Separation of Pluripotent Stem Cells and Early B Lymphocyte Precursors with Antibody Fall-3

By Christa E. Müller-Sieburg

From the Medical Biology Institute, La Jolla, California 92037

Summary

A major goal in the study of hematopoiesis is to obtain populations of primitive stem cells, free of restricted and mature cells. We previously showed that a small population of normal bone marrow, the Thy-1°Lin- cells, was highly enriched for pluripotent stem cells that repopulate lethally irradiated mice. These cells also differentiated along the B lymphocyte lineage in response to the stromal elements in Whitlock-Witte cultures. These two hematopoietic activities were entirely contained in and were enriched to similar extents in the Thy-1°Lin- population. Here we show for the first time that these two activities can be resolved functionally and phenotypically. The cells that respond to the stroma in lymphoid culture are more sensitive to the cytotoxic drug 5-Fluorouracil than are stem cells. Furthermore, we have derived a new monoclonal antibody, Fall-3, that detects primitive stem cells but does not label the B cell precursor. This indicates that the small Thy-1°Lin- population is heterogeneous, containing precursors restricted to the B cell lineage as well as pluripotent stem cells. Antibody Fall-3 defines a novel stem cell antigen, expressed on all primitive stem cells and thus, will be useful in the further characterization and isolation of both stem cells and B cell precursors.
CFU-S. Thus, both populations include in addition to stem cells, restricted myeloid-erythroid precursors. However, the question whether the Thy-1<sup>−</sup>Lin<sup>−</sup> population contains additional restricted precursors particularly, precursors committed to the lymphoid lineages, was not addressed in these reports.

Here we present evidence for further heterogeneity in the Thy-1<sup>−</sup>Lin<sup>−</sup> population. We show that the Thy-1<sup>−</sup>Lin<sup>−</sup> population contains a precursor restricted to the B cell lineage in addition to pluripotent stem cells. This B cell precursor is characterized by its capacity to establish lymphoid Whi tlock-Witte cultures in limiting dilution. B cell precursors and stem cells can be distinguished functionally and phenotypically. A monoclonal antibody, designated Fall-3 that we have derived, allows for the first time the separation of these cells and will be helpful in the further characterization and isolation of both stem cells and B cell precursors.

Materials and Methods

Animals. BALB/c and C57BL/6-Ly-5.2 mice were bred either at the Lilly Research facility (La Jolla, CA) or at the Medical Biology Institute (La Jolla, CA). C57BL/6-Ly-5.2 mice were obtained originally from Dr. E. Boyse (Sloan-Kettering Cancer Center, New York). 3- to 5 wk-old mice were injected i.v. with a single dose of 150 mg 5-Fluorouracil (5-FU) (Sigma Chemical Co., St. Louis, MO) per kilogram body weight in saline containing 0.1 M NaHCO<sub>3</sub>, where indicated. C57BL/6 mice, used as hosts in reconstitution experiments at 3-6 mo-of-age, were either purchased from Jackson Laboratories (Bar Harbor, ME) or bred at the Medical Biology Institute facility.

Derivation of Monoclonal Antibody Fall-3. Lewis Rats were injected i.p. and s.c. with 5 × 10<sup>8</sup> to 10<sup>9</sup> mouse bone marrow cells depleted of cells that express B220, Gr-1, or Mac-1. Cells were injected three times at 14-d intervals. 3 d after the last immunization spleen cells were fused to the mouse myeloma X63Ag8-653 (15). Supernatants of hybrids were screened on mouse bone marrow cells by immunofluorescence. mAb Fall-3 was detected with goat anti-rat-FITC antibodies (Caltag, South San Francisco, CA). After subcloning of antibody-producing hybrids, mAbs were prepared from serum-free culture supernatant by precipitation with 50% saturated ammonium sulfate. The isotype of mAb Fall-3 is IgM as determined in a double diffusion assay using a rat isotyping kit (ICN ImmunoBiologicals, Costa Mesa, CA).

Stem Cell Assay. To measure stem cells we used a combination of the radioprotection and long-term repopulation assays as described previously (5). Briefly, C57BL/6 mice were lethally irradiated with 1,140 rads (2 doses of 570 rads) and reconstituted i.v. with different numbers of bone marrow cells derived from pools of 3-5 Ly-5 congenic C57BL/6-Ly-5.2 mice. Age matched cohorts of 6-10 mice were injected for each cell dose. The extent of repopulation in surviving mice was determined 3 to 5 mo later by staining peripheral blood cells. Blood was collected into EDTA solution (3 mg EDTA per ml PBS) and incubated with 2% Dextran for 30 min at 37°C, followed by lysis of residual erythrocytes with Gey's solution. White blood cells were labeled with a biotinylated antibody specific for the donor type Ly-5.2 marker detected by Avidin-FITC. A cell line, A-20, secreting antibodies specific for Ly-5.2 was the generous gift of Dr. S. Kimuro, (Sloan-Kettering Cancer Center, NY). The Ly-5 marker is expressed on most hematopoietic cells with the exception of erythroid cells (16). To determine the restoration of individual lineages, white blood cells were contained with antibodies specific for B220 (mAb Ra3-6B2), Mac-1 (mAb M1/70), Gr-1 (mAb Ra3-8C5), and Thy-1 (mAb 31-11). In all mice tested, donor derived T, B, and myeloid cells were reconstituted in ratios comparable to that seen in unirradiated mice.

This assay does not readily allow the quantification of stem cell frequency. To compare stem cell level we introduce the "stem cell unit" which we define as the number of normal bone marrow cells needed to completely repopulate 50% of lethally irradiated animals. Therefore, 1 stem cell unit = 8 × 10<sup>8</sup> cells = 100% stem cell activity (see Fig. 1). Further, we calculate: % stem activity in 5-FU bone marrow = 1 stem cell unit/No. of cells for 50% reconstitution (5-FU bm); stem cell activity per femur = (cells per femur/1 stem cell unit) × % stem cell activity; % stem cell activity per femur = stem cell activity per femur 5-FU bone marrow/stem cell activity per femur normal bone marrow.

Cell Separations and Immunofluorescence Analysis. Bone marrow cells were incubated with a saturating concentration of mAb Fall-3 detected with goat anti-rat-FITC antibodies (Caltag). Cells were then separated into Fall-3<sup>+</sup> and Fall-3<sup>−</sup> subpopulations with a multi-laser cell sorter at the Flow Cytometry Laboratory at the Salk Institute for Biological Studies (La Jolla, CA). The background level for the staining was determined by using either an isotype matched antibody (Fall-84) detected with goat anti-rat-FITC antibodies or just the FITC-labeled second stage antibodies. The background levels determined with these controls did not differ significantly.

For two color immunofluorescence, mAb Fall-3 was detected with PE-labeled goat anti-rat antibodies (Caltag). Cells were washed and then incubated for 1 to 2 min with 25% normal rat serum (Cappel Laboratories, Cochranville, PA), followed by biotinylated lineage specific antibodies (4,5) detected with Avidin-FITC (Vector Labs, Burlingame, CA). The hybridoma cell line secreting antibody E13 161-1, specific for Sca-1 (6) was obtained from Dr. I. Weissman (Stanford University, CA).

Colony-Forming Assay/Colony-Forming Unit Culture (CFU-C) and Cytokines. Unseparated bone marrow cells or sorted subpopulations derived from 3- to 4-wk-old BALB/c or C57-Ly-5.2 mice were plated in 0.8% (w/v) methylcellulose, supplemented with IL-3 at 400 U/ml, as described previously (5). Recombinant IL-3 (17) in the form of supernatants of appropriately transfected Cos 7 cells was a generous gift of Dr. D. Rennick (DNAX Research Institute, Palo Alto, CA). Colonies consisting of 50 or more cells were scored after 9-11 d of culture. In some experiments colonies were counted again after 3 wk of culture.

Limiting Dilution Analysis for Stroma-Responsive Cells. This assay has been described in detail (4, 18, 19). Briefly, the monoclonal stromal cell line AC-3.5 or its subclone AC-3.GG was seeded into the wells of 96-well plates in RPMI supplemented with 5% FCS (J.R. Scientific, Woodland, CA) and 5 × 10<sup>-5</sup> M L-2 ME. Cultures were recharged with graded doses of bone marrow cells (one plate for each cell concentration) 2 to 4 d later and then were fed weekly. Wells that contained colonies of at least 10<sup>3</sup> small lymphoid cells were scored by phase microscopy after 10 to 14 d of culture according to morphological criteria. Some experiments were evaluated again 3 wk post reseeding. We showed previously that this assay is linear, and, in fact, detects B lineage cells as all colonies tested contained cells that expressed the B lineage specific marker B220 (4, 19).

Results

Stem Cell Content in Normal and 5-FU Treated Bone Marrow. 5-FU has been used previously to define hematopoietic differen-
It has been reported that mature cells and late precursors are more sensitive to this drug than primitive precursors or stem cells. We used a single injection of 5-FU to determine whether this treatment would reveal heterogeneity in the Thy-1<sup>+</sup>Lin<sup>-</sup> populations by differentially affecting two activities completely contained within this population: stem cell activity as assessed in a long-term repopulation assay and the capacity to respond to stromal cells with B cell differentiation. We reasoned that a differential effect of 5-FU on these activities would indicate that the activities are carried by different cells, co-enriched in the same population.

Since all stem cells are found in the Thy-1<sup>+</sup>Lin<sup>-</sup> population (5), stem cell content measured in unseparated bone marrow reflects stem cell content in the Thy-1<sup>+</sup>Lin<sup>-</sup> population. The level of stem cells in the bone marrow of normal mice and of mice at day 2, day 4, and day 10 after injection of 5-FU was measured in a long-term repopulation assay in lethally irradiated mice. Host mice were injected with graded doses of donor cells derived from mice that differed in the Ly-5 locus. Peripheral blood cells of surviving mice were stained with a mAB specific for donor type Ly-5 to determine the extent of repopulation. As previously described (27), a number of surviving animals that received low doses of stem cells showed considerable host-derived reconstitution. For easier comparison of stem cell activity, the percentage of mice that were completely reconstituted by donor cells (> 80%) are depicted in Fig. 1. To allow the survival of 50% of the lethally irradiated animals for at least 3 mo and to obtain > 80% reconstitution by donor type cells in these mice, 8 x 10<sup>4</sup> normal bone marrow cells per recipient were needed in these experiments. The level of pluripotent stem cells was reduced fourfold in 5-FU day 2 bone marrow, while the level of stem cells in 5-FU day 4 bone marrow was similar to that found in normal marrow. Bone marrow from mice treated 10 d previously with 5-FU contained a fourfold higher level of stem cells.

**B Cell Precursors in 5-FU Treated Bone Marrow.** Thy-1<sup>+</sup>Lin<sup>-</sup> cells also respond with high frequency to the microenvironment in Whitlock-Witte bone marrow cultures by differentiating along the B cell lineage. These stroma-responsive cells are also entirely contained within the Thy-1<sup>+</sup>Lin<sup>-</sup> population (4). Thus, the capacity to respond to the stromal cells in Whitlock-Witte cultures of unseparated bone marrow reflects this activity in the Thy-1<sup>+</sup>Lin<sup>-</sup> population. We wished to determine whether the capacity to give rise to B lineage cells in culture was affected by 5-FU to the same or different extent as the capacity to repopulate irradiated animals.

The frequency of cells that give rise to colonies of at least 10<sup>4</sup> small lymphoid cells in limiting dilution on the monoclonal stromal line AC-3.5 is decreased at least 10-fold in 5-FU day 2 and reduced six-fold in 5-FU day 4 bone marrow compared to normal marrow (Table 1). In contrast, 5-FU day 10 bone marrow contains a four-fold higher frequency of B cell precursors than normal bone marrow. The cultures were counted generally at 2 wk. To determine whether cells from 5-FU treated bone marrow have a delayed response to stromal cells we also evaluated the cultures at 3 wk (data not shown). In agreement with previous observations on normal bone marrow cells (18), we did not observe additional colonies.

**Differential Effects of 5-FU.** Both radioprotecting and stroma-responsive cells were affected by 5-FU. However, their kinetics of recovery was markedly different. Fig. 2 summarizes and compares the data presented in Table 1 and Fig. 1. Since the radioprotection assay does not allow readily to calculate frequencies we have used stem cell units for comparison (see Materials and Methods for calculations). The fre-

![Figure 1](image-url)
Kinetics of recovery of hematopoietic activities after a single injection of 5-FU. Data are compiled from Fig. 1 and Table 1. Activity in normal bone marrow was set at 100%. For calculation of stem cell units see Materials and Methods. (A) Comparison of the level or frequency of activities; (B) Comparison of activities based on numbers per femur taking into account the reduced cellularity in 5-FU bone marrow.

The kinetics of recovery after 5-FU treatment indicates that the hematopoietic activities found in the Thy-1^Lin^- population in normal bone marrow reflect heterogeneity in this population rather than multiple capacities of the same cell (Fig. 2). To define further the cells that carry these activities, we separated normal bone marrow with monoclonal antibody Fall-3. We derived this antibody from a fusion designed to detect subpopulations in bone marrow depleted of mature cells.

Antibody Fall-3 (IgM) stains 15 to 30% of normal bone marrow (Fig. 3). Cells from CBA/J, BALB/c, and C57BL/6 mice are detected to a similar extent. Only minor populations (~5%) of spleen, thymus, and Peyer's patches cells were stained above background. Two-color immunofluorescence analysis indicates that less than 5% of the mature bone marrow cells that express the lineage markers B220, Gr-1, Mac-1, Thy-1, or CD-8 bear also the Fall-3 determinant (data not shown). This suggests that mAb Fall-3 detects preferentially cells that are not mature granulocytes, macrophages, T or B lymphocytes.

Cells that expressed the cell surface determinant detected by mAb Fall-3 contain most if not all pluripotent stem cells that repopulate all lineages in irradiated mice (Fig. 4A). Only mice that received Fall-3^+ cells were stably reconstituted at >80% donor type cells in their blood. All mice that were...
Discussion

We have analyzed the interrelationship of two early hematopoietic activities contained exclusively in the Thy-1+Lin- population. The activities are radioprotecting stem cells and B cell precursors that initiate Whitlock-Witte cultures. Both activities are affected by 5-FU. However, these activities show markedly different kinetics of recovery and are affected to different degrees by 5-FU (summarized in Fig. 2). At 4 d post 5-FU treatment, cells that respond to the stroma in Whitlock-Witte cultures are depleted more strongly than the cells that repopulate irradiated animals. Furthermore, the former cells recover slower than radioprotecting stem cells after a single injection of 5-FU. This indicates that the capacity to respond to stromal cells with B cell differentiation is carried by restricted precursors contained in the Thy-1+Lin- population and not by stem cells themselves. Most importantly, B cell precursors and stem cells bear different cell surface antigens and can be separated with mAb Fall-3. Thus, the Thy-1+Lin- population is functionally and phenotypically heterogeneous and contains restricted precursors for the B cell lineage in addition to stem cells.

We compared B cell precursors and stem cells in 5-FU day 2, 4, and 10 bone marrow. The most pronounced difference between these two activities was observed at day 4 post 5-FU treatment. The higher incidence of stem cells at this time point could be a reflection of the less severe reduction of this activity earlier in the time course. Alternatively, it is tempting to speculate that the kinetics reflect a precursor progeny relationship. Stem cells may have to replenish themselves to a certain level before being able to generate the B cell precursors. A more detailed analysis involving more time points is necessary to resolve this issue.

Stem Cells in 5-FU Bone Marrow. The frequency of radioprotecting stem cells is enriched in 5-FU day 10 bone marrow when compared to normal marrow. However, we found that the reduction in cell number caused by 5-FU treatment outweighs this enrichment and the absolute number of stem cell units per femur is reduced in bone marrow of 5-FU treated mice over the whole time span tested. Nakano and colleagues reported that the cells that reconstitute erythropoiesis in anemic W/Wv mice were enriched in 5-FU day 2 bone marrow and depleted in 5-FU day 4 marrow when tested in the competitive repopulation assay (26). Using the same assay, Lerner and Harrison (28) did not find any effect of 5-FU on stem cells 2 to 15 d after injection. These results could be reconciled to ours by considering the ages of the 5-FU treated animals. We used 3- to 5-wk-old mice whereas the other studies used adult mice. It is conceivable that stem cells are expanding in young animals resulting in more 5-FU sensitive stem cells in young mice than in older mice.

Besides the difference in the age of the animals, different assay systems for stem cells were used. We define stem cells by their capacity to rescue and fully repopulated lethally irradiated animals. In the competitive repopulation assay the stem cells measured are not necessarily required for survival of the animals as an alternative (competitive) source of stem cells is provided. It is possible that the stringent radioprotection assay reveals damages in the stem cell compartment that could not be revealed with other assays. It has been reported that a single injection of 5-FU resulted in long-term impairment of the lymphocyte lineages; decreases in cellularity and
functional activities were seen for at least 3 wk (25). These results are compatible with a reduction of stem cells by 5-FU as seen in our study.

When we compared the capacity to repopulate mice and the ability to respond to stromal cells, we used two assay systems that required different time spans for completion. The B cell precursor activities were evaluated after 2 wk while the radioprotection assay required 3 mo to ascertain repopulation by stem cells. However, mice that did not receive stem cells died 2 to 3 wk after grafting, indicating that the radioprotection assay requires stem cells to be active at this time. The B cell precursor assay was carried up to 3 wk without revealing additional colonies. Thus, it seems unlikely that the difference in recovery rates of stem cells and B cell precursors was due to a delay in maturation induced by 5-FU but rather is a reflection of different cell types.

Another activity enriched in the Thy-1\(^{1+}\)Lin\(^{-}\) population, the capacity to form multilineage colonies in response to IL-3, is also affected by 5-FU. We found 0.2, 10, and 129% of the normal response in Thy-1\(^{1+}\)Lin\(^{-}\) cells sorted from 5-FU day 2, day 4, and day 10 bone marrow, respectively (5, data not shown). Consequently, 5-FU appears to affect the CFU-C even stronger than B cell precursors and radioprotecting stem cells. This indicates that these myeloid-erythroid precursors are distinct from B cell precursors and stem cells. Therefore, the "Thy-1\(^{1+}\)Lin\(^{-}\)" population that contains these activities is functionally heterogeneous. Treatment with 5-FU can reduce this heterogeneity. 5-FU day 4 bone marrow contains normal levels of stem cells but at least six-fold lower frequencies of B cell precursors and CFU-C (Fig. 2, data not shown). Thus, the combination of 5-FU treatment and cell sorting, particularly with mAb Fall-3, may result in improved enrichment for pluripotent stem cells.

Fall-3. The antigen defined by mAB Fall-3 is expressed on all pluripotent stem cells that stably repopulate the majority of blood cells for \(\geq 5\) mo. Thus, mAB Fall-3 defines a novel stem cell antigen in the same sense as Sca-1 (6) defines a stem cell antigen. However, Sca-1 (Ly 6A/E) and Fall-3 most likely detect different antigens. One notable difference between these antibodies is that mAB Fall-3 stains bone marrow of both Ly-6\(^{+}\) and Ly-6\(^{-}\) mice to a similar extent while Sca-1 is restricted to cells from strains that carry the Ly-6\(^{b}\) allele (29, 30). Sca-1 stains about 7 to 10% of bone marrow cells, most of which are B220 positive (31), while Fall-3 detects about 30% of bone marrow cells, most of which are immature cells. Unlike Sca-1, mAB Fall-3 stains only few cells in thymus, spleen or lymph nodes. Furthermore, two color immunofluorescence analysis on bone marrow cells with Fall-3 and Sca-1 shows that most cells that express one of the antigens lack the other. Only 1 to 2% of bone marrow cells co-express Fall-3 and Sca-1 (Fig. 5). This small population of cells that express both antigens can account easily for all pluripotent stem cells in bone marrow since stem cells comprise a very minor subset of bone marrow. Furthermore, the staining pattern and tissue distribution of Fall-3 suggest that this mAB is not identical to other mAbs that define antigens in the Ly-6 family (32), or to mAbs specific for Qa-m7, another antigen expressed on stem cells (8).

While most radioprotecting stem cell activity was found in the Fall-3\(^{+}\) population, a number of mice that received Fall-3\(^{-}\) cells survived and contained donor type cells in their blood 5 mo post reconstitution. In these mice, as in the Fall-3\(^{+}\) reconstituted mice, donor derived T, B, and myeloid cells were found. However, in all the mice that received Fall-3\(^{-}\) cells the level of reconstitution was low (Fig. 3A). Currently, we cannot discriminate whether this reflects a stem cell subset with limited proliferative capacity or whether this reconstitution is due to a few contaminating Fall-3\(^{+}\) cells in the Fall-3\(^{-}\) population. The level of the determinant detected by Fall-3 varies considerably on positive cells and staining with mAB Fall-3 does not result in discrete positive and negative populations (Fig. 2). If stem cells would express low levels of the Fall-3 antigen a few stem cells may have fallen within the negative population defined by our sorting gate. We are in the process of testing this hypothesis.

Fall-3 separates most, if not all, primitive stem cells from cells that respond to stromal cells in Whitlock-Witte cultures. Previously, we characterized in detail the Thy-1\(^{1+}\)Lin\(^{-}\) cells that can be detected in limiting dilution and initiate long-term (>6 wk) Whitlock-Witte cultures. The absence of B lineage specific markers, its extensive proliferative capacity, its failure to be transformed by Abelson virus in vitro (32), and its delayed differentiation into mature B cells places this cell very early in the B cell lineage (1). As we are able to separate this B cell precursor from primitive stem cells it will be possible to analyze these Thy-1\(^{1+}\)Lin\(^{-}\)Fall-3\(^{-}\) cells more thoroughly in vivo and in vitro.
Part of this work was done at Eli Lilly Research Laboratories, La Jolla Facility, and their support is gratefully acknowledged. I wish to thank Curtis Mazur and John Wineman for excellent technical assistance and Joseph Trotter for help with flow cytometry. I thank Drs. B. Adkins, C. Cowing, K. Dorshkind, D. Katz, R. Ogata, and H. B. Sieburg for critical reviewing of this manuscript and numerous suggestions and discussions. Especially, I am grateful to Dr. Cheryl Whitlock for introducing me to the field of early hematopoiesis.

This work was supported by National Institutes of Health grant DK-41214 and by an American Leukemia Society Scholar Award.

Address correspondence to Christa E. Müller-Sieburg, The Medical Biology Institute, 11077 North Torrey Pines Road, La Jolla, CA 92037.

Received for publication 14 January 1991 and in revised form 28 March 1991.

References

1. Abrahamson, S., R.G. Miller, and R.A. Phillips. 1977. The identification in adult bone marrow of pluripotent and restricted stem cells of the myeloid and lymphoid systems. J. Exp. Med. 145:1567.

2. Mintz, B., K. Anthony, and S. Litwins. 1984. Monoclonal derivation of mouse myeloid and lymphoid lineages from totipotent hematopoietic stem cells experimentally engrafted in fetal hosts. Proc. Natl. Acad. Sci. USA. 8:7835.

3. Keller, G., C. Paige, E. Gilboa, and E.F. Wagner. 1985. Expression of a foreign gene in myeloid and lymphoid cells derived from multipotent hematopoietic precursors. Nature (Lond.). 318:149.

4. Müller-Sieburg, C.E., C.A. Whitlock, and I.L. Weissman. 1986. Isolation of two early B lymphocyte progenitors from mouse bone marrow: a committed pre-pre-B cell and a clonalogenic Thy-1+ hematopoietic stem cell. Cell. 44:653.

5. Müller-Sieburg, C.E., K. Townsend, I.L. Weissman, and D. Rennick. 1988. Proliferation and differentiation of highly enriched mouse hematopoietic stem cells and progenitor cells in response to defined growth factors. J. Exp. Med. 167:1825.

6. Spangrude, G.J., S. Heimfeld, and I.L. Weissman. 1988. Purification and characterization of mouse hematopoietic stem cells. Science (Wash. DC). 241:58.

7. Visser, J.W.M., J.G.J. Bauman, A.H. Mulder, J.F. Eliason, and A.M. de Leeuw. Isolation of murine pluripotent hematopoietic stem cells. J. Exp. Med. 59:1576.

8. Bertoncello, I., S.H. Bartelmez, S.H. Bradley, E.R. Stanley, R.A. Harris, M.S. Sandrin, A.B. Krieger, I.K. McNiece, S.D. Hunter, and G.S. Hodgson. 1986. Isolation and analysis of primitive progenitor cells on the basis of differential expression of Qa-m7 antigen. J. Immunol. 136:3219.

9. Szilvassy, S.J., P.M. Landsdorp, R.K. Humphries, A.C. Eaves, and C.J. Eaves. 1989. Isolation in a single step of a highly enriched murine hematopoietic stem cell population with competitive long-term repopulation ability. Blood. 74:930.

10. Jordan, C.T., J.P. McKearn, and I.R. Lemischka. 1990. Cellular and developmental properties of fetal hematopoietic stem cells. Cell. 61:953.

11. Scheven, B.A.A., J.W.M. Visser, and P.J. Nijweide. 1986. In vitro osteoclast generation from different bone marrow fractions including a highly enriched hematopoietic stem cell population. Nature (Lond.). 321:79.

12. Sutherland, H.J., C.J. Eaves, A.C. Eaves, W. Dragowska, and P.M. Landsdorp. 1989. Characterization and partial purification of human marrow cells capable of initiation long-term hematopoiesis in vitro. Blood. 74:1563.

13. Paige, C.J., P.W. Kincade, L.A. Shinfeld, and V.L. Sato. 1981. Precursors of B lymphocytes. Physical and functional characterization and distinction from myeloid cells. J. Exp. Med. 153:154.

14. Jones, R.J., J.E. Wagner, P. Celano, M.S. Zicha, and S.J. Sharkis. 1990. Separation of pluripotent haematopoietic stem cells from spleen colony-forming cells. Nature (Lond.). 347:188.

15. Kearney, J.F., A. Radbruch, B. Liesegang, and K. Rajewsky. 1979. A new mouse myeloma cell line that has lost immunoglobulin expression but permits the construction of antibody-secreting hybrid cell lines. J. Immunol. 123:1548.

16. Scheid, M., and D. Triglia. 1979. Further description of the Ly-5 system. Immunogenetics. 9:423.

17. Rennick, D.M., F.D. Lee, T. Yokota, K.-I. Arai, H. Cantor, and G.J. Nabel. 1985. A cloned cDNA encodes a multilineage growth factor: multiple activities of interleukin 3. J. Immunol. 134:910.

18. Whitlock, C.A., G.F. Tidmarsh, C.E. Müller-Sieburg, and I.L. Weissman. 1987. Bone marrow stromal cells with lymphopoietic activity express high levels of a pre-B neoplasia associated molecule. Cell. 48:1009.

19. Whitlock, C.A., and C.E. Müller-Sieburg. 1990. Long-term B lymphoid cultures from murine bone marrow. Methods in Molecular Biology. 5:303.

20. Hodgson, G.S., and R.S. Bradley. 1979. Properties of hematopoietic stem cells surviving 5-fluorouracil treatment: evidence for a pre-CFU-S cell? Nature (Lond.). 281:381.

21. Williams, D.E., S.H. Boswell, A.D. Floyd, and H.E. Broxmeyer. 1985. Pluripotent hematopoietic stem cells in post-5-fluorouracil murine bone marrow express the Thy-1 antigen. J. Immunol. 135:1004.

22. Donowitz, G.R., and P. Quesenberry. 1986. 5-Fluorouracil effect on cultured murine stem cell progeny and peripheral leukocytes. Exp. Hematol. 14:207.

23. Bertoncello, I., S. Bartelmez, T.R. Bradley, and G.S. Hodgson. 1987. Increased Qa-m7 antigen expression is characteristic of primitive hematopoietic progenitors in regenerating marrow. J. Immunol. 139:1096.

24. Van Zant, G. 1984. Studies of hematopoietic stem cells spared by 5-fluorouracil. J. Exp. Med. 159:679.

25. Vetvicka, V., P.W. Kincade, and P.L. Witte. 1986. Effects of 5-Fluorouracil on B lymphocyte development. J. Immunol.
137:2405.

26. Nakano, T., N. Waki, H. Asai, and Y. Kitamura. 1989. Effect of 5-Fluoroacil on "primitive" hematopoietic stem cells that reconstitute whole erythropoiesis on genetically anemic W/Wv mice. *Blood.* 73:425.

27. Müller-Sieburg, C.E. 1989. Isolation and characterization of hematopoietic stem cells and progenitors. *Prog Immunol.* 7:331.

28. Lerner, C., and D.E. Harrison. 1990. 5-Fluorouracil spares hematopoietic stem cells responsible for long-term repopulation. *Exp Hematol.* 18:114.

29. Aihara, Y., H.-J. Bühring, M. Aihara, and J. Klein. 1986. An attempt to produce "pre-T" cell hybridomas and to identify their antigens. *Eur. J. Immunol.* 16:1391.

30. Spangrude, G.J., and R. Scollay. 1990. A simplified method for enrichment of mouse hematopoietic stem cells. *Exp Hematol.* 18:920.

31. Spangrude, G.J., and G.R. Johnson. 1990. Resting and activated subsets of mouse multipotent hematopoietic stem cells. *Proc Natl Acad Sci USA.* In press.

32. Tidmarsh, G.F., S. Heimfeld, C.A. Whitlock, I.L. Weissman, and C.E. Müller-Sieburg. 1989. Identification of a novel bone marrow-derived B cell progenitor population that coexpresses B220 and Thy-1 and is highly enriched for Abelson Leukemia virus targets. *Mol. Cell. Biol.* 9:2665.