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12. Product DNA was asymmetrically inserted into pGEM3zf(−) (Promega) as described (*9*). One microgram of the double-stranded DNA template was sequenced in both forward (M13-21 universal) and reverse (M13 reverse) directions with the use of dideoxynucleotide triphosphates (Dye-Deoxy terminators) (Applied BioSystems) and analyzed with a 373A automated DNA sequencing system (Applied BioSystems) as described previously (*9*).
13. The multiple aligned sequence editor program (D. Faulkner and Y. Jerka, Molecular Biology Computer Research Resource, Harvard University) was used for alignment similarity scores. Positions in which gaps were inserted to maintain the alignment were discounted.
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28. Supported by the National Institute of Child Health and Human Development under grant HD26619-01, the National Institute of Allergy and Infectious Diseases under contract AI-32535, and the National Institute of Neurological Diseases and Stroke under grant PO1-25569. We thank A. Leigh-Brown, J. Theiler, D. Balding, G. Myers, J. Levy, L. Stein, N. Bouck, and A. Rowley for advice and discussion. Sequences have been submitted to GenBank under accession numbers M76788 to M76973, and alignments are available from the Human Retroviruses and AIDS Database.

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Cytokine Stimulation of Multilineage Hematopoiesis from Immature Human Cells Engrafted in SCID Mice

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Severe combined immunodeficient (SCID) mice transplanted with human bone marrow were treated with human mast cell growth factor, a fusion of interleukin-3 and granulocyte-macrophage colony-stimulating factor (PIXY321), or both, starting immediately or 1 month later. Immature human cells repopulated the mouse bone marrow with differentiated human cells of multiple myeloid and lymphoid lineages; inclusion of erythropoietin resulted in human red cells in the peripheral blood. The bone marrow of growth factor–treated mice contained both multipotential and committed myeloid and erythroid progenitors, whereas mice not given growth factors had few human cells and only granulocyte-macrophage progenitors. Thus, this system allows the detection of immature human cells, identification of the growth factors that regulate them, and the establishment of animal models of human hematopoietic diseases.

THE HEMATOPOIETIC SYSTEM IS ORganized as a hierarchy, ranging from large numbers of mature differentiated cells to rare pluripotent stem cells capable of extensive self-renewal and differentiation (*1*). Much of our knowledge of the organization and regulation of the hematopoietic system is derived from studies in the mouse where short- and long-term reconstitution assays are available to detect stem cells (*1, 2*). In contrast, our understanding of the biology of the human hematopoietic system is less complete because of a lack of in vivo stem cell assays. Several different approaches for engrafting human hematopoietic cells into immune-deficient mice have been described that employ transplantation of adult bone marrow, mature lymphoid cells, or fetal organs (*3*). These mouse models have already been used for studying a variety of human diseases including leukemia, autoimmunity, and infectious diseases (*3*). However, analysis of the normal developmental program of human hematopoiesis has been limited because engraftment of the murine tissues is low and only lymphoid or macrophage lineages develop.

We have described a system closely modeled on conventional bone marrow transplantation assays employing intravenous injection of adult human bone marrow into immune-deficient mice conditioned with radiation (*4*). Human macrophage progenitors migrate to the murine marrow, increase in number, and are maintained in this environment for several months; however, no mature cells are detected (*4, 5*). Other studies also indicate that the microenvironment of irradiated mice can support the growth of normal human lymphoid cells (*6*) and primary leukemic cells and cell lines (*7*). Human progenitor cells (*8*) and some leukemic cell lines (*9*) can also be maintained on murine stromal cells in vitro.

Because hematopoiesis is regulated by many growth factors, the low engraftment and the absence of multilineage differentiation in immune-deficient mice transplanted

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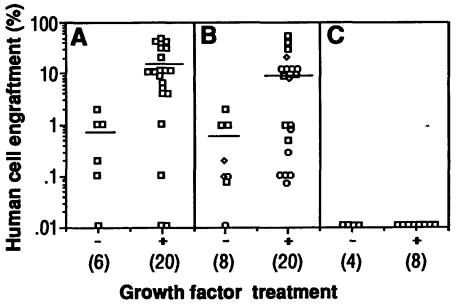

Fig. 1. Summary of the DNA analysis of the bone marrow of SCID mice transplanted with human hematopoietic cells with and without human growth factor treatment. The extent of human cell engraftment of the bone marrow was estimated as described (*13*). The results of 11 different experiments are presented. The horizontal line indicates the mean level of human cells from the number of mice shown (*n*) that were treated with individual or combinations of growth factors (+) or left without growth factors (−). (**A**) Sublethally irradiated SCID mice transplanted with human bone marrow and treated with growth factors for 30 to 40 days. (**B**) Sublethally irradiated SCID mice transplanted with human bone marrow, left untreated for 30 days, then treated with growth factors for an additional 30 days. Mice were transplanted with bone marrow cells from three different donors indicated by the different symbols. (**C**) Sublethally irradiated SCID mice transplanted with ficoll purified human PBLs or RBCs (4×10^7) and treated with growth factors for 21 to 30 days. No human cells were detected in the bone marrow of these mice.

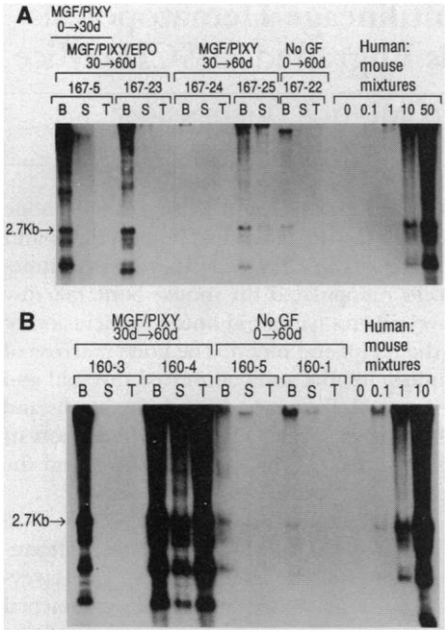

Fig. 2. DNA analysis of mice transplanted with human bone marrow cells and not given growth factors until 1 month later. The extent of human cell engraftment was determined as described (*13*). (**A**) Mice were transplanted with bone marrow and left for 1 month except 167-5, which received MGF/PIXY for the first month. Mice were then given MGF/PIXY/EPO (167-5 and 167-23) or MGF/PIXY (167-24 and 167-25) for the second month; mouse 167-22 did not receive factors for the entire period of time (60 days). (**B**) Mice transplanted with human bone marrow from a different donor and only given MGF/PIXY for the second month after the transplant. Mice 160-5 and 160-1 were not treated with growth factors for the entire period of the experiment.

with bone marrow may be due to the lack of appropriate concentrations of cross-reactive murine growth factors. We report that coadministration of erythropoietin (EPO) with hu-MGF (human mast cell growth factor; *kit* ligand) (*10*) or PIXY321 [a fusion protein of human interleukin-3 (IL-3) and human granulocyte-macrophage colony-stimulating factor (GM-CSF)] (*11*) into irradiated SCID mice transplanted with human bone marrow stimulated immature cells to repopulate the murine bone marrow with human progenitors and mature cells of the myeloid, lymphoid, and erythroid lineages.

Immune-deficient mice were transplanted with normal human bone marrow cells and treated with growth factors, incorporating several modifications to our previous protocol (*4, 12*). To quantitate the extent of human cell engraftment, we analyzed DNA extracted from the bone marrow, spleen, and thymus of transplanted SCID mice by Southern (DNA) blot (*13*). In the absence of cytokines, the extent of human cell engraftment ranged from less than 0.01% to about 1.0% depending on the donor marrow (Fig. 1). The majority of mice receiving MGF or PIXY321 alone had elevated numbers of human cells (>10%) compared to untreated mice. Mice injected with both MGF and PIXY321 had the best human cell engraftment (Fig. 1). In some mice treated with both factors, there was extensive repopulation of murine marrow with human cells (up to 50%) within 30 days (Fig. 1). Overall, 76% (41 of 54) of growth factor–treated mice had at least ten times more human cells than untreated mice; in some mice the stimulatory effect of growth factor treatment exceeded 100-fold (Fig. 1).

Mouse bone marrow was the preferred site of engraftment; however, a low number of human cells (0.1 to 1.0%) were usually present in murine spleen and thymus. Mice with the most human cells in the bone marrow often had enlarged spleens with ten times more splenocytes than normal, suggesting that repopulation of the bone marrow with human cells leads to enhanced extramedullary murine hematopoiesis. Untransplanted mice that were treated with growth factors did not show changes in bone marrow or spleen cellularity because these human growth factors are species-specific.

In order to confirm that immature cells in the human bone marrow and not contaminating mature peripheral blood lymphocytes (PBLs) in peripheral blood were responsible for engrafting SCID mice, we transplanted ten times more PBLs into irradiated SCID mice and treated with growth factors for 21 days. No human cells were detected in the bone marrow (Fig. 1) in accord with previous studies (*6, 14*).

We have detected low numbers of human cells in the bone marrow of transplanted mice for as long as 4 months (*5*). This observation predicts that if immature progenitor cells are still present in the bone marrow long after transplantation, they should still respond to exogenous human growth factors. Accordingly, a series of experiments were conducted where growth factor treatment was delayed for 1 or 3 months after transplantation (Fig. 2). Growth factor treatment only in the second month after transplant resulted in a large increase (10- to 100-fold) in the number of repopulating human cells compared to animals that did not receive growth factors for the entire time. Overall, 75% (15 of 20) of the mice treated by delayed addition of factor produced at least tenfold higher numbers of human cells compared to untreated control mice (Fig. 1). In one case, a tenfold increase was observed when factors were given from 3 to 4 months after transplant. Some mice (for example, 160-4) showed extensive repopulation of the thymus and the spleen in addition to the bone marrow (Fig. 2B).

Several of the most engrafted mice were examined by flow cytometry to determine which hematopoietic lineages were present (*15*) (Fig. 3). The bone marrow of mice treated with growth factors for 0 to 30 or 30 to 60 days contained immature $CD34^+$ and $CD33^+$ myeloid cells as well as mature $CD13^+$ granulocytes and monocytes. B cells bearing the early differentiation marker

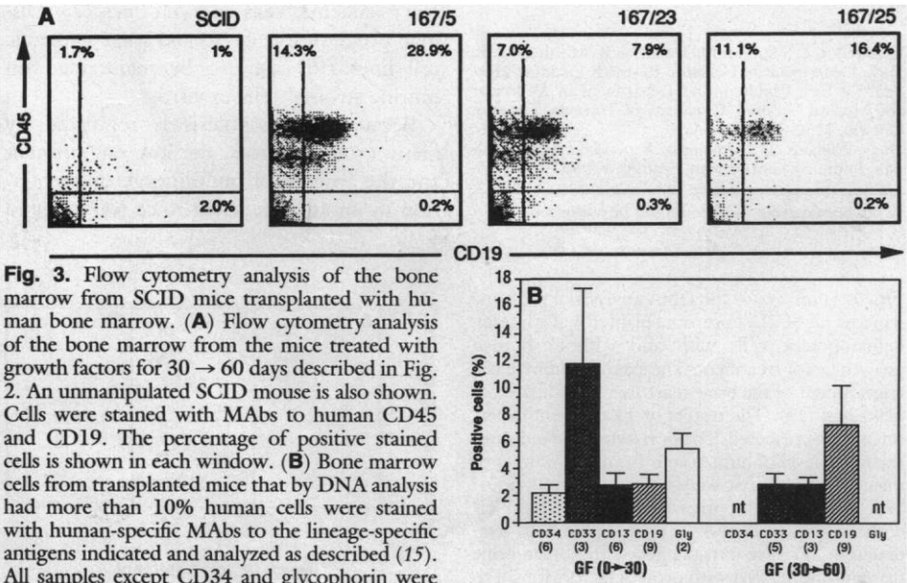

Fig. 3. Flow cytometry analysis of the bone marrow from SCID mice transplanted with human bone marrow. (**A**) Flow cytometry analysis of the bone marrow from the mice treated with growth factors for 30 → 60 days described in Fig. 2. An unmanipulated SCID mouse is also shown. Cells were stained with MAbs to human CD45 and CD19. The percentage of positive stained cells is shown in each window. (**B**) Bone marrow cells from transplanted mice that by DNA analysis had more than 10% human cells were stained with human-specific MAbs to the lineage-specific antigens indicated and analyzed as described (*15*). All samples except CD34 and glycophorin were also stained with MAbs to the pan-leukocyte marker CD45. Mice were treated with growth factors for 30 days starting immediately after the transplant (0 → 30) or after a 1-month delay (30 → 60). CD34 and glycophorin were not tested (nt) for the (30 → 60) group. The mean ± SE of single or double positive cells from the number of mice indicated (*n*) is shown. No human cells were detected in transplanted mice not treated with growth factors ($n = 7$).

Table 1. Multiple lineages of human progenitors are present in the bone marrow of SCID mice transplanted with human bone marrow and treated with growth factors. Bone marrow from transplanted SCID mice given the growth factors indicated was plated in methylcellulose cultures selective for the growth of human colonies (*16*). The total number of colonies was enumerated from duplicate plates.

Mouse	Growth factor treatment (days)	Progenitors per 2×10^5 cells plated			
		BFU-E	CFU-GM	CFU-GEMM	Blast
164-14	M/P (0 → 30)	26	50	0	2
167-15	P (0 → 30)	8	20	0	0
178-4*	P/E (30 → 60)	58	50	6	0
181-8*	P/E (30 → 60)	23	101	0	1
	None†	0	3	0	0

*Cultures from these mice contained two megakaryocyte colonies and >20 of the CFU-GM were composed only of eosinophils. †Averages of 15 untreated mice.

$CD19^+$ were detected in most of the mice analyzed and many were seen in several mice treated for 60 days (for example, mouse 167-5) or after delayed treatment with growth factors (167-23 and 167-25) (Fig. 3A). No $CD2^+$ T cells were found in the bone marrow of any mice tested. In addition to these myeloid and lymphoid cells, glycophorin-positive erythroid cells were present in the marrow of two mice (Fig. 3B). In contrast, no human cells were detected by flow cytometry in mice not given growth factors.

The detection of mature human hematopoietic cells of multiple lineages in the bone marrow of reconstituted mice indicated that human progenitors were also present. To quantify the progenitors, bone marrow cells from transplanted mice were plated in methylcellulose cultures that were completely selective for human progenitor cells (*16*). Compared to untreated mice that contained low numbers of only granulocyte-macrophage progenitors (CFU-GM), mice treated with growth factors had about ten times more total progenitor cells (Fig. 4A). Committed erythroid progenitors (BFU-E) were detected in the bone marrow only of treated mice (19 of 44). Regardless of the growth factor combination or whether growth factor treatment was delayed for 1 month, BFU-E comprised approximately 20% of the total human progenitors present in the murine bone marrow (Fig. 4A and Table 1). Only MGF alone was ineffective in giving rise to BFU-E. Many of the BFU-E yielded large macroscopic colonies composed of several small clusters typical of early erythroid progenitors (*17*) (Fig. 4B). The human origin of the BFU-E was confirmed by identification of human hemoglobin in individual colonies (below). In addition to both committed myeloid and erythroid progenitors, 5 of 19 mice also contained the multipotential progenitor CFU-GEMM (Fig. 4, C and D, and Table 1). CFU-GEMM is one of the most immature progenitors that can be assayed in culture (*18*), giving rise to a large colony that exhibits a differentiation profile typically seen in the bone marrow (*19*). The marrow of some highly repopulated mice also contained human cells capable of giving rise to small blast colonies; the blast nature of the cells was confirmed by Wright stain (Fig. 4E). Although these colonies were not tested, some blast colonies have high replating potential and may be derived from an earlier progenitor cell type (*20, 21*). Several representative highly engrafted mice, treated with different growth factor combinations, that contain these multiple lineages of progenitors are shown in Table 1.

The presence of human BFU-E in the bone marrow of growth factor–treated mice suggested the possibility that mature human red blood cells (RBCs) may be produced in the marrow and released into the peripheral blood. Blood from 2 of 18 mice treated with MGF/PIXY321 alone contained a low number of human RBCs (~1%), as detected by agglutination with antibodies directed to the human blood group antigens (*22*). Inclusion of hu-EPO in the growth factor cocktail resulted in an increase in the proportion of mice positive for human RBCs (22 of 37), in the number of human RBCs present in the blood (~5%), and in the total number of murine RBCs. The presence of human RBCs was independently confirmed by detecting human hemoglobin in blood lysates after electrophoretic separation on cellulose acetate plates; the two species of mouse hemoglobin migrate significantly slower than human hemoglobin (*23*). The two representative mice shown in Fig. 5A did not receive EPO for the first 30 days after transplant and were negative for human RBCs by agglutination. After treatment with growth factors plus EPO for a second month, the mice contained high numbers of BFU-E, the blood agglutinated strongly, and a distinct human hemoglobin band equivalent to approximately 5% human RBCs appeared (Fig. 5A, mouse 167-5). Human hemoglobin was also detected in the bone marrow from the crushed femur of a mouse transplanted with bone marrow

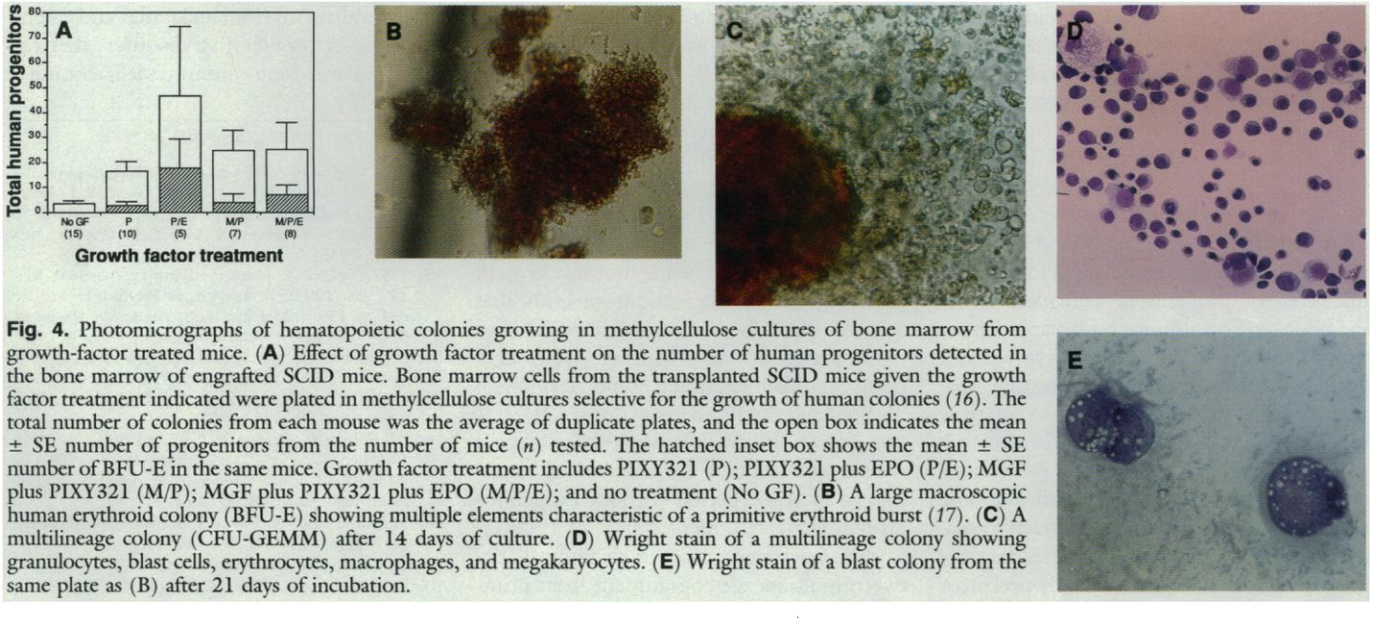

Fig. 4. Photomicrographs of hematopoietic colonies growing in methylcellulose cultures of bone marrow from growth-factor treated mice. (**A**) Effect of growth factor treatment on the number of human progenitors detected in the bone marrow of engrafted SCID mice. Bone marrow cells from the transplanted SCID mice given the growth factor treatment indicated were plated in methylcellulose cultures selective for the growth of human colonies (*16*). The total number of colonies from each mouse was the average of duplicate plates, and the open box indicates the mean ± SE number of progenitors from the number of mice (*n*) tested. The hatched inset box shows the mean ± SE number of BFU-E in the same mice. Growth factor treatment includes PIXY321 (P); PIXY321 plus EPO (P/E); MGF plus PIXY321 (M/P); MGF plus PIXY321 plus EPO (M/P/E); and no treatment (No GF). (**B**) A large macroscopic human erythroid colony (BFU-E) showing multiple elements characteristic of a primitive erythroid burst (*17*). (**C**) A multilineage colony (CFU-GEMM) after 14 days of culture. (**D**) Wright stain of a multilineage colony showing granulocytes, blast cells, erythrocytes, macrophages, and megakaryocytes. (**E**) Wright stain of a blast colony from the same plate as (B) after 21 days of incubation.

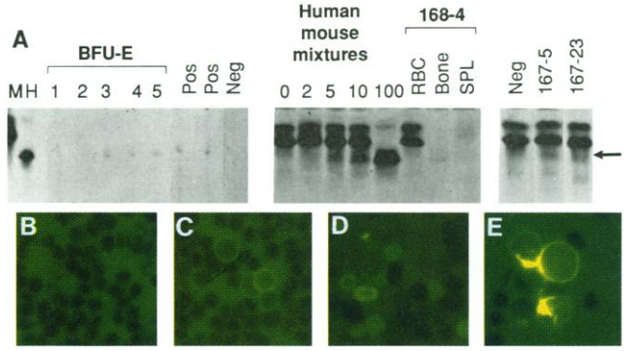

Fig. 5. Detection of human red blood cells (RBCs) in the peripheral blood and bone marrow of SCID mice transplanted with human bone marrow. (**A**) Cellulose acetate electrophoresis of the blood, bone marrow, and BFU-E of transplanted mice treated with growth factors including EPO. Blood was collected from mice 167-5 and 167-23 and tested for the presence of human hemoglobin after electrophoretic separation to distinguish mouse and human hemoglobin. Mouse 168-4 was transplanted with bone marrow from a patient with α-thalassemia minor and got less bone marrow cells because fewer cells were available. Thus the entire femur was crushed in lysis buffer, which made the sample much more dilute. After cystamine modification, mouse hemoglobin runs as two slower migrating species compared to a single human band. Mixtures of mouse and human blood (2, 5, 10, and 100%) were separated simultaneously, and the amount of human hemoglobin was quantitated by laser densitometry. The negative control was blood from an untransplanted animal. M, mouse blood; H, human blood. Five BFU-E from methylcellulose cultures of mouse 167-5 were also analyzed for the presence of human hemoglobin; the negative control was methylcellulose from the same plate. The positive controls were BFU-E from plates with human bone marrow. Blood (1 cm in a hematocrit tube) was lysed in cystamine buffer (50 μl) (*23*). In experiment 168, the bone was crushed in 100 μl of cystamine buffer. Samples were diluted, applied to cellulose acetate plates, and run at 300 V for 15 minutes. The plates were then stained in Ponceau S. Individual BFU-E were picked with a drawn-out pipette and lysed in 2 μl of the cystamine buffer before electrophoresis. (**B**) Fluorescence microscopy of blood from a control SCID mouse stained with FITC-labeled lectin that binds to the human blood group antigens. (**C**) A 1% mixture of human blood in 99% mouse blood. (**D**) Blood from a mouse treated for 1 month with growth factors plus EPO starting 1 month after the transplant of human cells. More than 1% of the cells stain positive to the lectin indicating the presence of mature human red cells. (**E**) Bone marrow from a highly repopulated mouse treated with growth factors plus EPO starting 1 month after the transplant of human cells. Blood was diluted 1:2 and mixed with FITC-labeled *Tetragonolobus purpureas* lectin directed to the O blood group antigen (Sigma). Photographs were taken under ultraviolet fluorescence microscopy using a Nikon Microphot FXA microscope. Original magnifications were ×400 except for (E), which was ×600.

from a patient with α-thalassemia minor (Fig. 5A, mouse 168-4). Mice transplanted with 4×10^7 RBCs and given growth factors including EPO (Fig. 1) had undetectable human RBCs in the blood 21 days after the transplant, suggesting that the half-life of human RBCs is much shorter in mice than humans. In order to directly visualize the human RBCs, we stained the peripheral blood and bone marrow from growth factor–treated mice with fluorescein isothiocyanate (FITC)–labeled lectins that bind to the human, but not mouse, red cell antigens (Fig. 5, B through E). Fluorescence microscopy shows positively stained human red cells in the bone marrow and peripheral blood.

Our data provide the first evidence that immature human cells engraft and survive in the murine microenvironment. First, no human cells or progenitors were detected in the bone marrow of mice transplanted intravenously with human PBLs with or without growth factor treatment, suggesting that mature cells in the donor bone marrow inoculum were not responsible for the multilineage engraftment. Second, the continued presence of myeloid, erythroid, and multipotent progenitors, in addition to mature cells, in treated mice indicates that the growth factors stimulate the early precursor that maintains the progenitor pool. And third, the strongest evidence that immature cells, earlier in ontogeny than CFU-GEMM, can engraft the murine microenvironment comes from experiments where growth factor treatment was delayed for 1 month after the transplant. Without exogenous growth factor treatment, human cells are about 1% of the bone marrow, only a few CFU-GM progenitors are present, no multipotential or erythroid progenitors can be detected, no human red cells are in the peripheral blood, and there are no B cells. Growth factor treatment of mice left untreated for 1 month after the transplant results in the production of large numbers of human cells of multiple lineages, including multipotential and committed progenitors. The maintenance of immature cells in the murine microenvironment indicates that some murine growth factors or cellular interactions, or both, that are important for human cell survival have cross-species activity. Murine MGF is a good candidate because it stimulates human myeloid and erythroid progenitors (*24*). It is not known if the immature human cells remain quiescent or are able to self-renew during the time period without exogenous growth factor treatment.

Our experiments do not distinguish between multilineage engraftment from primitive pluripotent stem cells or from separate immature precursor cells committed to each of these lineages. Conclusive characterization to distinguish between these two possibilities will require a detailed examination of the developmental potential and capacity for self-renewal of the immature engrafting cell types by combining retrovirus gene transfer techniques (*5*) with this transplantation system (*25*). Such genetic approaches will also provide an in vivo model to test gene therapy strategies targeted to stem cells rather than T cells (*26*).

Growth factor treatment of transplanted mice provides a rapid in vivo assay for factors that affect the differentiation and proliferation of early cells within the human hematopoietic stem cell hierarchy. Moreover, the appearance of erythroid cells in response to EPO suggests it may be possible to repopulate specific lineages selectively depending on the choice of growth factor.

Multilineage repopulation of SCID mice expands the range of human diseases that can now be studied in this animal model. For example, the production of mature red cells in the blood of mice transplanted with the bone marrow from a patient with α-thalassemia minor suggests that it may be possible to create animal models of human anemias and thalassemias. Cells from patients with Diamond-Blackfan anemia also engraft SCID mice and transplantation of cells from patients with more severe diseases such as B^0 thalassemia and sickle cell anemia should also be possible. Finally, we have established an animal model of human pre-B acute lymphoblastic leukemia that is analogous to the progression of the disease in humans (7); however, acute or chronic myeloid leukemias have proven difficult to grow in mice. In vivo cytokine treatment may stimulate myeloid leukemias and extend the types of proliferative disorders that can be transplanted into immune-deficient mice.

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12. Several modifications were made to the bone marrow transplant protocol reported earlier (*4*) in an attempt to obtain multilineage engraftment. First, SCID recipient mice were used rather than athymic *bg/nu/xid* mice because normal human T cells and human leukemic cells migrate to the murine thymus (*6, 7*). The SCID mice were bred and maintained under defined flora conditions at the Ontario Cancer Institute. Second, the bone marrow cell dose was increased to 2×10^7 to 4×10^7 cells. Third, cytokines were delivered by alternate daily intraperitoneal (IP) injection rather than osmotic minipump to permit the delivery of higher doses over a longer time. The quantities of growth factors given IP to each mouse on alternate days were PIXY321, 8 μg; MGF, 20 μg; and EPO, 20 U. PIXY321 at 0.8 μg was ineffective. Fourth, recipient SCID mice were sublethally irradiated with 400 cGy before transplantation. This radiation dose permits immune reconstitution of SCID mice with syngeneic bone marrow and at the same time nontransplanted mice survive provided they are maintained in a pathogen-free environment (*27*).
13. DNA (5 μg) was digested with Eco R1 and hybridized with a human chromosome 17–specific α-satellite probe (p17H8). To quantitate the amount of human cells present in these tissues, the intensity of the 2.7-kb band was compared to human/mouse DNA mixtures (0.1, 1.0, 10, and 50% human) by described procedures (*4, 7*). Multiple exposures of the autoradiographs were taken to ensure sensitivity to 0.01% human DNA.
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15. Flow cytometry was done by incubating bone marrow cells with saturating amounts of human-specific monoclonal antibodies (MAbs) directly conjugated with FITC or phycoerythrin to the early myeloid antigens, CD34 (10M34, Amac) and CD33 (MY9, Coulter); granulocytes and macrophages, CD13 (MY7, Coulter); early B cells, CD19 (B4, Coulter); and erythroid cells, glycophorin (Amac). Samples labeled with CD33, CD13, and CD19 were also stained with MAb CD45 (KC56, Coulter), which is expressed on most human hematopoietic cells. In this case only the double-labeled cell population was enumerated. Samples were run on an EPICS Profile Analyzer (Coulter). In each experiment, bone marrow cells from normal SCID mice were stained with the same MAbs as negative controls. In addition, an isotype control MAb was included. Background staining in the single or double positive window never exceeded 2% and was subtracted from the percentage of positive cells automatically calculated for the test samples. Human bone marrow and PBLs were used as positive controls and for gating of myeloid and lymphoid cells.
16. Bone marrow cells from transplanted mice (2×10^5) were plated in methylcellulose cultures (*5*). Briefly, the cultures contained tested lots of human plasma and hu-IL-3 (10 U/ml), hu-GM-CSF (1 U/ml), PIXY321 (5 ng/ml), MGF (50 ng/ml), and hu-EPO (2 U/ml). The specificity of the assay was confirmed by plating human and mouse mixtures and by PCR on individual colonies using primers specific for the human dystrophin gene (*5*). Morphological criteria and histological staining were used to identify colonies derived from colony-forming unit granulocyte-macrophage (CFU-GM), burst-forming unit erythroid (BFU-E), and colony-forming unit granulocyte-macrophage-erythroid-megakaryocyte-eosinophils (CFU-GEMM) progenitors. Blast colony-forming progenitors were detected after 21 days of culture and their identity was confirmed by histological analysis.
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22. Human RBCs were detected in transplanted, EPO-treated mice by agglutination reaction using commercial antibody reagents directed to the human blood group antigens (Baxter). Approximately 0.5 μl of blood was mixed with phosphate-buffered saline or antibodies to blood groups for 1 min at room temperature. Red cell agglutination was performed in triplicate and measured by low power microscopy. The extent of agglutination in the test samples was visually compared to test mixtures of human and mouse blood (1, 10, 100%). Control mouse blood did not agglutinate under these conditions.
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Form-Cue Invariant Motion Processing in Primate Visual Cortex

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The direction and rate at which an object moves are normally not correlated with the manifold physical cues (for example, brightness and texture) that enable it to be seen. As befits its goals, human perception of visual motion largely evades this diversity of cues for image form; direction and rate of motion are perceived (with few exceptions) in a fashion that does not depend on the physical characteristics of the object. The middle temporal visual area of the primate cerebral cortex contains many neurons that respond selectively to motion in a particular direction and is an integral part of the neural substrate for perception of motion. When stimulated with moving patterns characterized by one of three very diverse cues for form, many middle temporal neurons exhibited similar directional tuning. This lack of sensitivity for figural cue characteristics may allow the uniform perception of motion of objects having a broad spectrum of physical cues.

OBJECTS IN OUR VISUAL WORLD commonly differ physically, such as in brightness, texture, or distance from an observer. This variation generally bears little relation to the ways in which an object can move through space. Optimal detection of visual motion thus demands that the underlying neural apparatus disregard physical diversity among the cues that define moving objects. In this report I present neurophysiological evidence that motion-sensitive neurons in the middle temporal area (MT) of monkey visual cortex meet this demand by expressing form-cue invariance (*1*) in their selectivity for direction of motion.

Moving stimuli fall into two broad classes on the basis of the spatial characteristics of the defining figural cue. The first and most typical class consists of moving stimuli that are either brighter or darker than their surroundings. Adopting the terminology of Cavanagh and Mather (*2*), I refer to this as first-order motion (*3*). Consider, alternatively, the motion percept elicited by a traveling wave of flickering leaves arising from movements of a predator through a forest canopy. This complex but biologically significant temporal texture (*4*) is but one instance of a second class of visual motion that is clearly perceived despite the absence of any unique pattern of luminance traceable over space and time. More precisely, moving stimuli of this second class (i) are discriminable only by means other than luminance contrast (for example, spatial or temporal texture, stereoscopic disparity) and (ii) have a spatial luminance profile that varies during motion. Such stimuli have been labeled second-order motion (*2*).

The descriptive differences between these two classes of motion underscore important functional differences with respect to the mechanisms necessary for their detection. Owing to a pattern of luminance that is correlated over space and time, first-order motion can be detected in conventional spatiotemporal frequency or energy models for motion detection (*5, 6*). The computations utilized by these models may also underlie motion detection at early stages in the primate visual system, and there is abundant psychophysical (*5, 7*) and physiological (*8*) evidence in support of this possibility. Traditional motion models cannot account for detection of second-order motion (*9*). However, despite the substantial differences be-

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