Human Hematopoiesis in SCID Mice Implanted With Human Adult Cancellous Bone

By Jasbir S. Sandhu, Brian R. Clark, Erin L. Boynton, Harry Atkins, Hans Messner, Armand Keating, and Nobumichi Hozumi

The persistence of hematopoietic cells from human adult cancellous bone fragments implanted subcutaneously into CB-17 scid/scid mice was studied. Recipient mice received either no pretreatment (control group) or pretreatment with 3 Gy total-body irradiation and anti-asialo GM1 sera (ASGM1; pretreated group) before implantation. Pretreated severe combined immunodeficient (SCID) mice implanted with human bone were subsequently given ASGM1 every 7 days for the duration of the experiments. At 12 weeks postimplantation, flow cytometry of cells from pretreated and control animal tissues detected human CD45+ cells in the mouse spleen (mean, 7.8% and 3.4% positive cells, respectively), and bone marrow (BM; mean, 16.5% and 4.8% positive cells, respectively), and in the implanted human bone (73% and 8.9% positive cells, respectively). At 12 weeks, pretreated mice had human granulocyte-macrophage colony-forming cells (GM-CFC) and burst-forming units—erythrocyte (BFU-E) in the implanted human bone in the murine BM and in some of the spleens. The spleens also had extensive infiltration of human B cells and macrophages. Mean serum levels of human IgG in pretreated animals were 14 µg/mL during weeks 6 to 12, compared with trace levels (<1 µg/mL) in control mice. Bone from patients with acute myeloblastic leukemia (AML) was also implanted in pretreated SCID mice, and retrieved at 8 weeks for analysis. Comparison of preimplantation and implanted samples showed that the original histology was maintained, and massive infiltration of human CD68+ cells was observed in the mice spleens and BM. Implantation of AML bone in SCID mice facilitates analysis of in situ AML cell interaction with stromal cells in the leukemic state, and therapies against AML can be tested in this system, especially the selective killing of AML cells in the presence of other BM cells. Furthermore, this model requires no exogenous administration of cytokines to maintain human hematopoiesis with both normal or AML bone. Because the structure and function of both normal and diseased human adult bone is maintained, this animal model should facilitate investigation of both normal human hematopoiesis and hematopoietic malignancies.

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THE UNDERSTANDING of human hematopoiesis has been hampered due to a lack of a good animal model. Several approaches have been used to develop an animal model of human hematopoiesis, including the engraftment of human hematopoietic cells into severe combined immunodeficient (SCID) mice. But, using adult and fetal hematopoietic cells, only a limited degree of human hematopoiesis was observed. There may be at least two explanations for this: first, the human hematopoietic microenvironment (HHM) may be required for efficient human hematopoiesis; and second, the SCID mice residual nonspecific immune system (consisting of natural killer [NK] cells and macrophages) may be a barrier to the survival of human hematopoietic cells in the mouse. To investigate the effect of HHM on hematopoiesis, Kollmann et al transplanted into irradiated SCID mice both human fetal hematopoietic and adherent stromal cells and observed infiltration of human CD45+ cells in the mouse tissue. Kyoizumi et al addressed the issue of HHM by implanting human fetal bone into SCID mice, and detected fetal bone marrow (BM) cells in the implanted bone and a small number of circulating human CD45+ cells in the mouse blood, but no data were provided on the infiltration of early and mature human hematopoietic cells in other mouse tissues. Recently, Heike et al implanted adult bone in SCID mice, and also administrated human cytokines at the site of the bone implantation, and detected circulating human CD59+ cells in SCID mice only for the duration of cytokine administration. However, the regular administration of cytokines may skew the differentiation of human BM cell populations.

The immune reaction by SCID mice toward human hematopoietic cells remains an issue. For example, Nolta et al failed to engraft adult human BM cells in SCID mice and suggested that these animals produced an immune response against human hematopoietic cells possibly mediated by NK cells. Macrophage and NK cells have also been implicated in the graft-versus-host reaction during BM transplantation, and the administration of anti-NK-cell antibody to B6 mice was found to inhibit the rejection of transplanted mouse BM cells. Several approaches have been tried to overcome the SCID mouse immune barrier to human hematopoietic cells. First, upregulation of human hematopoiesis in mice by infusion of exogenous human cytokines has been attempted, and second, implantation of either highly active fetal BM cells or fetal bone in mice has achieved only limited success. Both of these approaches have limitations: cytokine administration may skew the differentiation of human BM cells, and engraftment of human fetal BM cells and bone in SCID mice is of limited value, since these tissues do not reflect adult BM hematopoietic conditions. Attempts to implant adult human bone in SCID mice in the absence of human cytokine administration have failed.

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To develop an animal model for adult hematopoiesis, we implanted adult human cancellous bone in pretreated SCID mice, using a recently developed novel protocol that has been used successfully to transplant human tissues\(^{16,17}\) and lymphocytes into SCID mice.\(^{18,19}\) Before transplantation of human tissues or cells, SCID mice were pretreated with radiation and anti-asialo GM1 rabbit sera (ASGM1); this serum specifically depletes only mouse macrophages and NK cells.\(^{19}\) Our investigations have clearly shown that the main barrier to the successful implantation of viable human tissues is the barrier to the successful implantation of viable human tissues (assessed by H&E; as described later).

To investigate the long-term effect of the SCID mouse environment on implanted bone, cancellous bone from five adult donors was implanted into both control (five animals from each donor) and pretreated SCID mice (five animals from each donor). The bone was harvested at 12 weeks after implantation and tissue sections were prepared from the bone implants. The sections were used for staining with H&E for immunohistochemical analysis (as described later). The spleens of pretreated Hu-bone-SCID mice were removed and processed for immunohistochemistry (as described later). The implanted bone and implanted bone samples were stained with antibody that recognized the human CD68 cell-surface marker.

**Materials and Methods**

**Antibodies.** Mouse antihuman CD45 antibody (Becton Dickinson, Mountain View, CA) conjugated with fluorescein isothiocyanate (FITC) was used to detect hematopoietic cells of human origin; Ly5.1 antibody (Cedarlane Laboratories, Hornby, Ontario, Canada) stains SCID mouse hematopoietic cells, and was used with the secondary antibody FITC-antimouse IgG2a (Cedarlane Laboratories). Mouse antihuman antibodies CD19, CD45, and CD68 (from Serotec, Oxford, UK) were used for immunohistochemical analysis.

Implantation of SCID mice with human bone. Cancellous bone was obtained from healthy adult patients (age range, 67 to 82 years) undergoing total hip joint replacement for treatment of degenerative osteoarthritis. Human bone specimens were obtained using protocols approved by the University of Toronto (Canada) Human Ethics Committee. Cancellous bone was obtained from the proximal femur, morcellized using a rongeur, and maintained under sterile conditions in RPMI (1640) medium (GIBCO-BRL, Burlington, Canada). To assess the state of the bone immediately before implantation, a representative sample of preimplantation bone specimen was processed for histological examination (as described later). Transplantation of bone into SCID mice was performed within 2 hours of procurement, under a general anesthetic (intramuscular administration of Xylazine, Oxford, UK) were used for immunohistochemical analysis.

Histology and immunohistochemistry of human bone implants and Hu-bone-SCID mouse spleens. Human bone specimens were harvested from pretreated and control Hu-bone-SCID mice, fixed in 10% (vol/vol) neutral buffered formalin, decalcified in 10% (vol/vol) formic acid, and embedded in paraffin. Specimens were cut into 5-μm sections and stained with H&E or with specific mouse antihuman antibodies (CD19, CD45, and CD68). Spleens from Hu-bone-SCID mice were also removed in some experiments and fixed in 10% (vol/vol) formalin, and embedded in paraffin. Specimens were cut into 5-μm sections and stained with specific mouse antihuman antibodies (CD19, CD45, and CD68).\(^{20}\)

**Colony-forming Assays.** Ten pretreated SCID mice were implanted with human bone from two donors. At 12 weeks, the Hu-bone-SCID mice were killed by cervical dislocation, and BM from both femurs and spleen cells were obtained by standard procedures. The implanted human bone fragments were excised and cells recovered by the procedure described by Kyoizumi et al.\(^{21}\) Human colony-forming cells (CFCs) in Hu-bone-SCID mice BM, spleen, and implanted human bone were assayed for human progenitor cells.\(^{22}\) BM cells from SCID mice not implanted with human bone were also assayed for human progenitor cells.\(^{22}\) Cells were plated in triplicate in Iscove’s modified Dulbecco’s medium containing 10% (vol/vol) bovine serum albumin solution (Boehringer Mannheim, Mannheim, Germany), 30% (vol/vol) fetal bovine serum (batch-selected: Hyclone, Logan, UT), 10 ng/mL recombinant human interleukin-3 (rh-IL-3; Cangene, Mississauga, Canada), 50 ng/mL recombinant human mast-cell growth factor (rh-MGF; Immunix, Seattle, WA), 2 U/mL cromyhoepoietin (rh-Epo; Ortho Pharmaceuticals, Raritan, NJ), and 1.25% (wt/vol) methylcellulose (Stem Cell Technologies, Vancouver, Canada). Cultures were incubated at 37% in a humidified atmosphere of 5% CO\(_2\), 95% air and scored after 14 days. Colonies (>50 cells) were enumerated as described.\(^{22}\)

Characterization of human cells in Hu-bone-SCID mice. Pretreated \((n = 24)\) and control \((n = 24)\) SCID mice were implanted with human bone from eight donors. Bone from each donor was implanted in three pretreated and three control SCID mice. At 12 weeks, the Hu-bone-SCID mice tissues and the implanted human bone were analyzed using flow cytometry for human CD45\(^+\) cells. Cells were prepared from Hu-bone-SCID mice spleens, peripheral blood, BM and implanted human bone. Red blood cells were lysed.
with hypotonic saline as previously described. Cells from various tissues were incubated with mouse antibody specific for human CD45, conjugated with FITC. Cells (1 to 2 × 10^6 cells per sample) were incubated with antibody for 45 minutes on ice, followed by three washes with PBS containing 2% fetal calf serum (FCS). At least 5 × 10^5 cells were counted in an EPIC-C flow cytometer (Couter Electronics, Hialeah, FL).

Splenocytes, BM cells, and lymphocytes from SCID mice, not implanted with human bone, were also stained and analyzed by flow cytometry. These cells were uniformly negative for staining with anti-human lymphocyte CD45 antibody.

Cells from the bone implanted in pretreated Hu-bone-SCID mice (n = 24) were also stained with Ly5-1 followed by the secondary antibody FITC-antimouse IgG2a and analyzed by flow cytometry. In each experiment, isotype-matched FITC-conjugated antibodies were included as negative controls.

**Analysis of Hu-bone-SCID sera for human Ig.** Blood samples were taken from pretreated (n = 10) and control (n = 10) Hu-bone-SCID mice (implanted with human bone from single donor) at 2-week intervals. Serum samples from Hu-bone-SCID mice were tested for human IgG using an enzyme-linked immunosorbent assay (ELISA; as described by Sandhu et al.6). Briefly, microcells were coated with the anti-human goat antibody (0.2 µg per well; Caltag Laboratories, San Francisco, CA), diluted in carbonate buffer (pH 9.6), and adsorbed on to microtiter plates (Immunlon 2; Dynatech, Chantilly, VA). The wells were washed with PBS and Tween (Sigma, St Louis, MO), and blocked with 5% wt/vol freeze-dried fetal calf serum (FCS). At 2 hours on ice, followed by 2 mol/L sodium hydroxide was added to each plate to stop the reaction, and absorbance was measured with an automated photometer at 410 nm. Positive-control human sera from volunteers in each assay were used to ensure reproducibility between plates and assays. Standard curves were made using purified human IgG (Sigma).

**RESULTS**

**Histology of the bone implants.** Histological analysis of adult human bone implants from control and pretreated Hu-bone-SCID mice was performed, at 3 and 12 weeks postimplantation (Fig 1A through E). Compared with preimplantation tissue (Fig 1E), bone specimens from control SCID mice at 3 weeks were largely necrotic and had fibrous changes in the BM and bone trabeculae (Fig 1C), with no major areas of viable BM, the 3-week specimens from pretreated mice also had few viable cells (Fig 1A), but the necrotic and fibrous changes observed in the BM from these specimens were less severe than the implants from control animals (Fig 1C).

By 12 weeks all of the human bone implants from pretreated SCID mice had recovered and looked morphologically similar to the preimplantation tissue (Fig 1B and E); in contrast, bone specimens from control mice remained necrotic and fibrous, with no significant recovery of the BM, and the histology of these implants was similar to that of the 3-week specimens (Fig 1D and E).

**Hematopoietic progenitor-cell activity in the implanted bone and Hu-bone-SCID tissue.** Twelve weeks after bone implantation, human hematopoietic progenitor-cell activity in Hu-bone-SCID mice BM, spleen, and the implanted human bone was measured, using a methylcellulose-based assay. Under the conditions used for analysis of human CFCs, only an occasional murine colony (determined by karyotypic analysis) appears by days 4 to 6, but they remain small (typically <30 cells) and are clearly dying by day 12 of culture. Thus, these colonies are easily distinguished from the human colonies (>50 cells) scored on day 14 on the basis of colony morphology and degenerative changes. BM from mice not transplanted with human bone formed few colonies under the conditions used, showing perhaps the strict species-specificity of human IL-3 to promote predominantly human, but not murine hematopoiesis. The results show (Table 1) that human progenitor cells with colony-forming capacity granulocyte-macrophage CFCs (GM-CFCs) and burst-forming units—erythroid (BFU-E) were present in all of the mouse BM and implanted human bone specimens. However, only some of the spleen of the Hu-bone-SCID-mice had detectable human progenitor-cell activity. The lack of human colony-forming units—erythroid (CFU-E) in these mice is consistent with absence of cross-reactivity of murine erythropoietin on human cells and the strict erythropoietin dependence of CFU-E.

**Characterization of human leukocytes in the bone implants and SCID mice tissue.** At 12 weeks, cells from pretreated (n = 24) and control (n = 24) Hu-bone-SCID mice tissues (spleen, femurs, and blood) and bone implants were isolated and stained with mouse antihuman CD45 antibody and analyzed by flow cytometry (Fig 2A through H). Human bone implants from pretreated SCID mice had an average of 73% human CD45+ cells, whereas bone implants from control SCID mice had an average of only 8.9% CD45+ cells (Table 2). A similar pattern was observed for tissues from control and pretreated Hu-bone-SCID mice. Pretreated Hu-bone-SCID mice BM had a substantially higher number of human CD45+ cells (16.5%) than BM from control animals (3.4%; Table 2). Splenocytes from pretreated animals had an average of 7.8% human CD45+ cells, whereas control animals had an average of 3.4% human CD45+ cells (Table 2).

Peripheral blood from pretreated animals had a mean of 5.5% circulating human CD45+ cells, while control animals had less than 2% human CD45+ cells (Table 2).

At 12 weeks, the majority of cells in the bone implants from pretreated SCID mice were of human origin, as shown by flow cytometry analysis using antihuman CD45 or antiserum Ly 5.1 antibodies (specific for human and mouse hematopoietic cells, respectively; Fig 3A and B). At 12 weeks, the 24 bone implants from eight donors had more than 70% of BM cells that were positive for the human CD45 marker (Table 2 and Fig 3A), and a small percentage of cells (range, 5% to 10%; mean ± SD, 8% ± 5%) that stained with the Ly 5.1 antibody (Fig 3A).

**Immunohistochemistry of bone implants and Hu-bone-SCID mice spleens.** At 12 weeks, immunohistochemistry for human cells was performed on bone implants and spleens.
of pretreated Hu-bone-SCID mice. The spleen and bone tissue sections were stained with mouse antihuman antibodies that recognized human CD45, CD68, and CD19 cell-surface markers (Fig 4A to F). The largest proportion of cells in the implanted bone was human CD45⁺ cells, followed by human B cells (CD19) and macrophages (CD68; Fig 4A through C). A similar pattern was observed in Hu-bone-SCID mice spleens (Fig 4D through F).

These data show that the implanted bone is viable at 12 weeks and can sustain human B cells, macrophages, and human CD45⁺ cell populations, and extensive infiltration of these cells was observed in the spleens of pretreated SCID mice (Fig 4D through F). However, it is not clear if these cells originate from the implanted bone or if there are other sites of human hematopoiesis in the Hu-bone-SCID mice that give rise to these cell populations.
HUMAN HEMATOPOIESIS IN Hu-BONE-SCID MICE

Analysis for human CFCs was performed on the implanted bone, mice were observed in BM from normal SCID mice; however, murine and spleen, and BM (under the conditions used, small number of colonies were observed in BM from normal SCID mice; however, murine and human colonies could be clearly distinguished). Colony values are given as human GM-CFC/BFU-E/CFU-E per 10^6 nucleated cells plated.

Abbreviations: ND, not determined; GM-CFC, granulocyte-macrophage colony-forming cell (CFC); BFU-E, burst-forming unit-erythroid; CFU-E, colony-forming unit-erythroid.

Human bone was implanted in pretreated SCID mice for 12 weeks. Analysis for human CFCs was performed on the implanted bone, mice spleen, and BM (under the conditions used, small number of colonies were observed in BM from normal SCID mice; however, murine and human colonies could be clearly distinguished). Colony values are given as human GM-CFC/BFU-E/CFU-E per 10^6 nucleated cells plated.

Human IgG in the Hu-bone-SCID mice blood. Peripheral blood was taken from Hu-bone-SCID mice at various time intervals and analyzed for human IgG (Fig 5). The data show that pretreated Hu-bone-SCID mice have detectable levels of circulating human IgG. The IgG concentration increased during the first 8 weeks after bone implantation and then reached a plateau. In control Hu-bone-SCID mice, only trace levels of human Ig (<1 μg/mL) were detected over the same interval.

Implantation of human leukemic bone in SCID mice. Although normal human bone can be maintained in pretreated SCID mice, its not clear if bone from adults with disease can be implanted in this animal model. Therefore, bone from each patient (n = 6) diagnosed with AML was implanted into single pretreated SCID mice (n = 6), for 8 weeks, to determine whether the diseased state of the engrafted bone can be maintained. The results are shown in Fig 6A and B. Spleens of the Hu-bone-SCID mice were also stained with mouse antihuman CD68 antibody, and the results are shown in Fig 6C.

Immunohistochemistry for the human cell-surface marker CD68 was performed on preimplantation and implanted bone. Spleens of Hu-bone-SCID mice were also stained for human CD68^+ cells. The results (Fig 6) show that a significant number of human CD68^+ cells were present in the bone specimens (Fig 6A and B) and in the Hu-bone-SCID mice spleens. Not only was the histology of the implanted bone maintained, but a massive infiltration of leukemic cells into the mouse tissue was observed (Fig 6C). Infiltration of human leukemic cells was also detected in the liver and lungs of Hu-bone-SCID mice (data not shown). Mice with the AML bone at 8 weeks had ruffled fur, were lethargic, and had stopped growing; such features were not observed in mice with normal bone implants.

Table 1. Analysis of Implanted Human Bone and Hu-Bone-SCID Mice Spleen and BM for Human CFCs

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Table 2. Expression of Human CD45 Surface Marker on Cells From Implanted Bone and Hu-Bone-SCID Mice Tissues

<table>
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<th>Tissue</th>
<th>Pretreated (n = 24)</th>
<th>Control (n = 24)</th>
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<tbody>
<tr>
<td>Implanted human bone</td>
<td>73.0 ± 10</td>
<td>8.9 ± 3</td>
</tr>
<tr>
<td>Mice spleen</td>
<td>7.8 ± 3</td>
<td>3.4 ± 2</td>
</tr>
<tr>
<td>Mice femur</td>
<td>16.5 ± 4</td>
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<td>Mice blood</td>
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Bone from 8 donors (67 to 82 years of age) was implanted in control (3 mice for each donor) and pretreated (3 mice for each donor) SCID mice. After 12 weeks, the implanted human bone and the mice tissues (spleen, femur, and blood) were analyzed by flow cytometry for human cells expressing the CD45 marker.

Fig 2. Representative example of phenotypic characterization of human cells isolated from implanted human bone and Hu-bone-SCID mouse tissue at 12 weeks. Cells from pretreated Hu-bone-SCID mice tissues and bone implants were stained with fluorescein isothiocyanate (FITC)-conjugated antihuman CD45 monoclonal antibody and analyzed by flow cytometry; implanted human bone (A; 76% CD45^+ cells), mouse BM (B; 15% CD45^+ cells), spleen (C; 9% CD45^+ cells), and blood (D; 4% CD45^+ cells). Flow cytometry analysis of cells from control Hu-bone-SCID mice tissues and bone implants; implanted human bone (E; 9% CD45^+ cells), mouse BM (F; 3% CD45^+ cells), spleens (G; 2% CD45^+ cells), and blood (H; no cells detected). (-----) Staining with a control antibody.
Fig 3. Representative example of phenotypic characterization of human and murine cells in implanted human bone from SCID mice at 12 weeks. Cells from implanted human bone from pretreated Hu-bone SCID mice were characterized by flow cytometry after staining with either Ly5.1 antibody for mouse cells (A; 7% positive cells) or antihuman CD45 antibody for human cells (B; 74% positive). (-----) Staining with a control primary antibody or isotope-matched FITC anti-Ly5.2 antibody.

DISCUSSION

Several reports on human hematopoiesis in the SCID mouse, after implantation of either human fetal bone or injection of fetal BM cells, have been published. However, embryonic and adult hematopoietic progenitors differ with respect to cycling rates, response profiles to hematopoietic factors, and differentiation capacities. These and other features peculiar to fetal hematopoiesis require consideration when data are interpreted and conclusions extrapolated for adult hematopoiesis.

Previous attempt to transplant adult human bone into athymic nude mice and SCID mice were unsuccessful. For example, Heike et al reported that human hematopoiesis cells were only viable in SCID mice if human cytokines

Fig 4. Immunohistochemistry of implanted human bone and Hu-bone-SCID mice spleens. Implanted human bone from pretreated Hu-bone-SCID mice was harvested at 12 weeks and fixed. Specimens were cut and sections stained with antibodies specific for the following human cell surface markers, CD45 (A), CD19 (B), and CD69 (C). Spleens from these animals were also removed and fixed, and the sections were stained with the following antihuman antibodies: CD45 (D), CD19 (E), and CD68 (F).
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Fig 5. Human IgG production in Hu-bone-SCID mice. Pretreated and control SCID mice (5 animals in each group) were implanted with human bone. The mice were bled at 2, 4, 6, 8, 10, and 12 weeks, after bone implantation. Data are mean ± SEM values for Hu-bone-SCID mice pretreated with radiation and ASGM1 antisera (■) and control mice (●).

were administered at the site of the bone implantation, and in athymic mice, the human bone was resorbed by the animal. This is the first report to describe the successful implantation of viable human adult bone into pretreated SCID mice. We have shown previously that SCID mice macrophage and NK cells are a major barrier to the efficient engraftment of human lymphocytes and tissues. The SCID mice macrophage may also be involved in the absorption of human bone implants (unpublished data, July 1994). The ASGM1 antibody removes both of these SCID mice cell populations and facilitates long-term engraftment of human adult bone in SCID mice.

In these studies, both the short- (3 weeks) and long-term (12 weeks) effects of pretreatment on the adult human BM and hematopoiesis were analyzed. Histology of the human bone specimens from pretreated and control SCID mice shows that the human BM underwent a crisis phase at 3 weeks in both groups of animals (Fig 1A and C). By 12 weeks, BM in specimens from pretreated animals had recovered (Fig 1B), but no recovery of BM was observed in bone implants from control mice (Fig 1D). In pretreated animals, a similar pattern of cell viability loss (at 3 weeks) and recovery (by 12 weeks) was observed for the human cells involved in human bone synthesis.

At 12 weeks, pretreated and control Hu-bone-SCID mice tissues cells were analyzed by flow cytometry for human CD45+ cells, and the pretreated animals had consistently higher infiltration of human CD45+ cells than the control group (Fig 2 and Table 2); also human bone implants from pretreated animals had a higher population of human CD45+ cells compared with implants from the control group. The bone implants were also analyzed for mouse hematopoietic

Fig 6. Implantation of human bone in pretreated SCID mice from patients with AML. A portion of the bone sample was taken for preimplantation (A) immunohistochemical analysis (stained with antihuman CD68), and the remainder was implanted in pretreated SCID mice. At 8 weeks, human bone grafts (B) and Hu-bone-SCID mice spleen (C) were removed and processed for immunohistochemical analysis and stained with antihuman CD68 antibody.
cells at 12 weeks, and only 5% to 10% of the cells were of murine origin, and more than 70% of the cells were human CD45+ (Fig 3). Immunohistochemical analysis of bone implants and spleens from pretreated Hu-bone-SCID mice showed that human leukocytes (CD45), macrophages (CD68), and B cells (CD19) are sustained long term (at least 12 weeks) in these tissues (Fig 4A through F) due to pretreatment of the mice.

BM is a major site of Ig production. In fact, more than half of serum IgG is produced by B cells in the BM. The high level of human IgG in pretreated Hu-bone-SCID mice blood suggests that human B cells are functional in the implanted human bone and mice tissue. In contrast, blood from the control group had only a trace amount of human IgG, which may indicate a minimal number of human B cells present in the animal tissue and implanted bone (Fig 5).

In pretreated Hu-bone-SCID mice at 12 weeks, cells of multiple lineage in different stages of maturation were present in the implanted bone. Human progenitor cells of multiple lineages were also detected (Table 1). Every pretreated mouse examined had human CFCs in femoral marrow, and three had occasional human CFCs in the spleen. Human GM-CFCs were more abundant than erythroid CFCs in the mice marrow, but examination of cells in the implanted bone fragment indicated the presence of both GM-CFCs and erythroid CFCs. Clearly, human hematopoietic cells from the implanted bone fragment were capable of colonizing and persisting in the murine hematopoietic environment. The fact that human progenitor cells were consistently found in mouse BM but only three spleens may be a reflection of the different hematopoietic environment of the two tissues.

Extrapolation of the numbers of human CFCs obtained from the femoral BM of mice examined indicate that an average of 1,900 human GM-CFCs are present per 10⁷ femoral BM cells, suggesting an average of 38,000 human GM-CFCs per mouse (assuming a femora is one twentieth of the total marrow volume, and equal seeding). However, it is unclear from this study whether human CFCs are constantly generated in the human bone fragment and migrate to the murine femora, or whether human hematopoietic progenitors colonize murine marrow and are able to undergo proliferation therein.

Bone from patients with AML was also implanted in pretreated SCID mice to determine if this model could be used to investigate human hematopoietic malignancy. The preimplantation bone and the 8-week AML bone grafts from SCID mice had similar histology. Immunohistochemical analysis of preimplanted and postimplanted AML bone specimens showed an abundance of human CD68+ cells, suggesting that the cell populations in the AML implanted bone were maintained in the pretreated SCID mouse environment (Fig 6A and B). Immunohistochemical analysis of Hu-bone-SCID mice spleens showed massive infiltration of human CD68+ cells (Fig 6C). These data clearly show that not only can the pretreated SCID mice maintain the structure and function of the normal human bone, but can also maintain the characteristics of the diseased bone. Interestingly, by 8 weeks, Hu-bone-SCID mice implanted with AML bone appeared to be sick, and three of these mice died at 2, 4, and 5 weeks postimplantation. The cause of the death was not clear; however, pretreated SCID mice implanted with normal adult bone are normally healthy and have a mortality rate of almost zero at 7 weeks postimplantation. This high mortality rate of SCID mice with AML bone may be due to high levels of infiltration of human myeloid cells found in mouse tissues, since the spleens (Fig 6C), lungs, liver, and BM (data not shown) were highly infiltrated with myeloid cells. Further studies are in progress to clarify these issues.

Lapidot et al reported that efficient engraftment of human AML cells into SCID mice required the administration of exogenous cytokines; however, these cytokines are likely to skew the differentiation of the human hematopoietic stem cells or enhance the survival of selected populations. For example, Nolta et al transplanted both human CD34+ cells and IL-3-producing human stroma in bnx mice, and detected no mature human B cells derived from CD34+ progenitor cells in the mice. Thus, the IL-3 cytokine skewed the normal human hematopoiesis in this model. Implantation of human adult bone into SCID mice can overcome these limitations, since our model facilitates human hematopoiesis of both normal and AML bone in the absence of cytokine administration, because, in both normal and AML bone, the stromal cells provide the requisite growth factors required for hematopoiesis.

Namikawa et al injected BM cells from eight patients with myeloid leukemias directly into human fetal bone in SCID-hu mice. Although cells from seven patients grew in the fetal bone, no infiltration of AML cells in mouse tissue was detected. In contrast, our Hu-bone-SCID mice implanted with AML bone had extensive infiltration of AML cells in mouse spleen (Fig 6C), lungs, BM, and liver (data not shown). Therefore, the depletion of mouse NK cells in our SCID mice allows implantation of human AML bone and infiltration of human AML cells in the mouse tissues. The AML bone implanted in SCID mice also allows examination in situ of stromal cell–stem cell interactions in the leukemic state, which is not possible by engrafting AML cells in fetal bone. Thus, our Hu-bone-SCID mouse system allows development and testing of anti-neoplastic or tumor-directed immunotoxins-based therapies for AML that selectively kill AML cells in the presence of normal BM and stromal elements in the human bone. Therefore, the effect of the therapy on other BM cell population can be characterized, which is not feasible by simply engrafting AML cells into SCID mice.

Nolta et al were unable to engraft BM cells from adults in nonpretreated SCID mice and Kollmann et al reported that, in the absence of radiation, no human fetal BM cells were detected in SCID mice tissues. We found that both ASGM1 and radiation pretreatment are essential for efficient engraftment of human cells and tissue in SCID mice. Our Hu-bone-SCID mice model offers distinct advantages over other methods used to engraft human hematopoietic cells in SCID mice. First, it is simple and requires only subcutaneous implantation of adult cancellous bone, which provides the HHM essential for long-term human hematopoiesis. Second, our pretreated mice provide the requisite environment...
for the long-term viability of the human bone and facilitate infiltration of human hematopoietic cells in the mouse tissue.

Pretreated SCID mice implanted with adult bone may provide a tool by which to study human hematopoietic disorders. Preliminary data from AML bone implantations studies suggest that this model will facilitate investigation of malignancy directly in the context of a human BM microenvironment, a model system more relevant than systems in which human malignancies are investigated in the context of mouse BM. For human gene therapy trials, this model may also serve as a valuable in vivo system to test long-term expression of exogenous genes introduced into human hematopoietic progenitor cells.

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Human hematopoiesis in SCID mice implanted with human adult cancellous bone

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