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Retrovirus-mediated gene transfer to purified hemopoietic stem cells with long-term lympho-myelopoietic repopulating ability

(bone marrow transplantation/hemopoiesis)

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ABSTRACT **Despite recent advances in marrow stem cell purification, controversy about the nature and heterogeneity of cells with the potential for long-term repopulation of lymphoid and myeloid tissues remains. Essential to the resolution of these questions is the use of strategies to track the progeny produced *in vivo* from individual hemopoietic stem cells in purified populations. We have used a procedure for obtaining highly enriched populations of stem cells with competitive repopulating ability from male mice (pretreated with 5-fluorouracil), and in this paper we present the results of studies in which small numbers (150–2000) of these cells were exposed to supernatant containing a helper-free recombinant retrovirus carrying the neomycin-resistance gene and then were transplanted together with 2 × 10^5 "compromised" female marrow cells into irradiated female recipients. Male cells—i.e., progeny of purified stem cells—were found in one or more of the tissues examined (peripheral blood, marrow, spleen, and thymus) in 28 of 28 mice evaluated at various times between 35 and 196 days after transplantation. In 20 of these mice (71%), the neomycin-resistance gene was also detected, although not always at a level that correlated with the proportion of male cells. Analysis of spleen colonies (day 12) generated in secondary recipients confirmed that viral integration was confined to male repopulating cells. In three mice direct evidence of a common clone in both lymphoid and myeloid tissues was also obtained. These results show the feasibility of retrovirus-mediated gene transfer to highly purified populations of lympho-myelopoietic stem cells with long-term (6 months) repopulating potential by using a supernatant infection protocol. This approach should facilitate further analysis of hemopoietic stem cell control *in vivo* and find future applications in the evolving use of bone marrow transplantation for hemopoietic rescue and gene therapy.**

The biological properties of the most primitive hemopoietic cells capable of long-term blood cell production *in vivo* are not well defined. Analysis of the clonal progeny of mouse marrow cells carrying unique chromosomal (1) or retroviral (2–4) markers has provided strong evidence of the presence in normal adult bone marrow of stem cells that are individually capable of regenerating and maintaining both lymphoid and myeloid systems for many weeks after transplantation. The existence of human hemopoietic stem cells with these potentialities has also been indicated recently by the demonstration of clonal populations of mature blood cells of multiple lineages in normal bone marrow transplant recipients (5). Progeny analyses have further suggested the existence of hemopoietic stem cells that can function *in vivo* for extended periods of time but that express more restricted differentiation potentialities or that are less competitive in generating mature progeny of a particular lineage (3, 4, 6), raising the possibility of considerable heterogeneity at this level. Further characterization of the most primitive blood cell elements would be greatly facilitated by the development of procedures for their purification and subsequent use in lineage-mapping studies. Recent reports have suggested the feasibility of obtaining highly purified populations of mouse marrow stem cells with *in vivo* repopulating ability, although the extent to which these may be detected as CFU-S (colony-forming unit-spleen: cells capable of generating macroscopic spleen colonies visible 9–14 days after transplantation) (7) appears to differ markedly according to the procedure used (8–10). Moreover, to our knowledge, direct evidence that current purification procedures selectively enrich for stem cells with lymphopoietic as well as myelopoietic potential has not yet been reported.

We have recently reported (10) a procedure for the single-step isolation of a population of primitive hemopoietic stem cells from 5-fluorouracil (5-FU)-treated mouse bone marrow; 1 in 4 of these cells are day 12 CFU-S, and at least 1 in 85 are capable of competitive long-term marrow repopulation when cotransplanted into lethally irradiated recipients with 2 × 10^5 twice serially transplanted marrow cells. In the present study, we have used this purification procedure in combination with retrovirus-mediated gene transfer to demonstrate the long-term lympho-myelopoietic repopulating ability of individual purified stem cells.

MATERIALS AND METHODS

Animals. Six- to 12-week old (C57BL/6J × C3H/HeJ)F_1 (B6C3F_1) male and female mice bred and maintained in the animal facility of the British Columbia Cancer Research Centre from parental strain breeders originally obtained from The Jackson Laboratories were used in all experiments. B6C3F_1 mice are homozygous for the *Thy-1.2* allele and are of the $H\text{-}2K^b/H\text{-}2K^k$ haplotype.

Stem Cell Purification Procedure. Cells with competitive long-term repopulating ability were purified from the marrow of male B6C3F_1 mice injected intravenously 4 days previously with 5-FU as described (10). Briefly, marrow cells were indirectly labeled with anti-Thy-1.2 and anti-H-$2K^b$ monoclonal antibodies, and then cells with high forward and intermediate-to-high orthogonal light scattering properties that expressed low levels of Thy-1.2 and high levels of H-$2K^b$ antigens were isolated in a single step with a fluorescence-activated cell sorter (FACS 440; Becton Dickinson).

Infection and Assay of Purified Marrow Cells. A replication-defective recombinant retrovirus, TKneo19, which carries the bacterial gene for neomycin resistance (neo^r) under the

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Abbreviations: CFU-S, colony-forming unit-spleen; neo^r, neomycin resistance; 5-FU, 5-fluorouracil.

control of the herpes simplex virus (HSV) thymidine kinase gene (*tk*) promoter (Fig. 1) was derived from a *myc/neo* retrovirus provided by B. Vennstrom (11, 12) by deletion of *myc* sequences and inversion of the TKneo insert (13). Helper-free TKneo19 viral producer clones were generated in the ψ-2 ecotropic packaging cell line (14) using published procedures (15). The ψ-2 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) with 10% (vol/vol) calf serum. The clone selected produced TKneo19 virus at a titer of $>5 \times 10^5$ per ml as assayed by generation of G418-resistant colonies on NIH 3T3 cells. Virus-containing supernatant from cultures of these cells was found to be negative for the production of helper virus as assessed by attempts to serially transfer TKneo19 on NIH 3T3 cells (16).

The infection protocol was based on preliminary studies with unenriched bone marrow collected 4 days after injection (i.v.) of 150 mg of 5-FU per kg of body weight that yielded gene transfer efficiencies of 70% in day 12 CFU-S without preselection (C.C.F., S.J.S., C.J.E., and R.K.H., unpublished data). In brief, aliquots of 150–2000 purified marrow cells were placed into microcultures containing 0.3 ml of supernatant from logarithmic-phase TKneo19-producing ψ-2 cell cultures with 4 μg of Polybrene per ml, 10% (vol/vol) pokeweed mitogen-stimulated mouse spleen cell-conditioned medium (17), and 10% (vol/vol) agar-stimulated human leukocyte-conditioned medium (18). After 6–8 hr at 37°C, half of the medium was replaced with an equal volume of freshly prepared virus-containing medium, and the cultures were incubated a further 12–14 hr. Cells from each well were then collected separately, washed, and injected into irradiated (8–8.5-Gy x-rays) female $B6C3F_1$ mice (one well per mouse) together with (experiment 1, mice 1.1–1.8, and experiment 2, mice 2.1–2.19) or 2 hr after (experiment 3, mice 3.1–3.10) another injection of 2×10^5 syngeneic "compromised" female marrow cells that had been subjected to two cycles of serial marrow transplantation and regeneration (10, 19). Such cells contain approximately normal numbers of most types of clonogenic hemopoietic cells (10, 20) but are unable to override the ability of 100-fold fewer unenriched normal or day 4 5 FU-treated marrow cells to contribute significantly to long-term (>5 weeks) marrow repopulation (10).

Spleen Colony Analysis. Irradiated (8–8.5 Gy) female $B6C3F_1$ mice were injected intravenously with 5×10^4 marrow cells from competitively repopulated mice and sacrificed 12 days later. Well-isolated macroscopic spleen colonies were dissected for DNA extraction.

Separation of Marrow Macrophage and Splenic Lymphocyte Subpopulations. Suspended marrow cells (2.5×10^6) were placed for 24 hr at 37°C in 60-mm² tissue culture dishes containing 5 ml of RPMI 1640 medium with 10% fetal calf serum, 1% pokeweed mitogen-stimulated mouse spleen cell-conditioned medium, and 5% EMT6 cell-conditioned medium as a source of growth factors (17, 21). Nonadherent cells were removed by washing, and the adherent macrophages were amplified in the same medium by culture for 1–2 weeks. Harvested cells were frozen until DNA extraction.

Spleen cells were first fractionated by adding 5×10^7 cells in 1 ml of RPMI 1640 medium with 5% fetal calf serum to a 3-ml nylon wool column, which was then incubated for 1 hr at 37°C prior to elution by extensive washing of the nonadherent fraction (primarily T lymphocytes) (22). Adherent cells were detached by gently agitating the nylon wool plug for 5–10 min in PBS containing 10 mM EDTA, and B lymphocytes were isolated by panning of these cells for 1 hr at 37°C in 100-mm-diameter plastic dishes ($\leq 10^8$ cells per dish) precoated with unpurified rabbit anti-mouse immunoglobulin (23). After washing away most of the nonspecifically bound cells, the adherent B lymphocytes were removed and frozen as for other cell samples.

DNA Analyses. DNA was purified from $NaDodSO_4$/proteinase K-digested cells by phenol/chloroform extraction (24). After dialysis against 1× TE buffer (3 mM Tris/0.2 mM EDTA, pH 7.5), DNA was digested with *Pvu* II, *Bam*HI, *Hin*dIII, or *Eco*RI (BRL) at 2–5 units/μg of DNA for 4–12 hr at 37°C. After ethanol precipitation, DNA was dissolved in 20 μl of TE buffer, electrophoresed through a 1% agarose gel, and transferred to nitrocellulose (Schleicher & Schuell) or nylon (Zeta-Probe; Bio-Rad) membranes (24). Blots of *Pvu* II- or *Hin*dIII-digested DNA were probed with the pY2 plasmid, which contains a 720-base-pair (bp) *Mbo* I fragment of the Y chromosome from male BALB/c mice cloned into the *Bam*HI site of pBR322 (25). pY2 probe was ^{32}P-labeled to high specific activity by nick-translation with a kit purchased from BRL. Blots of *Bam*HI-, *Hin*dIII-, or *Eco*RI-digested DNA were probed with a 2.3-kbp *Bam*HI subfragment of the TKneo19 retrovirus containing only the neo^r gene and the *tk* promoter [^{32}P]oligonucleotide-labeled by using the multiprime labeling method with a kit purchased from Amersham. Filters were washed at a final stringency of 0.1% $NaDodSO_4$ containing 0.1× SSC (0.15 M NaCl/0.015 M sodium citrate, pH 7) and 0.1% sodium pyrophosphate at 65°C (24). Autoradiography was performed at −70°C with a Kodak XAR-5 film for 24–72 hr. In some cases, blots were stripped for reprobing by twice gently agitating them for 15 min in 0.1× SSC/0.5% $NaDodSO_4$, which was boiled and allowed to cool to room temperature.

RESULTS

Transfer of the neo^r Gene to Purified Stem Cells. In three experiments, a total of 37 mice were transplanted with 150–2000 purified male marrow cells that had been exposed to TKneo19. To ensure activation of the most primitive stem cells in the purified population and to facilitate short-term

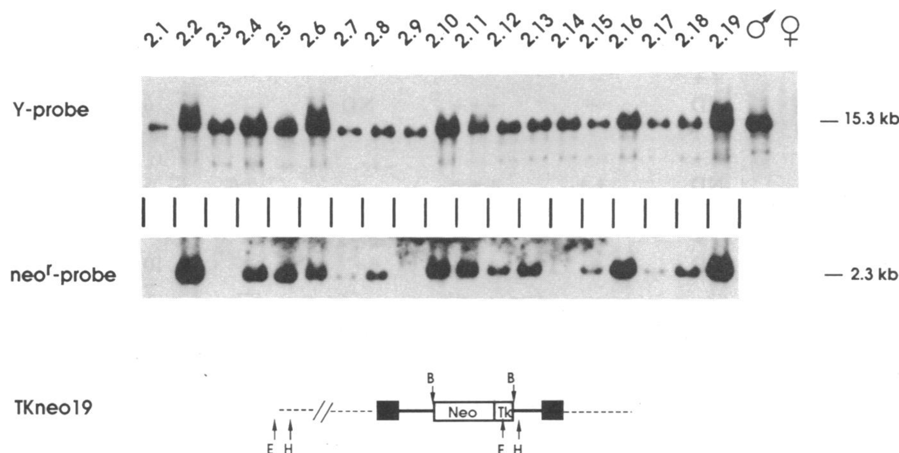

FIG. 1. Southern analysis of DNA from peripheral blood leukocytes isolated from 19 female mice 35 days after transplantation with 300 (mice 2.1–2.15) or 500 (mice 2.16–2.19) TKneo19-infected, purified, male, day 4 5-FU-treated marrow cells together with 2×10^5 "compromised" female marrow cells. (*Top*) Hybridization with the Y-specific probe. Uninfected normal male and female DNA samples are shown as controls. Each lane was loaded with ≈5 μg of *Bam*HI-digested DNA. (*Middle*) Identical blot reprobed with the neo^r-specific probe. (*Bottom*) Structure of the TKneo19 provirus with *Bam*HI (B), *Hin*dIII (H), and *Eco*RI (E) restriction sites; the dashed line represents target genomic sequences.

survival of these animals, each recipient was also injected with 2×10^5 "compromised" female marrow cells prepared as described. In all of the 28 recipients that were evaluated for this study, male cells were determined by Southern analysis to comprise >50% of the cells in at least one of the various hemopoietic tissues analyzed, but, as noted previously with a similar protocol (10), the proportion of male cells often varied considerably between different tissues of the same animal. Results for peripheral blood leukocytes from 19 mice (experiment 2) sampled 35 days after transplantation are shown in Fig. 1. Analysis of the same DNA with a neo^r gene-specific probe revealed that 16 of these 19 animals had circulating neo^r-positive leukocytes. Assessment of marrow, spleen, and thymus cell DNA from 8 other mice (experiment 1) sacrificed on day 35 after transplantation showed 3 animals to be both male and neo^r-positive (Table 1). Another mouse (experiment 3), sacrificed on day 140 after transplantation, was found to contain male and neo^r-positive cells in the spleen but not in the marrow or thymus (Table 1). Gene transfer to purified repopulating stem cells was thus detected in 20 of the 28 (71%) recipients analyzed. The neo^r-specific signal varied considerably among these reconstituted animals, consistent with a variable proportion of marked male stem cells contributing to hemopoiesis at any given time.

To obtain definitive evidence that retrovirally marked cells were derived from the transplanted purified male stem cells, marrow cells from three primary mice (1.7, 2.4, and 2.10) in which neo^r-positive cells were detected were used to generate macroscopic spleen colonies (day 12) in secondary female recipients. These were then individually excised for DNA analysis. Results for seven spleen colonies generated from the marrows of mice 2.4 and 2.10 sacrificed 49 days after the initial transplant of purified stem cells are shown in Fig. 2. Overall, 10 of 16 day 12 spleen colonies analyzed with pY2 were male and therefore must have originated from a stem cell with competitive long-term repopulating ability in the original purified population. The detection of some female spleen colonies is consistent with the male–female chimerism seen in many recipients of the type of mixed transplants used here (10). Five of 10 male spleen colonies also contained unrearranged provirus with an integration pattern (data not shown) identical to that of the donor marrow. The neo^r gene was not detected in any of the female spleen colonies.

Analysis of Individual neo^r-Marked Clones. To determine the number and distribution of unique proviral integration sites in different lymphoid and myeloid cell lineages, DNA was digested separately with *Hin*dIII and *Eco*RI (enzymes that cut only once within the retroviral sequence; Fig. 1). Of the 20 neo^r-positive mice identified from all three experiments, 12 were selected for this type of analysis on the basis of an initial demonstration of a relatively high proportion of male and retrovirally marked cells. Results for 6 mice are shown in Figs. 3 and 4 and are summarized together with the results for the other 6 mice in Table 1. For convenience they are presented according to the interval between the time of transplantation and the time of sacrifice. All mice showed the presence of some retrovirally marked cells at the time of sacrifice in at least one tissue; when only one tissue was involved (3 mice only), this was always the spleen. Although the variable content of male cells in many mice indicated that hemopoietic reconsti-

Table 1. Summary of 12 mice transplanted with retrovirally marked purified male repopulating stem cells

Mouse	Purified cells transplanted,* no.	Time of assessment, days after Tx	Proportion of male cells†				Retroviral fragment size‡, kbp		
			PB	bm	spl	thy	bm	spl	thy
1.3	1000	35	ND	+	++	+++	—	5.2	5.2
1.4	250	35	ND	++	+	−	5.8	5.8	—
							8.9	8.9	—
2.4	300	35	+++						
		49	ND	+++	+++	+++	8.1	8.1	8.1§
							10.9	10.9	10.9§
2.10	300	35	+++						
		49	ND	+	+++	++	—	7.1	7.1
							—	8.8	8.8
1.7	2000	61	+++						
		98	+++						
		121	ND	+++	+++	+++	7.2	7.2	7.2
3.10	150	140	ND	−	+++	−	—	7.1	—
2.5	300	35	++						
		144	ND	+++	++	+++	—	5.8	—
2.6	300	35	+++						
		144	ND	+++	+++	+++	—	12.8	ND
2.13	300	35	++						
		144	ND	++	+++	+	ND	21.0	ND
2.8	300	35	++						
		196	ND	+	+++	ND	—	6.1	6.1
							—	6.8	6.8
2.15	300	35	++						
		196	ND	++	+++	+	5.6	5.6	5.6
							7.2	7.2	7.2
2.18	500	35	++						
		196	ND	+	+++	+++	—	10.2	—
							—	7.1	—

Tx, transplantation; PB, peripheral blood; bm, bone marrow; spl, spleen; thy, thymus; ND, not determined.
*All mice were cotransplanted with purified male, day 4 5-FU-treated marrow cells and 2×10^5 compromised female marrow cells.
†Estimated proportion of male cells: −, 100% female; +, <10% male; ++, 10–80% male; +++, >80% male.
‡Determined after digestion with *Hin*dIII or *Eco*RI (mouse 2.4). All integrations were also verified by analysis of DNA digested with *Eco*RI or *Hin*dIII (mouse 2.4).
§Weakly detected in thymus on the original autoradiogram.

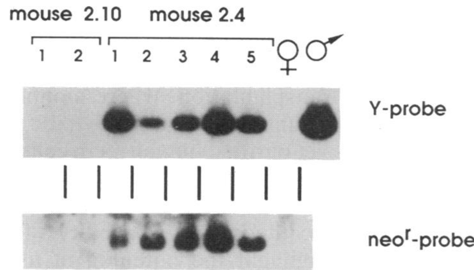

FIG. 2. Southern analysis of DNA from individual day 12 spleen colonies generated from the marrows of mice 2.4 and 2.10 sacrificed 49 days after transplantation. Uninfected normal male and female DNA samples are shown as controls. (*Upper*) *Pvu* II-digested DNA (10 μg per lane) hybridized to the Y-specific probe. (*Lower*) *Bam*HI-digested DNA (30 μg per lane) hybridized to the neo^r-specific probe.

tution was typically oligoclonal, only one or two integration sites were detected in neo^r-positive tissues. This type of preliminary analysis cannot distinguish between single clones marked by two retroviral integration events and two prominent clones each marked by a single unique integration event; however, it is obvious that the conditions used enabled the assessment of the competitive repopulating potential of individual retrovirus-infected stem cells.

The first 2 mice shown in Fig. 3 (experiment 1, mice 1.3 and 1.4) were sacrificed 35 days after transplantation. In both, the proportion of male cells in the marrow, spleen, and thymus was different in each tissue, but in each case was correlated with the intensity of neo^r-specific hybridization. In mouse 1.3, a single 5.2-kbp proviral fragment was found in both the spleen and thymus but was not detectable in the marrow. Because of the smear present in the bone marrow lane, however, we cannot rule out the presence of neo^r-specific hybridization to DNA fragments > 5 kbp. In mouse 1.4, a 5.8-kbp and an 8.9-kbp proviral fragment were present at the same level in the marrow and spleen but were not detectable in the thymus. Mouse 2.4 (experiment 2) was sacrificed 49 days after transplantation. At that time the marrow, spleen, and thymus were all predominently male. Common 8.1-kbp and 10.9-kbp proviral fragments were clearly evident in marrow and spleen DNA analyzed by *Eco*RI digestion. These were also detectable in the thymus but at very low levels (not reproduced from the original autoradiogram) in Fig. 3. The presence of a common pair of bands in marrow and spleen and seen weakly in thymus was verified by analysis with *Hin*dIII (data not shown). Mouse 1.7 (experiment 1) was sacrificed 121 days after transplantation, and the marrow, spleen, and thymus were also all found to be predominantly male. A single 7.2-kbp proviral fragment was seen in each of these tissues as well as in lymph node cells and in separately isolated bone marrow macrophages, splenic B lymphocytes, and splenic T lymphocytes, which were also predominantly male (Fig. 4). Mouse 2.5 (experiment 2) was sacrificed on day 144 after transplantation. The marrow, spleen, and thymus all contained a high, albeit variable, proportion of male cells, but a single 5.8-kbp proviral fragment was detected in only the spleen of this animal. Mouse 2.15 (experiment 2) was sacrificed on day 196 after transplantation. This animal contained decreasing proportions of male cells in the spleen, marrow, and thymus; however, a 5.6-kbp and a 7.2-kbp proviral fragment were evident in all three tissues. Overall, at least 3 of the 12 mice had clearly been repopulated by a stem cell with lymphoid as well as myeloid differentiation potential, since they showed the same integration pattern in both marrow and thymus (mice 1.7, 2.4, and 2.15) at the time of sacrifice.

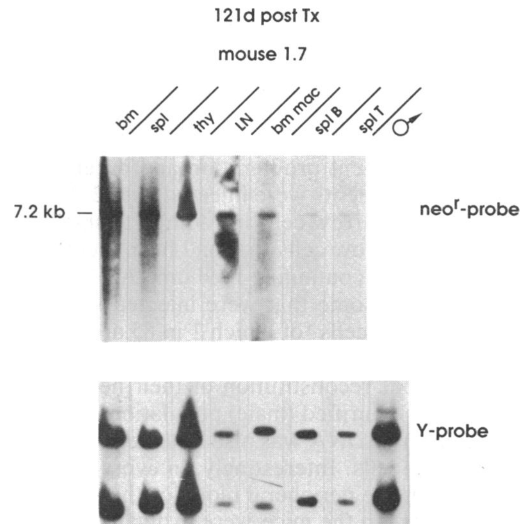

FIG. 4. Presence of a common retrovirally marked clone in bone marrow (lane bm), spleen (spl), thymus (thy), lymph node (LN) and separately isolated marrow macrophage (bm mac), splenic B (spl B), and T (spl T) lymphocytes from mouse 1.7 sacrificed 121 days (121d) after transplantation (post Tx). (*Upper*) *Hin*dIII-digested DNA (lanes bm, spl, and thy: 25 μg per lane; lanes LN, bm mac, spl B, and spl T: ≈5 μg per lane) hybridized to the neo^r-specific probe. (*Lower*) Identical blot reprobed with the Y-specific probe. Uninfected normal male DNA (25 μg) is shown as a control.

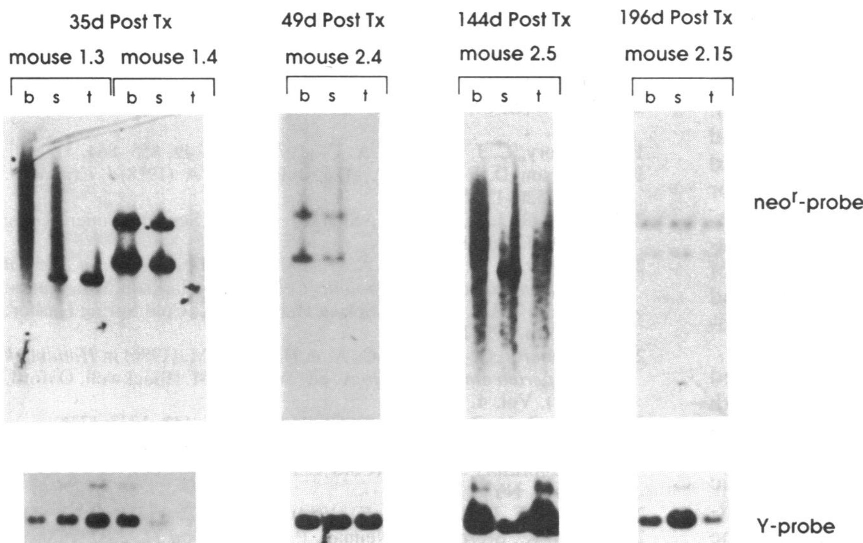

FIG. 3. Southern analysis of DNA from the bone marrow (lanes b), spleen (lanes s), and thymus (lanes t) of five female mice sacrificed 35–196 days (35d–196d) after transplantation (Post Tx) with 1000 (mouse 1.3), 250 (mouse 1.4), or 300 (mice 2.4, 2.5, and 2.15) TKneo19-infected, purified, male, day 4 5-FU-treated marrow cells together with 2×10^5 "compromised" female marrow cells. (*Upper*) *Hin*dIII (mice 1.3, 1.4, 2.5, and 2.15)- or *Eco*RI (mouse 2.4)-digested DNA (30 μg per lane) hybridized to the neo^r-specific probe. (*Lower*) *Pvu* II-digested DNA (10 μg per lane) hybridized to the Y-specific probe. Proviral fragment sizes are described in Table 1.

DISCUSSION

We have developed a simple procedure that allows small numbers of purified hemopoietic stem cells from mouse bone marrow to be stably and efficiently infected with a neo^r-containing retrovirus without any apparent effect on their potential for subsequent proliferation and differentiation *in vivo*. All female recipients were injected with 2×10^5 "compromised" female marrow cells in addition to small numbers of purified male marrow cells that had been incubated overnight with TKneo19-containing medium. All 28 such mice analyzed, including some that were injected with, at most, 150 purified marrow cells [of which 1 in 85 are estimated to be capable of competitive long-term repopulation (10)], showed subsequent reconstitution of their hemopoietic tissues by cells in the purified (male) population. In 20 of these mice (71%), proviral DNA was also detected in the regenerated hemopoietic cells. Interestingly, in every case this was seen in cells from the peripheral blood or the spleen regardless of the time when the mice were evaluated (1–6 months after transplantation), but not necessarily in the marrow or thymus. These findings clearly show that the cells isolated by the purification procedure used are capable of sustaining hemopoiesis for extensive periods of time after transplantation, even in the presence of a competing graft that could, if injected alone, reconstitute these same animals (S.J.S., R.K.H., A.C.E. & C.J.E., unpublished data).

In one mouse the neo^r-containing restriction fragment demonstrated in DNA from marrow, spleen, and thymus 121 days after transplantation was also identified in purified subpopulations of marrow macrophages and splenic T and B lymphocytes, thus establishing the multilineage differentiation potential of the parent stem cell. Since all of these populations were also predominantly male, the cell from which the marked clone arose must have been present in the purified, day 4 5-FU-treated marrow cell population. Similar results were obtained in 2 other mice. In each of these, myeloid (marrow) as well as lymphoid (thymus) tissues contained the same two proviral inserts. The equivalent intensity of the two bands (relative to one another) in all tissues suggested that a single clone consisting of cells containing a double integration was present, rather than two clones each having retrovirus integrated at a single unique site. Evidence of clones with double integrations was also obtained in 4 other mice, although the tissue distribution of these clones at the time of analysis was not as broad. This does not necessarily mean that the differentiation potential of the original stem cells in these latter animals was more restricted, since multiple parameters as yet not understood likely influence the commitment and amplification of individual lympho-myelopoietic stem cells transplanted *in vivo*. Moreover, the cell turnover kinetics of different lineages may prevent simultaneous detection of multilineal clones as their contribution to a particular lineage modulates with time (4). Thus, although simultaneous involvement of lymphoid and myeloid cells was not obtained in 9 of the 12 mice repopulated with neo^r-positive clones, it is quite possible that many, or even all, were derived from lympho-myelopoietic stem cells. More extensive longitudinal studies of purified subpopulations from individual mice injected with limiting numbers of purified stem cells will be necessary to characterize and quantitate more precisely the cell types present in our purified populations.

The present experimental system thus appears well suited for further studies of purified hemopoietic stem cells with retroviral markers to identify individual clones regenerated in irradiated recipients. However, it should be noted that under the conditions used, reconstitution of the entire hemopoietic system was usually oligoclonal. Many of the recipients exhibited male–female chimerism in at least one tissue, and the prevalence of a marked clone did not always correlate with the proportion of male cells. This situation appears to correspond well to that obtained in the clinical setting of allogeneic bone marrow transplantation, where monoclonal reconstitution may be encountered but appears to be relatively uncommon (5, 26). As yet purified human stem cells have not been transplanted as an alternative to whole or T-lymphocyte-depleted marrow for clinical purposes. The present findings suggest the theoretical feasibility of such an approach. They also highlight the potential of using gene transfer to purified human hemopoietic stem cells for analyzing the value and importance of various hemopoietic cell subpopulations in human marrow for marrow rescue and their candidacy as targets for gene therapy.

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