Red cell composition was determined by electrophoresing red cell lysates on cellulose acetate membranes, staining for GPI activity and estimating the DBA/B6 ratio by the relative strengths of the two characteristic isozyme bands.

to red cells, was lost in allophenic no. 20 within the first 5 mo and in concordance with its coat color, red cells thereafter were solely supplied by DBA stem cells. Third, striking and consistent changes in chimerism occurred in four of six allophenics during the second year of life (22 of 27 in the entire group). In each case, the red cell pool reflected greater contribution by B6 stem cells; in three of the four chimeras in Fig. 4, red cells became wholly B6.

A consistent, but not universal, finding in the group of 27 allophenics studied over a long period was that if initial red cell chimerism was initially greater than ~80% DBA (<20% B6), the B6 red cell contributions did not become predominant during later life (e.g., allophenic no. 30).

Of the total group of 64 allophenics bled periodically during the first year of life, ~10% (seven) were characterized by dramatic fluctuations in red cell chimerism and are therefore referred to as "unstable" chimeras, in contrast to the predominant "stable" phenotype described above (Fig. 4). Fig. 5 depicts the results of multiple bleedings

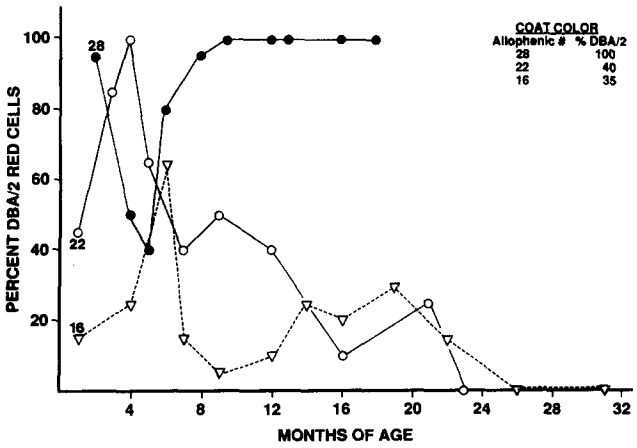



FIGURE 5. Genotypic composition of red cells in a group of "unstable" allophenic mice. Red cell composition was determined by electrophoresing red cell lysates on cellulose acetate membranes, staining for GPI activity, and estimating the DBA/B6 ratio by the relative strengths of the two characteristic isozyme bands.

of three of the seven "unstable" chimeras, and it can be seen that the fluctuations are large (>40%), they usually occur only once in the first 8 mo of life, they may occur in either direction, and they are transient.

The arbitrary distinction between "stable" and "unstable" chimeras is based on red cell chimerism in the first year of life, and as can be seen in Fig. 5, the ascendancy of B6 red cells late in life (allophenics nos. 22 and 16) occurs in the "unstable" phenotype, as well as in the "stable" phenotype (Fig. 4). Allophenic no. 22 in Fig. 5 is noteworthy because, after an approximately equal contribution of B6 and DBA stem cells initially, red cell composition shifted to 100% DBA at 4 mo and ultimately to a wholly B6 red cell pool at 23 mo.

Table I compares the genotypic contributions to various organs, tissues, and blood cell types in "stable" allophenic mice necropsied at two different ages: 3–5 mo and 22–37 mo. The four allophenics analyzed at the earlier time show reasonably good concordance (no significant differences by rank sums analysis) in the composition of all organs, tissues, and blood cell types within each mouse, even though the four allophenics differed markedly in their overall composition. In contrast, a difference can be noted between hemato-lymphoid tissues and other organs analyzed in the four allophenics necropsied at 22–37 mo. At this time splenic, thymic, marrow, and blood cell composition was 10–50% more B6 than was brain, heart, kidney, liver, or coat color, and the differences were statistically significant ($p < 0.01$) in each of the four mice by rank sums analysis (12). This demonstrates that, with aging, the ascendancy of the B6 genotype in the red cell pool noted in Figs. 4 and 5 is also reflected in blood platelets, granulocytes, and lymphocytes as well as in bone marrow, spleen, and thymus, and suggests that the stem cell population involved was truly pluripotent, supplying both the lymphoid and myeloid arms of differentiation.

*Long-term Studies of Allophenic Marrow Engrafted into Lethally Irradiated Hosts.* A second aim of these studies was to determine whether or not genotype-limited differences in stem cell population kinetics would affect hematopoietic repopulation. To this

TABLE I

*Nonconcordance between Hematolymphoid Cells and Other Organs during Aging of Allophenic Mice*

| | Age of allophenic at necropsy | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 3-5 Mo (% DBA) | | | | 22-37 Mo (% DBA) | | | |
| Tissue | No. 25 | No. 37 | No. 33 | No. 50 | No. 21 | No. 22 | No. 43 | No. 46 |
| Coat color | 80 | 20 | 65 | 25 | 10 | 45 | 35 | 50 |
| Brain (cerebral cortex) | 73 | 18 | 73 | 36 | 12 | 53 | 40 | 59 |
| Heart | 68 | 25 | 70 | 36 | 10 | 59 | 38 | 57 |
| Kidney | 76 | 33 | 78 | 41 | 11 | 56 | 48 | 52 |
| Liver | 70 | 22 | 67 | 32 | 9 | 56 | 42 | 67 |
| Spleen | 82 | 17 | 71 | 35 | 0 | 35 | 11 | 37 |
| Thymus | 81 | 29 | 60 | 29 | 0 | 37 | 0 | 27 |
| Bone marrow | 73 | 24 | 65 | 38 | 0 | 37 | 0 | 37 |
| Blood: | | | | | | | | |
| Red cells | 83 | 36 | 72 | 43 | 0 | 44 | 0 | 46 |
| Platelets | 87 | 28 | 75 | 40 | 0 | 45 | 0 | 44 |
| Lymphocytes | 63 | 21 | 62 | 30 | 0 | 37 | 0 | 34 |
| Granulocytes | 61 | 16 | 63 | 33 | 0 | 26 | 0 | 23 |

end we adopted the idea of engrafting allophenic marrow in lethally irradiated hosts. Hematopoietic and lymphoid repopulation, under these conditions, may put stringent demands on the stem cell populations and thus uncover genotypic differences in repopulating abilities as well as aging patterns.

We used $BDF_1$ (C57BL/6 × DBA/2)$F_1$ mice as hosts for the transplantation experiments, reasoning that the mutual tolerance of cells of the two genotypes within the allophenic graft (13) would obviate graft vs. graft and graft vs. host disease and permit successful engraftment with stem cells of both genotypes.

That this is the case is shown in Fig. 6. Groups of 10–11 $F_1$ mice were injected with $1 \times 10^5$, $1 \times 10^6$, or $6 \times 10^6$ marrow cells from a "stable" allophenic 5 mo of age whose marrow chimerism was 55% DBA, 45% B6. The lower cell dose was included to explore the effects of limiting stem cell dilutions on repopulation, and it can be seen that despite a graft nearly evenly split between DBA and B6 cells, B6 stem cells failed to make significant contributions at any time during the study. Although we have not (and can not) directly assayed the number of stem cells of each genotype engrafted, we assume that the the overall marrow chimerism is a good index of relative stem cell numbers. Our previous work showed no difference between stem cell (CFU-S) concentration in the marrows of inbred B6 and DBA mice (7).

In this study, DBA was the predominant red cell genotype initially, but the proportion of DBA red cells declined with time. At the $1 \times 10^5$ cell dose, 7 of 11 mice survived the duration of the experiment and after 2 mo the predominant genotype was the host ($F_1$). The two highest cell doses afforded >80% host survival and portray two themes. First, as the cell dose increased, $F_1$ contributions decreased and were replaced by the B6 genotype. Secondly, the DBA genotype was predominant throughout the study but its proportion declined steadily in the face of rising contributions from the other two genotypes, but particularly from B6. Finally, at the highest cell dose and after 16 mo, the cell population closely approximated the initial makeup of the graft used to repopulate the hosts.

White blood cell chimerism, in addition to the red cell composition, was determined at the last four blood sampling points of this experiment and the results are shown in Fig. 7. In concordance with the red cell picture, white cells of $F_1$ origin decreased as the size of the graft was increased. Similarly, DBA contributions to the white cell pool declined with time after transplant concomitantly with increasing contributions from $F_1$ stem cells ($1 \times 10^5$ cell dose) or B6 stem cells (at the two highest cell doses). A striking difference between red and white cell chimerism is discernible at all three graft sizes, but is most pronounced and statistically significant ($p < 0.001$) at the highest cell dose. The difference is a repetitive pattern at each bleeding in which DBA contributions to erythropoiesis were greater than DBA contributions to myelopoiesis; the pattern was reversed in the case of B6. The skewing of lineage-specific differentiation in a genotype-restricted manner was nearly identical, both qualitatively and quantitatively, to previous results of studies of allophenic mice themselves (Table I) (7).

Table II shows the chimerism of representative hosts from the experiment in Figs. 6 and 7 necropsied 16–18 mo after engraftment. The $1 \times 10^5$ cell dose was chosen because stem cells should be near limiting dilution (14), and this is reflected in the variation (standard error of the mean) of the red and white cell chimerism among hosts within this group (Figs. 6 and 7), and in the variation in the lymphoid and

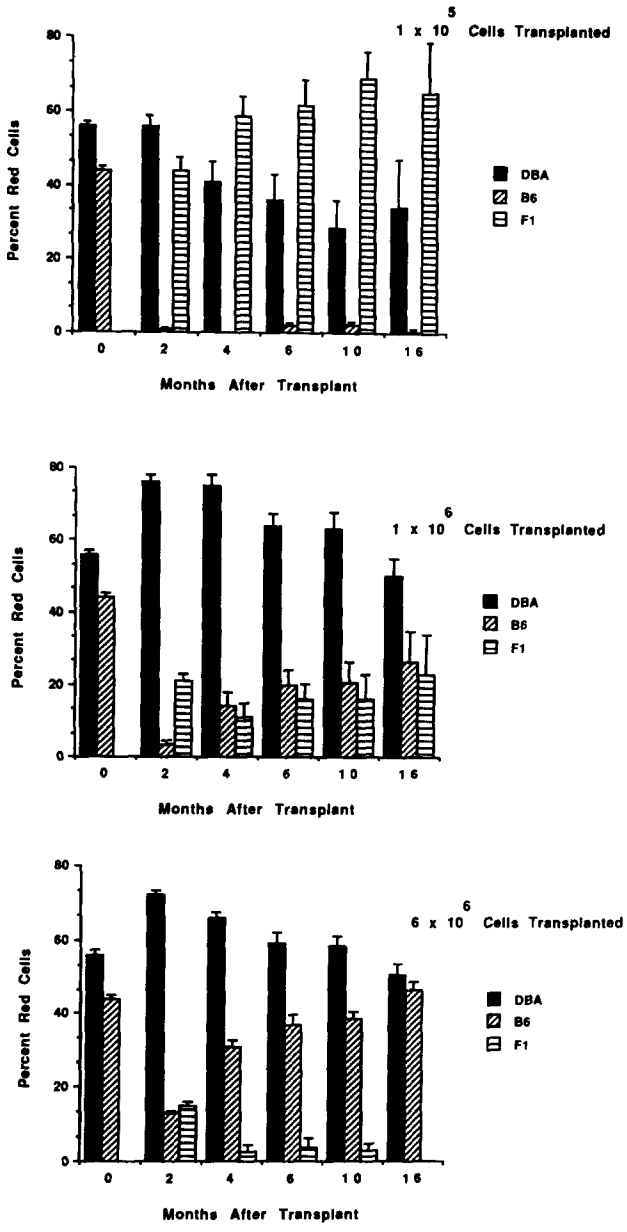



FIGURE 6. Effect of graft size on long-term erythropoiesis in radiation chimeras. Red cell composition was determined by electrophoresing red cell lysates on cellulose acetate membranes, staining for GPI activity and determining the relative DBA, B6, and $BDF_1$ contributions by image analysis of the gel as described in Materials and Methods. The time 0 point represents the composition of the allophenic marrow used to engraft. The error bars at time 0 represent the range in variation of red cell composition of the donor allophenic mice over three previous blood samplings. Bars at all other time points represent the means ± 1 SEM of groups of hosts (10–11) lethally irradiated (12 cGy) and injected with $1 \times 10^5$, $1 \times 10^6$, or $6 \times 10^6$ allophenic marrow cells.

hematopoietic composition of hosts 6 and 1 (Table II *A*). Host 6 was apparently not engrafted by donor hematopoietic stem cells of either genotype. However, cells of the lymph node were one-third DBA and this suggests that a lymphoid-restricted precursor of DBA origin may have been supplying lymphocytes.

Because of the potential importance of the marrow stroma in influencing hematopoiesis (15), we established stromal cell cultures from the marrows of the hosts and

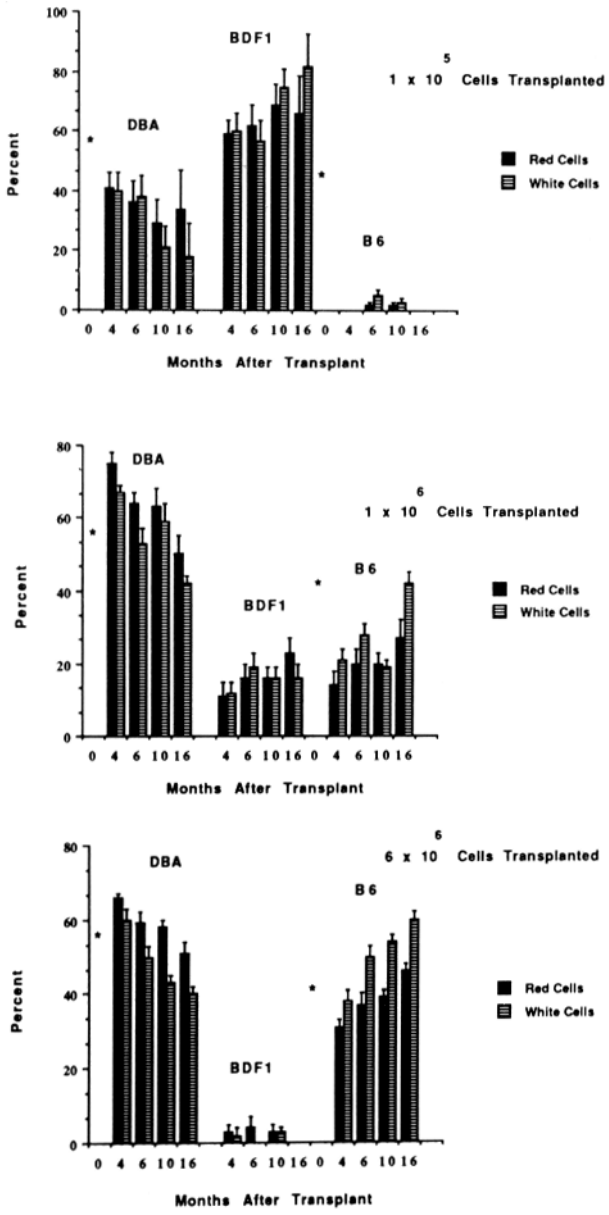



FIGURE 7. Comparison of red and white blood cell populations in groups of radiation chimeras engrafted with different numbers of allophenic marrow cells. White cell composition was determined, as for red cells, by electrophoresing white cell lysates on cellulose acetate membranes, staining for GPI activity, and determining the relative DBA, B6 and $BDF_1$ contributions by image analysis of the gel as described in Materials and Methods. White cells (~70% lymphocytes, 20% granulocytes) were obtained from the interface of a one-step Percoll cut (1.095 g/ml). Bars represent the white and red cell means ± 1 SEM of groups of hosts (10–11) lethally irradiated (12 cGy) and injected with $1 \times 10^5$, $1 \times 10^6$, or $6 \times 10^6$ allophenic marrow cells. The stars at time 0 for DBA and B6 represent the composition of the allophenic graft; there is no $BDF_1$ component to the graft and therefore no time 0 point for the hybrid.

determined the genotypic composition using GPI. After 5 wk of growth, endothelial cells, fat cells, macrophages, and fibroblast-like cells were present. Stromal cell cultures established with bone marrow of host 6 were comprised of 5% DBA cells and 95% cells of $F_1$ origin.

Host 1 demonstrated engraftment by DBA but not B6 stem cells, as well as host contributions. The extent of DBA:$F_1$ chimerism varied widely from organ to organ and among blood cell lineages, ranging from 17% DBA platelets to 84% DBA thymocytes. Stromal cells were about one-third DBA and two-thirds $F_1$.

TABLE II

*Genotypic Composition of Blood and Lymphoid Cells in Hosts Receiving Different Sized Grafts*

| | (A) 1 × $10^5$ Cells injected | | | | | | (B) 1 × $10^6$ Cells injected | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mouse 6 | | | Mouse 1 | | | Mouse 4 | | | Mouse 11 | | |
| | DBA | F1 | B6 | DBA | F1 | B6 | DBA | F1 | B6 | DBA | F1 | B6 |
| Platelets | 0 | 100 | 0 | 17 | 83 | 0 | 22 | 64 | 14 | 46 | 0 | 54 |
| Lymphocytes | 0 | 100 | 0 | 57 | 43 | 0 | 24 | 54 | 21 | 44 | 0 | 56 |
| Granulocytes | 0 | 100 | 0 | 57 | 43 | 0 | 30 | 54 | 15 | 35 | 0 | 65 |
| Red cells | 0 | 100 | 0 | 74 | 26 | 0 | 36 | 54 | 11 | 51 | 0 | 49 |
| Bone marrow | 0 | 100 | 0 | 55 | 45 | 0 | 29 | 37 | 34 | 42 | 0 | 58 |
| Spleen | 9 | 84 | 8 | 60 | 40 | 0 | 34 | 42 | 24 | 36 | 26 | 38 |
| Thymus | ND | ND | ND | 84 | 16 | 0 | 49 | 9 | 42 | 21 | 13 | 66 |
| Lymph node | 33 | 67 | 0 | 54 | 46 | 0 | 52 | 4 | 44 | 19 | 37 | 44 |
| Marrow stroma | 5 | 95 | 0 | 31 | 69 | 0 | ND | ND | ND | ND | ND | ND |

Hosts 4 and 11 in Table II *B* received $10^6$ allophenic marrow cells. At this cell dose B6 stem cells contributed to long-term engraftment, and in the case of host 11, blood cells were exclusively derived from stem cells of donor origin. In host 4, all three genotypes were represented in all tissues; however, host contribution to thymus and lymph node was nearly nonexistent, whereas it comprised fully half of all blood cell types.

One of the important findings of the previous study was that DBA stem cells apparently had an initial competitive advantage during host repopulation. A possible explanation is that hybrid resistance of host effector cells to cells of B6 origin accounts for the apparent DBA advantage. To address this possibility, we injected $F_1$ mice with anti-asialo GM1, an antibody cytotoxic to the effectors of hybrid and natural resistance, NK cells, and cytotoxic T cells (16, 17). A second group of $F_1$ hosts was injected with normal rabbit serum as a control and both groups were lethally irradiated and engrafted with the same pool of allophenic marrow cells. The results are shown in Fig. 8 and demonstrate that the dynamics of donor cell engraftment were virtually identical in the two groups.

The composition of the allophenic marrow grafts used in the graft size experiment was 55% DBA. To test the ability of DBA stem cells to compete during engraftment when they were at a severe numerical disadvantage, we engrafted hosts with allophenic marrow consisting of only 6% DBA cells. Fig. 9 shows that DBA cells did not make significant contributions to erythropoiesis at either graft size used. At the lower cell number (8 × $10^5$), the red cell pool was composed predominantly of host cells throughout the 6-mo study. In contrast, with the larger graft size (8 × $10^6$ cells), B6 erythroid progenitors made significant contributions at the 1-mo time point, and at later bleedings, the red cell pool was increasingly of B6 origin. After 6 mo 75% of the red cells were B6.

*Short-Term Studies of Allophenic Marrow Engrafted into Lethally Irradiated Hosts.* A recurring finding in the long-term studies (with the exception of Fig. 9) was the predominance of DBA hematopoiesis in the first weeks after engrafting $F_1$ hosts. To more carefully document the dynamics of hematopoietic chimerism during this critical time, we engrafted irradiated $F_1$ hosts with allophenic marrow and necropsied groups of hosts at intervals during the first 3 mo (12, 32, 55, and 88 d; Table III).

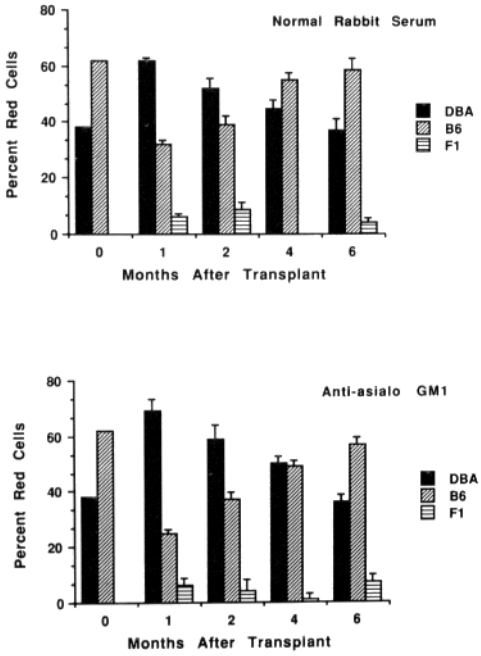



FIGURE 8. Hybrid resistance does not account for genotype-dependent repopulation patterns in radiation chimeras. Allophenic marrow which had the composition shown at the time 0 point was injected ($2 \times 10^6$ cells) into two groups of lethally irradiated (12 cGy) hosts (10 mice/group). One group received 50 $\mu$l of anti-asialo GM1 intravenously 2 d before irradiating and engrafting; the control group received 100 $\mu$l of normal rabbit serum. Red cell composition at the indicated times was determined by electrophoresing red cell lysates on cellulose acetate membranes, staining for GPI activity and determining the relative DBA, B6, and $BDF_1$ contributions by image analysis of the gel as described in Materials and Methods. Paired *t*-tests of DBA and B6 contributions between the control and experimental groups showed no significant differences at any of the time points.

A principal aim of these studies was to determine the extent of chimerism in the erythroid and myeloid progenitor cell populations of the bone marrow. Changes in these populations should provide an early index of changes that would only be obvious days to weeks later in the mature blood cell populations. To this end erythroid progenitor cells (CFU-E and BFU-E), myeloid progenitors (CFU-GM), and mul-

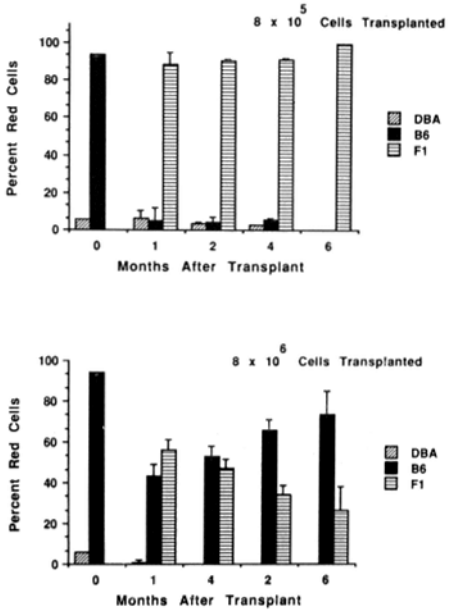



FIGURE 9. Effect of allophenic graft composition on genotypic contributions to repopulation of radiation chimeras. Red cell composition was determined by electrophoresing red cell lysates on cellulose acetate gels, staining for GPI activity and determining the relative DBA, B6, and $BDF_1$ contributions by image analysis of the gel as described in Materials and Methods. The time 0 point represents the composition of the allophenic marrow used to engraft. Bars at all other time points represent the means ± 1 SEM of groups of hosts (10) lethally irradiated (11 cGy) and injected with $8 \times 10^5$ or $8 \times 10^6$ marrow cells.

TABLE III

*Necropsy of Short-Term Engrafted Hosts*

| | Days After Engraftment | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 12 | | | 32 | | | | | | 55 | | | | | | 88 | | | | | | |
| | DBA | F1 | B6 | DBA | | F1 | | B6 | | DBA | | F1 | | B6 | | DBA | | F1 | | B6 | |
| | 1* | 1 | 1 | 1 | 2* | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 |
| Red Cells | 19 | 73 | 8 | 44 | 64 | 56 | 36 | 0 | 0 | 38 | 64 | 62 | 4 | 0 | 32 | 50 | 60 | 48 | 0 | 2 | 40 |
| Platelets | 58 | 42 | 0 | 39 | 76 | 61 | 0 | 0 | 24 | 34 | 56 | 61 | 3 | 4 | 41 | 22 | 55 | 75 | 0 | 3 | 45 |
| Granulocytes | 61 | 39 | 0 | 50 | 51 | 50 | 0 | 0 | 49 | 31 | 57 | 69 | 2 | 0 | 41 | 34 | 44 | 66 | 0 | 0 | 56 |
| Lymphocytes | 53 | 47 | 0 | 56 | 52 | 44 | 0 | 0 | 48 | 23 | 54 | 73 | 6 | 4 | 40 | 19 | 53 | 67 | 0 | 14 | 47 |
| Bone marrow | 57 | 40 | 3 | 35 | 50 | 64 | 0 | 1 | 50 | 44 | 52 | 43 | 3 | 13 | 45 | 13 | 54 | 85 | 0 | 2 | 46 |
| Spleen‡ | 19/25 | 1/25 | 5/25 | 35 | 29 | 65 | 34 | 0 | 37 | 42 | 44 | 51 | 12 | 7 | 44 | 37 | 54 | 63 | 0 | 0 | 46 |
| Thymus | 38 | 0 | 62 | 61 | 56 | 39 | 0 | 0 | 44 | 40 | 35 | 28 | 10 | 32 | 55 | 56 | 50 | 41 | 0 | 3 | 50 |
| Lymph node | 26 | 74 | 0 | 27 | 14 | 73 | 43 | 0 | 43 | 48 | 26 | 45 | 25 | 7 | 49 | 30 | 48 | 63 | 0 | 7 | 52 |
| Stromal Cells | 32 | 68 | 0 | 31 | 27 | 66 | 51 | 3 | 22 | 24 | 24 | 62 | 48 | 15 | 27 | 22 | 30 | 78 | 47 | 0 | 23 |
| Marrow-derived: | | | | | | | | | | | | | | | | | | | | | |
| CFU-E | | | | ND | ND | ND | ND | ND | ND | 73 | 61 | 0 | 0 | 27 | 39 | 18 | 67 | 82 | 0 | 0 | 33 |
| BFU-E | | | | 95 | 48 | 5 | 3 | 0 | 49 | 63 | 72 | 26 | 0 | 11 | 28 | 21 | 70 | 79 | 0 | 0 | 30 |
| CFU-GM | | | | 64 | 52 | 33 | 0 | 3 | 48 | 72 | 78 | 28 | 0 | 0 | 22 | 29 | 86 | 71 | 10 | 0 | 4 |
| CFU-GEMM | | | | 52 | 74 | 48 | 0 | 0 | 26 | 62 | 73 | 38 | 0 | 0 | 27 | 16 | 81 | 84 | 0 | 0 | 19 |
| Spleen-derived: | | | | | | | | | | | | | | | | | | | | | |
| CFU-E | | | | | | | | | | | | | | | | 14 | 56 | 48 | 6 | 9 | 38 |
| BFU-E | | | | | | | | | | | | | | | | 14 | 67 | 86 | 10 | 0 | 24 |
| CFU-GM | | | | | | | | | | | | | | | | 35 | 63 | 60 | 5 | 5 | 32 |
| CFU-GEMM | | | | | | | | | | | | | | | | 36 | 88 | 64 | 0 | 0 | 12 |

* Two graft sizes were used: *1* designates hosts that received $2 \times 10^5$ allophenic marrow cells; *2* designates hosts that received $3 \times 10^6$ cells.

‡ "Spleen" data at the 12-d time represent GPI analyses of individual spleen colonies (12 d CFU-S). At all other times GPI analyses were of a spleen fragment. The allophenic marrow graft was 40% DBA.

tipotent progenitors (CFU-GEMM) were grown from marrow in semi-solid medium in vitro and their genotypic derivation was determined by GPI analysis. A method was devised whereby individual colonies, each derived from a single progenitor, were picked from cultures, and each one's genotype was determined. The sensitivity of this new method permitted genotyping of colonies of 100 cells or more. In addition to the in vitro colonies, we analyzed in vivo spleen colonies obtained from a group of hosts necropsied 12 d after engraftment. Spleen colonies were derived from multipotential stem cells (CFU-S), and along with analysis of CFU-GEMM, may provide a picture of the earliest events after transplant. At 32, 55, and 88 d, two hosts each from groups of mice that had received either $2 \times 10^5$ or $3 \times 10^6$ allophenic marrow cells were necropsied. The data for the 12-d point represent averages of four hosts receiving the smaller sized graft; the data at the other time points are from one of the two mice sampled per group. The allophenic marrow source of the graft was 40% DBA, 60% B6; stromal cells cultured from this marrow were 42% DBA and 58% B6.

12-d spleen colonies were overwhelmingly of DBA origin (19 of 25), whereas mature blood cells were comprised of large numbers of host cells. Those blood cells with shorter lifespans (e.g., platelets and granulocytes) first reflected increasing DBA hematopoiesis, while red cells with a longer lifespan showed only modest DBA contributions. Stromal cells derived from this set of hosts showed only DBA and host components in approximately a 1:3 ratio.

At the later time points, as before, two main themes developed depending on the size of the graft. The $2 \times 10^5$ cell dose first showed major DBA representation, but with time the $F_1$ cells became predominant. The B6 genotype was never represented to any significant degree. Although only one host is shown per time point, it is apparent that the progenitor cell populations, and especially the CFU-GEMM, herald the increasing $F_1$ contributions. As early as 32 d, half of the CFU-GEMM were of $F_1$ origin, whereas 95% of erythroid precursors (BFU-E) were DBA. At 88 d ~80% of the marrow progenitors were $F_1$, while in the blood the red cells remained 50% DBA. CFU-GM and CFU-GEMM grown from spleen showed a generally larger DBA representation than the same progenitors grown from bone marrow. In all of the colony analyses presented here genotypic results are summarized from a total of 19–34 individual colonies analyzed per point.

Hosts receiving the larger graft size were not studied at 12 d because the larger number of injected stem cells resulted in confluent spleen growth and thus precluded analysis of individual colonies. At 32 days, however, the pattern was clear: only donor-derived genotypes were present in the progenitor cell populations. By this time the blood cells, except for red cells, were also exclusively of donor origin. Because of their 45-d lifespan the cohort of host red cells present at the time of engraftment had not yet been eclipsed by donor-derived cells. A second pattern, that of diminishing DBA blood cell contributions and concomitant increases in B6 blood cells, was subtle in this group of hosts but nonetheless apparent, particularly in the red cell population. The B6 contribution rose from 0 at 32 d to 40% at 88 d. The erythroid progenitors (CFU-E and BFU-E) at 32 d were already of nearly half B6 origin, thus heralding the changes in blood composition a month later. Stromal cell populations grown from marrow at each of the time points showed no change with time; at 1 mo all three genotypes contributed in roughly a 1:2:1 (DBA:$F_1$:B6) ratio, and there they

remained through 3 mos. The CFU-GM and CFU-GEMM populations in both marrow and spleen remained largely (~80%) of DBA origin up to 88 d.

## Discussion

*Changing Genotypic Contributions during Aging of Allophenic Mice Reveal Intrinsic Stem Cell Differences.* During aging of DBA/2 ←→ C57BL/6 allophenic mice, the B6 stem cell population eclipsed its DBA counterpart. This is evidenced by a shift toward the B6 genotype in all blood cell lineages and lymphoid populations, but not other tissues. The use of allophenic mice permits the simultaneous study of two stem cell populations under identical conditions and our results using this experimental model demonstrate intrinsic differences in aging of genetically distinct stem cells. It may or may not be a coincidence that the B6 strain is significantly longer lived than is the DBA strain (8). In the mouse, and by extension in humans, longevity is certainly related to the ability to resist disease and neoplasia. One might imagine that longevity and functional abilities of hematopoietic stem cells would have a direct bearing on longevity of the organism.

The mouse strains used to construct the embryo aggregates have previously been studied and it was found that their stem cell populations differed with respect to cell cycle kinetics; the population that waned in the allophenics (DBA) was the one normally with a larger proportion in cell cycle (7). Regulation of this phenotype was proposed to reside in a genetic locus designated *Stk*, for stem cell kinetics. Our current results raise the possibility that the number of divisions of which a stem cell is capable is finite and subject to the constraints on cell proliferation proposed by Hayflick (18) for fibroblasts. An alternative explanation for our findings is that during aging the mutual immune tolerance of the composite cells breaks down and the DBA genotype is selectively eliminated, and the B6 progenitor cells thus have a selective growth advantage. Such a mechanism has been proposed (19), but its existence remains controversial. Arguing against this explanation are numerous studies of Mintz and her colleagues demonstrating that in allophenics constructed of several inbred strains, tolerance, measured by a number of immunological criteria, was permanent (13, 20), despite a wide variety of antigenic differences (including H-2 incompatibility) between the partner strains.

Behringer et al. (21) have studied the blood cell composition of allophenic mice constructed using two congenic B6 inbred strains differing at only a few loci, including *Fv-2*, *Gpi-1*, and the minor histocompatibility loci *H-7* and *H-24*. Over the 14-wk period of study there was virtually no fluctuation in the chimerism of red cells, platelets, and lymphocytes in any of the 16 allophenic mice. The stability of chimerism of these allophenics stands in contrast to the fluctuations in blood cell chimerism reported in this paper and in results of Warner et al. (22) in which allophenics were compounded from embryos with many genetic differences, including ones at *H-2*. It is a formal possibility that the reproducible changes in blood cell chimerism reported here both in long-term studies of allophenics and in radiation chimeras engrafted with allophenic marrow were due to differences in alleles important in determining histocompatibility. Our hypothesis, however, is that other genetic differences, notably a difference in *Stk* alleles, is responsible at least in part for our results. In order for histocompatibility differences to account for our results in the radiation chimeras, one would have to invoke first a suppression of B6 stem cell clones

early during engraftment followed by a suppression of DBA stem cell clones later; this is to account for the early DBA predominance in recipients followed by B6 preeminence. Perfectly consistent with our hypothesis involving the importance of putative *Stk* alleles are the recent results of Schaefer and Dewey (23) in which a mixture of H-2 matched marrow cells from a congenic DBA/2 strain and a recombinant inbred strain, BXD-31 derived from (C57Bl/6 × DBA/2)$F_1$ hybrids, were engrafted in lethally irradiated H-2-matched hosts. Shifting patterns of hematopoiesis in the recipients favored cells derived from the DBA/2 stem cell pool over BXD-31 stem cells, findings that we interpret to suggest that the BXD-31 strain inherited the C57BL/6-derived *Stk* alleles, thus providing a competitive advantage to the DBA/2 stem cells in the graft.

*Allophenic Marrow Transplantation Reveals Genotype-restricted Growth Advantages at Different Times during Engraftment.* Transplantation of allophenic bone marrow into irradiated hosts has served to reveal another important difference in the functioning of DBA and B6 stem cells: DBA stem cells have an initial competitive advantage in the repopulation process. This advantage is seen irrespective of the extent of chimerism of the graft, except when the DBA component is less than ~10% (Fig. 9). These results are consistent with, but do not prove, the notion that a stem cell population characterized by a cell-autonomous proliferative advantage also has an advantage during initial phases of repopulation. However, as in the allophenics themselves, the B6 stem cell pool had a long-term advantage in maintaining hematopoiesis.

It should be noted that $F_1$ stem cells express the same *Stk* phenotype as DBA stem cells; i.e., the allele(s) regulating the high cycling rate appear to be dominant (7). This may hold at least part of the explanation as to why B6 stem cells are at a selective disadvantage when competing against both DBA and $F_1$ stem cells under conditions when the graft size was small (Figs. 6, 7, and 9).

*Progenitor Cells Assayed In Vitro Predict Changing Blood Chimerism after Engraftment.* A third point concerns the short-term studies of hematopoiesis in radiation chimeras. These studies represent the first detailed genetic analysis of chimeric progenitor populations at the level of individual progenitor cells. 12 d after transplant the majority of colonies on the spleen surface were of DBA origin, in keeping with the predominately DBA picture we would subsequently see in hematopoietic progenitors of the bone marrow and spleen, and in blood cells after several weeks. Analysis of the genotypes of myeloid and erythroid precursor cells grown in vitro reflects the early DBA predominance, and after a month, shows an increasing number of B6 progenitors that herald the shift toward B6 of blood cells at later times. It is noteworthy that CFU-GEMM, a multilineage colony forming cell and presumably more primitive than the erythroid (BFU-E and CFU-E) and myeloid (CFU-GM) committed cells, is the first population to show changes in genotypic composition. This finding, to our knowledge, is the first demonstration that CFU-GEMM assayed in vitro provide a direct reflection of the status of in vivo hematopoiesis. It is somewhat surprising that the CFU-GM population does not quickly predict changes in myeloid composition in light of the fact that granulocytes turn over rapidly in the circulation and granulocytes were skewed toward the B6 genotype (Fig. 7). One might have predicted that genotype-specific lineage skewing would have been reflected in the numbers of CFU-GM of B6 origin. It may be that the skewing is due to genotype-restricted differ-

ences in granulocyte differentiation and maturation distal in the developmental pathway to the CFU-GM precursors.

*Hybrid Resistance Is Abrogated when Allophenic Marrow Is Engrafted.* Of major concern to us when we initially adopted the idea of transplanting allophenic marrow into $F_1$ hosts was the known rejection of parental strain hematopoietic cells through the mechanism of hybrid resistance (reviewed in reference 24). B6, and to a lesser extent DBA, marrow cells are subject to rejection by $BDF_1$ hosts via this mechanism, and in pilot studies using a range of graft sizes we proposed to use in our allophenic work, we confirmed that inbred strain B6 cells did not engraft well, whereas DBA marrow cells from an inbred mouse did. As seen throughout this report, we found that B6 cells from an allophenic source engrafted well. In fact, in most experiments, they were the predominant stem cell source over the long term. To directly demonstrate that hybrid resistance was not a factor in the dynamic patterns of chimerism in the engrafted hosts, we injected a cytolytic antibody (anti-asialo GM1) against the cellular effectors of hybrid resistance to abrogate any potential effects of this rejection mechanism. The protocol used, including the dosage of antibody, has previously been shown to eliminate natural resistance to allogeneic marrow grafts (25). We do not at this point understand the mechanism by which B6 cells in an allophenic graft are rendered invisible to hybrid resistance or, alternatively, how an allophenic graft inactivates the rejection mechanism.

*Stromal Cells in Radiation Chimeras Are of Both Donor and Host Origin.* Our results with stromal cells deserve further comment. The literature is replete with reports supporting (26) and refuting (27) the notion that stroma may be donor derived. Under the conditions used, our results clearly show donor cell representation in long-term stromal cell cultures. We did not attempt to analyze the genotypic composition of the cell types comprising the stromal monolayer in vitro and it is therefore possible that one population, macrophages for example, were routinely donor derived, whereas other components (adipocytes, endothelial cells) may have been exclusively of host origin. Perkins and Fleischman (28) have shown that endothelial cell and adipocyte components of the stroma remain of host origin, whereas the macrophage component may be of donor origin. Nonetheless, donor-derived components of the stromal microenvironment may play an important role, perhaps even an instructional one, in in vivo hematopoiesis of radiation chimeras.

## Summary

We have studied contributions to hematopoiesis of genetically distinct stem cell populations in allophenic mice. Chimeras were made by aggregating embryos of inbred strains known to differ with respect to stem cell population kinetics. One partner strain (DBA/2) has previously been shown to normally have a stem cell (CFU-S) population of which 24% are in S-phase of the cell cycle, whereas the homologous population of the other partner strain (C57BL/6) was characterized by having only 2.6% in cycle (7). Contributions of the chimeric stem cell population to mature blood cell pools were studied throughout the life of the mice and intrinsic differences in stem cell function and aging were reflected in dynamic patterns of blood cell composition. The DBA/2 stem cell population was eclipsed by stem cells of the C57BL/6 genotype and, after 1.5–3 yr, the hemato-lymphoid composition of 22 of 27 mice

studied for this long had shifted by at least 25 percentage points toward the C57BL/6 genotype. 8 of the 27 had hematolymphoid populations solely of C57BL/6 origin.

To test whether or not a population of stem cells with an inherently higher cycling rate (DBA/2) might have a competitive advantage during repopulation, we engrafted allophenic marrow into lethally irradiated (C57BL/6 × DBA/2)$F_1$ recipients. DBA/2 hematopoiesis was predominant early, far outstripping its representation in the marrow graft. Perhaps as a consequence of inherently greater DBA/2 stem cell proliferation, the populations of developmentally more restricted precursor populations (CFU-E, BFU-E, CFU-GM, CFU-GEMM) showed an overwhelming DBA/2 bias in the first 2–3 mo after engraftment. However, as in the allophenic mice themselves during the aging process, the C57BL/6 genotypic representation was ascendant over the subsequent months. The shift toward C57BL/6 genotype was first documented in the marrow and spleen precursor cell populations and was subsequently reflected in the circulating, mature blood cells. Bone marrow–derived stromal cell cultures from engrafted mice were studied and genotypic analyses showed donor representation in stromal cell populations that reflected donor hematopoietic contributions in the same recipient. Results from these studies involving two in vivo settings (allophenic mice and engraftment by allophenic marrow) are consistent with the notion that a cell autonomous difference in stem cell proliferation confers on one population a competitive repopulating advantage, but at the expense of longevity.

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