Previously undetected human hematopoietic cell populations with short-term repopulating activity selectively engraft NOD/SCID-β2 microglobulin-null mice

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Increasing use of purified or cultured human hematopoietic cells as transplants has revealed an urgent need for better methods to predict the speed and durability of their engraftment potential. We now show that NOD/SCID-β2 microglobulin-null (NOD/SCID-β2m−/−) mice are sequentially engrafted by two distinct and previously unrecognized populations of transplantable human short-term repopulating hematopoietic cells (STRCs), neither of which efficiently engraft NOD/SCID mice. One is predominantly CD34+CD38+ and is myeloid-restricted; the other is predominantly CD34+CD38− and has broader lymphomyeloid differentiation potential. In contrast, the long-term repopulating human cells that generate lymphoid and myeloid progeny in NOD/SCID mice engraft and self-renew in NOD/SCID-β2m−/− mice equally efficiently. In short-term expansion cultures of adult bone marrow cells, myeloid-restricted STRCs were preferentially amplified (greater than tenfold) and, interestingly, both types of STRC were found to be selectively elevated in mobilized peripheral blood harvests. These results suggest an enhanced sensitivity of STRCs to natural killer cell–mediated rejection. They also provide new in vivo assays for different types of human STRC that may help to predict the engraftment potential of clinical transplants and facilitate future investigation of early stages of human hematopoietic stem cell differentiation.


Introduction

Blood cells are generated throughout adult life from a tiny subpopulation of undifferentiated stem cells. In adults, these stem cells are concentrated in the bone marrow (BM), although at birth they are also present in the blood in relatively high numbers (1–4). Because hematopoietic stem cells can enter the BM from the circulation at high efficiency (5, 6) the intravenous transplantation of adult BM cells and, more recently, of mobilized peripheral blood (mPB) and cord blood (CB) cell harvests have become an important therapeutic modality for patients with a broad spectrum of malignant and genetic disorders. Nevertheless, in many instances of either allogeneic or autologous stem cell therapy, undesirable patterns of hematologic recovery are obtained (7). It is therefore critical to identify the various types of human hematopoietic cells that are transplantable and to determine how changes in a given inoculum will affect the kinetics and durability of engraftment it will give so that these parameters can be predicted with confidence, particularly for transplants that have been manipulated previously ex vivo to expand, purge, or genetically modify the cells originally present.

Previous studies in mice have distinguished several distinct classes of hematopoietic cells with different engraftment properties. Long-term repopulating cells (LTRCs) have lifelong ability to produce all blood cell types and generate progeny that display similar potentials upon transfer to secondary and even tertiary recipients (8–11). Other cells with similar differentiation potentials may reconstitute both myeloid (M) and lymphoid (L) compartments, but typically for less than 4 months (9, 12). Additional types of short-term repopulating cells (STRCs) that are either myeloid or lymphoid restricted have also been identified in the mouse (13–15).

In vitro studies have provided considerable evidence of parallel hierarchical structures in the human and murine hematopoietic systems (16), and recent studies of sheep transplanted in utero with human hematopoietic cells have suggested the existence of distinct populations of transplantable human cells (17). However, both in vitro approaches and human-sheep xenografts have limitations, and neither has yet proven to be useful clinically. The ability of human hematopoietic cells from multiple sources to engraft the BM of sublethally irradiated NOD/LtSz-Prkd
**Methods**

Hematopoietic cells. Normal adult BM cells from cadaveric sources were obtained from the Northwest Tissue Center (Seattle, Washington, USA). Samples of CB from normal, full-term infants delivered by caesarian section in the animal facility of the British Columbia Cancer Research Centre (Vancouver, British Columbia, Canada) in microisolator cages and provided with autoclaved food and water. Mice were irradiated at 8–10 weeks of age with 350 cGy of $^{137}$Cs x-rays and thereafter received acidified water containing 100 mg/L ciprofloxacin (Bayer AG, Leverkusen, Germany). Test cells were injected intravenously with $^{10}$G irradiated (15 Gy) normal human BM cells as carrier cells within a few hours after the mice were irradiated. The presence of human cells in the BM of mice was determined using FACS analysis of cells harvested from the femurs and tibias after 10–16-week-old abortus material after dispase digestion as described (29). The procurement and use of all human cells was undertaken according to approved protocols, including obtaining appropriate informed consent. Low-density cells (<1.077 g/ml) were cryopreserved (19) and the lin− fraction obtained from thawed cells using StemSep columns (StemCell Technologies, Vancouver, British Columbia, Canada). Cells expressing CD34 were isolated from mobilized peripheral blood samples before cryopreservation using the CliniM ACS device (Miltenyi, Moenchen Gladbach, Germany). In some experiments, highly purified propidium iodide-negative (PI−) CD34+CD38− and CD34+CD38+ BM cells were isolated from the lin− fraction using a FACStar Plus cell sorter equipped with a 5-W argon laser and a 30-mW helium neon laser (Becton Dickinson, San Jose, California, USA) as described previously (3).

Short-term suspension cultures. Purified CD34+CD38− and CD34+CD38+ BM cells were cultured for 5 days in serum-free medium (BIT; StemCell Technologies) supplemented with $10^{-4}$ M 2-mercaptoethanol (Sigma Chemical Co., St. Louis, Missouri, USA), 40 μg/ml LDLs (Sigma Chemical Co.), and the following five purified recombinant human growth factors: 20 ng/ml IL-3 (Novartis, Basel, Switzerland), 20 ng/ml IL-6 (Cangene Corp., Mississauga, Ontario, Canada), 20 ng/ml G-CSF (StemCell Technologies), 100 ng/ml Steel factor (SF) (purified from media conditioned by COS cells that had been transiently transfected with human SF cDNA), and 100 ng/ml Flt3-ligand (FL) (Immunex Corp., Seattle, Washington, USA). After 48 hours, the cultures were diluted twofold by adding an equal volume of fresh medium and the same five growth factors. CD34+ CB cells were cultured for 16 hours in serum-free medium supplemented with 50 ng/ml thomboietin (Genetech Inc., Palo Alto, California, USA), washed twice, and then cultured for an additional 4 days in serum-free growth factor–supplemented medium to reproduce conditions described previously (30).

Isolation of Gμ/Gδ and S/Gδ/M cells from cultures of CB cells. DNA staining of viable cells was performed as described previously (30). Briefly, after being washed in HBSS with 2% FCS (HF), cells were stained for 90 minutes at 37°C in HF containing 10 μmol/L Hoechst 33342 (Molecular Probes Inc., Eugene, Oregon, USA), washed twice again in HF plus 10 μmol/L Hoechst 33342 and 1 μg/ml PI, resuspended in HF plus 10 μmol/L Hoechst 33342, and the Gμ/Gδ and S/Gδ/M cells were then sorted according to their DNA content (as illustrated in Figure 3).

Xenotransplantation of hematopoietic cells. NOD/SCID and NOD/SCID-$β^2m^{−/−}$ mice were bred and maintained in the animal facility of the British Columbia Cancer Research Centre (Vancouver, British Columbia, Canada) in microisolator cages and provided with autoclaved food and water. Mice were irradiated at 8–10 weeks of age with 350 cGy of $^{137}$Cs x-rays and thereafter received acidified water containing 100 mg/L ciprofloxacin (Bayer AG, Leverkusen, Germany). Test cells were injected intravenously with $^{10}$G irradiated (15 Gy) normal human BM cells as carrier cells within a few hours after the mice were irradiated. The presence of human cells in the BM of mice was determined using FACS analysis of cells harvested from the femurs and tibias after first blocking Fc receptors with human serum and an antimouse Fc receptor Ab (2.4G2), then by staining with mAb's against human CD34 (8G12), CD71 (OKT9), glycoporphin A (10F7; kindly provided by P.M. Landsorp), CD15, CD19, CD20, CD45 (from Becton Dickinson),
and CD41a and CD66b (from Pharmacia Biotech, Baie d'Urfe, Quebec, Canada), as described (23). Levels of nonspecific staining were established by parallel analyses of cells incubated with irrelevant isotype-matched control Ab's labeled with the same fluorochromes. Positive events were counted using gates set to exclude more than 99.99% of events in the negative-control analyses. Poisson statistics and the method of maximum likelihood were used to calculate frequencies of human repopulating cells from proportions of negative mice using the L-calc software (SternCell Technologies).

Statistical analyses. Comparisons were made using Student's t test.

**Results**

Human lin– BM cells engraft NOD/SCID-β2m–/– and NOD/SCID mice with different kinetics. As shown in Figure 1a, when normal adult lin– BM cells were transplanted into sublethally irradiated NOD/SCID-β2m–/– and NOD/SCID mice, more human cells were present in the BM of the NOD/SCID-β2m–/– mice at all times, analyzed up to 13 weeks after transplant (P < 0.03). However, the difference between the levels of engraftment obtained was not constant and was most pronounced (approximately 30-fold; P < 0.01) at the earliest time point examined (3 weeks after transplant). By 6 weeks after transplant this difference had decreased to be eightfold and by 13 weeks was only fourfold. The large difference seen at 3 weeks after transplant was due primarily to the presence in the NOD/SCID-β2m–/– mice of a much larger population of human glycophorin A+ erythroid cells, CD41+ megakaryocytic cells, and CD15/66b+ granulopoietic cells (P < 0.01; Figure 1, b–d). However, similar differences were seen when the numbers of human CD34+ cells and CD19/20+ B-lymphoid cells were compared, although at this time the latter were detectable only in occasional mice of either genotype. Subsequently, human B-lymphoid cells became predominant, and maturing human erythroid cells were rarely detected in either type of recipient. These findings suggested an enhanced ability of human STRCs with myeloid-restricted differentiation potential to engraft NOD/SCID-β2m–/– mice.

Different types of human cells engraft NOD/SCID-β2m–/– mice and NOD/SCID mice. Two approaches were used to address this question. In the first, we compared the frequencies of cells responsible for the engraftment seen in the two different types of recipients 3, 6, and 13 weeks after transplant by using Poisson statistics to calculate the frequency of CD34+ cells in the injected lin– BM cells. As shown in Table 1, the frequency of cells able to repopulate NOD/SCID-β2m–/– hosts for 3 weeks was approximately 30-fold higher than the frequency of cells able to repopulate NOD/SCID mice assessed after the same period (P < 0.01), i.e., a factor similar to that seen when total engraftment levels in the two recipient genotypes were compared (Figure 1a). Moreover, approximately half of the 3-week engrafted mice had been injected with low numbers of any type of human repopulating cell (RC) contained only human myeloid cells (erythroid, megakaryocytic, and granulopoietic, i.e., no human lymphoid cells), whereas the other half of the engrafted mice in these groups contained both. In contrast, at later times most of the mice in this analysis that were engrafted with human cells contained myeloid and lymphoid cells, although the frequency of human cells with this potential was much higher (P < 0.05) when NOD/SCID-β2m–/– mice were used as recipients, and NOD/SCID-β2m–/– mice were also more frequently engrafted only with B-lymphoid human cells.

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**Figure 1**

Different engraftment kinetics of human cells in NOD/SCID-β2m–/– and NOD/SCID mice. Groups of recipients were sacrificed 3, 6, and 13 weeks after transplantation, and the types and numbers of human cells present in the BM were determined by FACS analysis. (a) Total human CD45/71+ cells in NOD/SCID-β2m–/– mice (filled symbols, 13–14 mice/point) and NOD/SCID mice (open symbols, 15–16 mice/point) were calculated from data pooled from two independent experiments. (b) Absolute numbers of cells belonging to different human lineages present at different times. (c) Relative distribution of different types of human cells present after different periods in NOD/SCID-β2m–/– (filled bars) and NOD/SCID mice (open bars, same experiments as in b). Data for the 3-week NOD/SCID mice are not shown in this panel because of the low numbers of human cells present in these mice at this early time point. (d) Representative FACS profile of cells harvested from the BM of a NOD/SCID-β2m–/– mouse 3 weeks after transplantation of 2.5 × 10^6 human lin– BM cells. Note the high number of human erythroid (glycophorin A+) and megakaryocytic (CD41+) cells. Values for all figure parts are the mean ± SEM. ▲Significant difference, P < 0.05.
As a second approach we asked whether the cells that engraft NOD/SCID-β2m−/− mice after 3 and 8 weeks could be distinguished phenotypically. For this we analyzed the repopulating activity of the CD38+ and CD38− subsets of lin− CD34+ cells since NOD/SCID RCs are known to be CD34+CD38− primarily (3, 21). As shown in Figure 2, the CD38+ subset of lin− CD34+ adult human BM cells was responsible for 85% of the 3-week repopulating activity in NOD/SCID-β2m−/− mice. Conversely, 90% of the human cells present after 8 weeks was generated from CD34+CD38− cells. Limiting dilution analysis of the frequency of 3-week NOD/SCID-β2m−/− RCs yielded a value of 8 ± 2 per 106 CD34+CD38− adult BM cells.

The seeding efficiency and subsequent expansion in vivo of cells that repopulate NOD/SCID-β2m−/− mice for 6 weeks is similar in NOD/SCID-β2m−/− and NOD/SCID mice. We next sought to determine whether NOD/SCID-β2m−/− mice might simply be more efficient in their ability to support the engraftment and proliferation of the same type of lymphoid RCs that engraft NOD/SCID mice. For this we first compared the seeding efficiency in NOD/SCID-β2m−/− and NOD/SCID mice of human cells that repopulate NOD/SCID mice for 6–8 weeks. The very low frequency of these cells in adult human BM (Table 1 and refs. 4 and 23) precluded the use of adult BM. Because of the relatively higher numbers (and self-renewal activity) of NOD/SCID RC in human fetal liver (23), the latter source was used for these particular experiments. Accordingly, in each of three experiments 2 × 107 low-density human fetal liver cells were injected into groups of NOD/SCID-β2m−/− and NOD/SCID mice (six each). Twenty-four hours later, the BM cells from the femurs and tibias of all mice in each group were harvested and pooled. FACS analysis of the small aliquot consistently showed less than 0.01% human CD45/71+ cells to be present, as has been reported previously (20). The majority of the cells in each pool were injected into a total of six secondary NOD/SCID recipients (three with 30% each, three with 3% each). Six weeks later, four of nine (30% dose) and one of nine (3% dose) of the secondary recipients of cells from primary NOD/SCID mice contained both human lymphoid (CD34+CD19+20+) and myeloid (CD15/66b+) cells (greater than five each per 106 events analyzed). The corresponding results for the NOD/SCID recipients of cells from primary NOD/SCID-β2m−/− mice were six of nine and one of nine positive mice. The number of NOD/SCID lymphoid/megakaryocytic (CD41+) cells, and 16 ± 4% granulopoietic (CD15/66b+) cells.

As a third approach we asked whether the cells that engraft NOD/SCID-β2m−/− mice after 3 and 8 weeks could be distinguished phenotypically. For this we analyzed the repopulating activity of the CD38+ and CD38− subsets of lin− CD34+ cells since NOD/SCID RCs are known to be CD34+CD38− primarily (3, 21). As shown in Figure 2, the CD38+ subset of lin− CD34+ adult human BM cells was responsible for 85% of the 3-week repopulating activity in NOD/SCID-β2m−/− mice. Conversely, 90% of the human cells present after 8 weeks was generated from CD34+CD38− cells. Limiting dilution analysis of the frequency of 3-week NOD/SCID-β2m−/− RCs yielded a value of 8 ± 2 per 106 CD34+CD38− adult BM cells.
ence of human lymphoid and myeloid cells in their marrow 6 weeks later to provide a measure of the number of NOD/SCID lymphomyeloid RC originally injected into each group of mice used for the seeding efficiency determinations. The pooled data from all three experiments showed the seeding efficiency values to be similar for the two different types of primary recipients (i.e., 1.4% and 2.5% in the primary NOD/SCID-β2m−/− mice and NOD/SCID mice, respectively).

We also compared the ability of NOD/SCID lymphomyeloid RC to expand their numbers in the two different host genotypes. In this case, low-density human fetal liver cells (containing approximately 10^5 CD34+ cells per recipient) were again injected into primary NOD/SCID-β2m−/− mice or NOD/SCID mice (three each in two experiments), but secondary transplants into NOD/SCID mice (only) were performed 4 weeks rather than 24 hours later (7% 1.3% and 2% of the contents of six femurs and six tibias per secondary mouse, nine secondary mice per group in total). Six weeks later the numbers of secondary mice that contained both human lymphoid and myeloid cells were six of nine versus five of nine, one of nine versus one of nine, and zero of eight versus zero of nine, respectively, for the different cell doses. From these data, the frequency and hence the number of 6-week NOD/SCID lymphomyeloid RC regenerated in the two types of primary hosts were again found to be similar (three and four per 10^5 CD34+ cells injected into NOD/SCID and NOD/SCID-β2m−/− primary recipients, respectively; P > 0.05). Thus both the seeding efficiency and the self-renewal behavior of human stem cells that engraft NOD/SCID mice is duplicated, but not enhanced, in NOD/SCID-β2m−/− mice. Therefore, the higher numbers of human lymphoid and myeloid cells seen in NOD/SCID-β2m−/− mice for up to 13 weeks after transplant are more likely to be indicative of a second type of human RC with lymphomyeloid differentiation potential and one that is poorly able to repopulate NOD/SCID mice.

Additional evidence to support this hypothesis was provided by experiments with human CB cells that had been stimulated to proliferate in vitro. Previous studies have shown that proliferating murine STRCs and LTRCs differ in their ability to engraft syngeneic hosts as they progress through the cell cycle, the engraftment ability of LTRCs being severely compromised during their passage through S/G2/M, whereas proliferating STRCs are little affected (31–33). We have shown recently that proliferating human CB stem cells also show a lack of repopulating activity in NOD/SCID mice as they transit S/G2/M (30). However, in the present studies we found no such deficiency when proliferating CB cells were assessed for their ability to engraft NOD/SCID-β2m−/− mice for 6 weeks. Thus, as shown in Figure 3, the 6-week NOD/SCID-β2m−/− lymphomyeloid repopulating activity (both in terms of the relative proportions of engrafted mice, 56% versus 44%, and in the levels of engraftment attained, 7.2% versus 4.2%) was distributed between the G0/G1 and S/G2/M fractions equally for the two different types of primary recipients (i.e., 7.2% and 4.2% in the primary NOD/SCID-β2m−/− mice and NOD/SCID mice, respectively).

Table 2

<table>
<thead>
<tr>
<th>Source of cells</th>
<th>Total number of human cells regenerated (10^4)</th>
<th>3 weeks (% glycophorin A)</th>
<th>6–8 weeks (% CD34 CD19 20)</th>
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<tbody>
<tr>
<td>mPB</td>
<td>10 ± 0.6 (100)</td>
<td>3.8 ± 2.4 (87)</td>
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<tr>
<td>BM</td>
<td>1.2 ± 0.3 (62)</td>
<td>7.6 ± 2.1 (62)</td>
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<tr>
<td>CB</td>
<td>7.0 ± 2.1 (69)</td>
<td>23.8 ± 7.7 (79)</td>
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*aCD34+ cell-enriched samples from the different sources indicated. *Number of human CD34+T1+ cells per mouse BM per 10^6 CD34+ human cells injected. Values represent the mean ± SEM from three mPB, two BM, and four CB experiments.
The engraftment ability of LTRCs is restricted to the G0/G1 phases of cells (22, 26) as well as cells expressing CD34 but not CD38 (3, 21).

Figure 4
Model indicating a hierarchy of transplantable human hematopoietic cells with distinct biological properties. LTRCs include CD34 CD38– cells (22, 26) as well as cells expressing CD34 but not CD38 (3, 21). The engraftment ability of LTRCs is restricted to the G0/G1 phases of the cell cycle (30) and are the only human cells that efficiently engraft NOD/SCID mice. STRC-ML are CD34+CD38–, and their ability to engraft NOD/SCID-β2m–/– mice is not cell cycle-restricted. Most freshly isolated STRC-M express both CD34 and CD38.

their content of cells that repopulate NOD/SCID mice for 6–8 weeks (4, 34). It was, therefore, of interest to compare the levels of engraftment obtained after 3 and 6–8 weeks in NOD/SCID-β2m–/– mice transplanted with CD 34+ cell–enriched populations isolated from these three different sources of human cells. As shown in Table 2, in all groups the level of human engraftment was higher at the later time point, although the difference between 3 and 6–8 weeks was specific for each source of cells. In addition, in all groups most of the human cells seen after 3 weeks were again primarily erythroid (glycophorin A+), whereas after 6–8 weeks, all engrafted mice contained both lymphoid and myeloid human cells.

Selective expansion of human stem cells with short-term repopulating activity in short-term cultures of human BM. Currently, much effort is focused on the identification of culture conditions that will allow the pace of hematologic recovery in recipients of cultured transplants to be accelerated. To determine the extent to which human cells with rapid repopulating activity may be expanded in vitro and to characterize the phenotype of their precursors, CD34+CD38– and CD34+CD38+ cells were isolated from adult human lin– BM cells using FACS, and then aliquots were transplanted into NOD/SCID-β2m–/– mice before and after being maintained in a serum-free expansion culture for 5 days (with FL, SF, IL-3, IL-6, and G-CSF). This resulted in an overall tenfold increase in early (3-week) NOD/SCID-β2m–/– mouse engrafting activity (P < 0.01), approximately half of which was generated from the CD34+CD38– subset (Figure 2). In contrast, the level of engraftment achieved after 8 weeks from the cultured human BM cells was maintained in one experiment and declined approximately 30-fold in the other. In both instances the expanded NOD/SCID-β2m–/– RC were generated primarily, albeit not exclusively, from the population of initially CD34+CD38– cells.

Discussion
The use of xenogeneic hosts for analyzing the transplantable compartment of human hematopoietic cells is of pivotal importance to future clinical and experimental studies. Here we show that sublethally irradiated NOD/SCID-β2m–/– mice allow the efficient engraftment of two previously undescribed populations of human STRCs that engraft very poorly in the more widely used NOD/SCID mouse. We have designated these as STRC-M and STRC-ML to reflect the different lineage potentials these cells display in addition to their transient engrafting activity in NOD/SCID-β2m–/– mice (Figure 4). It is important to note that because of the reduced terminal differentiation and poor peripheralization of all human hematopoietic cells that mature in the BM of either of these mouse strains, the characterization of their precursors requires analysis of the progeny they generate in the murine BM microenvironment.

Evidence for multiple classes of human stem cells with engrafting potential detectable in NOD/SCID-β2m–/– mice. Time-course studies of a large series of NOD/SCID-β2m–/– mice transplanted with multiple sources of human hematopoietic cells, including both fresh and cultured cells, provided the first indication that these mice support a broader range of transplantable human cells than those that reconstitute the closely related NOD/SCID mouse. A hallmark of human STRC-M is the large and rapid but transient burst of erythroid cells they produce in the first 3 weeks after transplant, although analyses of oligoclonally repopulated mice showed that these cells also consistently produce detectable numbers of granulocytes and megakaryocytes (but not lymphoid cells). Most STRC-Ms isolated directly from normal adult human BM were shown to express CD38 and could be rapidly amplified in short-term (5-day) cultures from CD34+CD38+ as well as CD34+CD38– precursors. These features indicate a stage of human hematopoietic stem cell differentiation characterized by a lack of self-renewal activity and lymphopoietic potential, most likely analogous to murine progenitor in mice recently described (15).

The evidence for a second type of human STRC (with lymphoid as well as myeloid differentiation potential, but distinct from the human cells that regenerate the lymphoid and myeloid progeny seen after 6 weeks in NOD/SCID mice) is derived from a different set of observations. The first of these indicated a difference in the kinetics of human lymphomyeloid engraftment of NOD/SCID-β2m–/– and NOD/SCID mice. This reached a much higher peak after 6–8 weeks in the NOD/SCID-β2m–/– hosts, which then declined more rapidly so that the total level of engraftment in the two strains was increasingly similar by 13 weeks. In addition, the frequency
of 8- to 13-week human RC was different in the two types of recipients despite the fact that cells able to engraft NOD/SCID mice were found to home to the marrow of NOD/SCID-β2m–/– mice with similar efficiency. We also found that the amplification of NOD/SCID RC is not enhanced in NOD/SCID-β2m–/– mice. Finally, we showed that the ability of STRC-MLs to engraft NOD/SCID-β2m–/– mice is not altered as these cells progress through the different phases of the cell cycle. This latter finding contrasts dramatically with the transient loss of engrafting ability that accompanies the S/G2/M transit of lymphomyeloid cells that repopulate NOD/SCID mice (30, 36). Parallel differences between short and prolonged durability of engraftment and low and high sensitivity to cell cycle progression have been reported for murine RCs (31-33). It is also interesting to note that clonal analyses of gene-marked autografts in nonhuman primates have recently revealed exclusively myeloid clones (containing both erythroid and granulopoietic cells) during the first 24 weeks after transplant with lymphomyeloid clones first becoming detectable after that time (37). However, since most transplant with lymphomyeloid clones first becoming granulopoietic cells) during the first 24 weeks after transplant with lymphomyeloid clones first becoming detectable after that time (37). However, since most transplant with lymphomyeloid clones first becoming granulopoietic cells) during the first 24 weeks after transplant with lymphomyeloid clones first becoming detectable after that time (37). However, since most transplant with lymphomyeloid clones first becoming granulopoietic cells) during the first 24 weeks after transplant with lymphomyeloid clones first becoming detectable after that time (37). 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