we should expect great variability in zooxanthellae density. Hence, bleaching events in corals within such lagoons may be frequent and part of the expected cycle of variability.

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8. The data were collected from a lagoon near a small coastal village, Trou aux Biches, in the northwest of the island of Mauritius (latitude 20°S). The lagoonal area is about 4.1×10^6 m^2 with an average depth of 2.5 m. There is a high degree of eutrophication in the lagoon with only 20% live coral cover (80 to 90% *Acropora*, and a few occurrences of *Pocillopora* and *Porites*), algal proliferation (50% *Padina*, 10% *Sargassum*, 5% *Turbinaria*, 5% *Valonia*, 5% *Galaxaura*, and 25% other), and a considerable degree of anthropogenic activity (swimming, boating, fishing, snorkeling, water-skiing). A colony was selected that lay in about 2 m of water, depending on the state of the tide. Coral samples were collected by breaking off one live coral tip (2 to 3 cm) from a randomly selected part of the same colony each week.
9. Dissolved oxygen and surface water temperature (at depths of 0.5 to 1.0 m) were taken in situ, and nitrate and phosphate concentrations were determined from water samples in the laboratory. Zooxanthellae were extracted from live coral tips according to Drew's technique (*13*) after decalcifying in 5% HCl. Aliquots of homogeneous extracts were placed on a hemocytometer (improved Neubauer counting chamber, depth 0.1 mm), and zooxanthellae cells were counted under an inverted microscope at ×400. The aluminum foil method of Marsh [J. A. Marsh, *Ecology* **55**, 255 (1970)] was used to calculate the surface area of the coral tip from which the zooxanthellae were extracted. Data on amounts of solar radiation and rainfall in the area were obtained from the Mauritius meteorological office.
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12. To test for density dependence, we assumed that the change in density was linearly related to the present density. The following model [B. Dennis and M. L. Taper, *Ecol. Monogr.* **64**, 205 (1994)] was fitted:

$$\ln(N_t/N_{t-1}) = a + bN_{t-1} + \sigma Z_t$$

where N_t is the density of zooxanthellae at time t, a and b are constants, σ is a positive constant, and Z_t has a normal distribution with a mean of 0 and a variance of 1, so that σZ_t is the term representing density-independent factors (random shocks). The time between successive points, t and $t + 1$, is 1 week. A density-dependent model has a value for b significantly different from zero. The maximum likelihood estimates for the parameter values are $a = 0.15$, $b = -0.10$, and $\sigma^2 = 0.84$; the probability of the null model being rejected is $P = 0.016$ (as calculated by parametric bootstrapping).
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16. The sea surface temperature shows a seasonal fluctuation, with maximum temperatures in the summer period (December to April) just exceeding 30°C. The minimum temperature was 22.8°C (August 1993) and the maximum was 30.8°C (April 1994). The concentrations of dissolved oxygen (mean = 7.8 ppm, SD = 2.6 ppm), nitrate (mean = 26.9 μg liter^{-1}, SD = 45.7 μg liter^{-1}), and phosphate (mean = 20.0 μg liter^{-1}, SD = 44.1 μg liter^{-1}) all fluctuated greatly over the study period (n = 147).
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18. Because dissolved oxygen in the water column is continuous with water in the coelenteron, it may lead to increased oxygen concentration within the coral. High concentrations of oxygen within the coral can precipitate bleaching [M. P. Lesser, *Coral Reefs* **16**, 187 (1997)].
19. Because the data collected were from the tip of the coral and because growth is expected to be greatest at this point [E. H. Gladfelter, *Biol. Bull. (Woods Hole)* **165**, 811 (1983); F. P. Wilkerson, D. Kobayashi, L. Muscatine, *Coral Reefs* **7**, 29 (1988)], the observed variability may be attributable in part to a combination of coral growth and zooxanthellae division to exploit the newly available space [R. J. Jones and D. Yellowlees, *Philos. Trans. R. Soc. London Ser. B* **352**, 457 (1997)].
20. Some of this variability is undoubtedly because different parts of the same colony were sampled and the orientation of the coral branch to incident light is known to affect zooxanthellae density [L. R. McCloskey and L. Muscatine, *Proc. R. Soc. London Ser. B* **222**, 215 (1984); Z. Dubinsky, P. G. Falkowski, J. W. Porter, L. Muscatine, *ibid.*, p. 203]. In addition, it is possible that different strains of zooxanthellae exist in different parts of the colony [R. Rowan, N. Knowlton, A. Baker, J. Jara, *Nature* **388**, 265 (1997)]. Thus, the data collected reflect the normal degree of zooxanthellae variability expected over an entire colony. However, this cannot be the only cause of variation, as the test for density dependence specifically examined and rejected the hypothesis that the sole cause of the variability through time is just random sampling from some distribution of zooxanthellae abundance.

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Dependence of Human Stem Cell Engraftment and Repopulation of NOD/SCID Mice on CXCR4

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Stem cell homing and repopulation are not well understood. The chemokine stromal cell–derived factor-1 (SDF-1) and its receptor CXCR4 were found to be critical for murine bone marrow engraftment by human severe combined immunodeficient (SCID) repopulating stem cells. Treatment of human cells with antibodies to CXCR4 prevented engraftment. In vitro CXCR4-dependent migration to SDF-1 of CD34$^+$CD38$^{-/low}$ cells correlated with in vivo engraftment and stem cell function. Stem cell factor and interleukin-6 induced CXCR4 expression on CD34$^+$ cells, which potentiated migration to SDF-1 and engraftment in primary and secondary transplanted mice. Thus, up-regulation of CXCR4 expression may be useful for improving engraftment of repopulating stem cells in clinical transplantation.

Stem cells within the bone marrow microenvironment actively maintain continuous production of all mature blood cell lineages throughout life. These rare primitive cells are functionally defined by their ability to home to the bone marrow and to durably repopulate transplanted recipients with both myeloid and lymphoid cells (*1, 2*). Several groups have established in vivo models for engrafting human stem cells (*3–8*). We developed a functional in vivo assay for primitive human SCID repopulating cells (SRCs) based on their ability to repopulate the bone marrow of intravenously transplanted SCID or nonobese diabetic SCID (NOD/SCID) mice with high levels of both myeloid and lymphoid cells (*5, 6, 8*).

Chemokines are cytokines that are best known for their ability to selectively attract subsets of leukocytes to sites of inflammation (*9*). The role that chemokines and their receptors play in homing and repopulation of human stem cells is not fully understood. The

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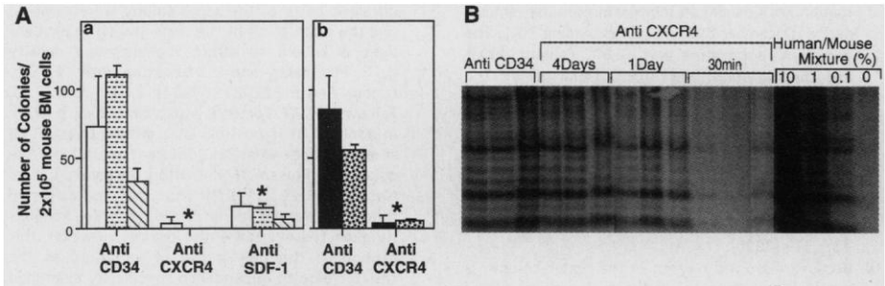


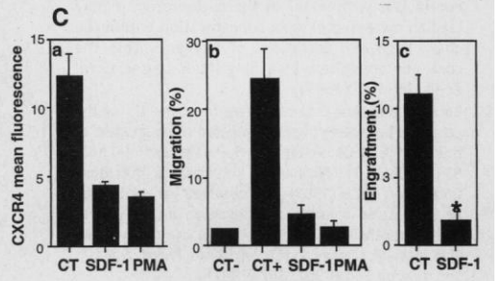
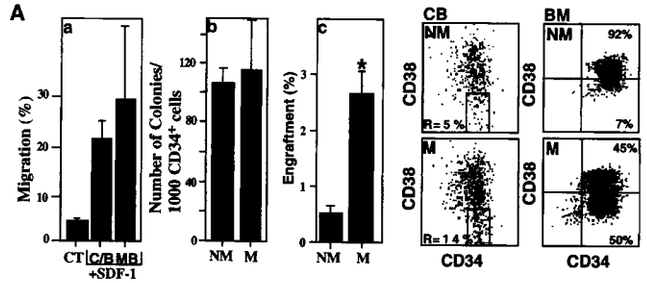


Fig. 1. Effect of antibodies to CXCR4 and SDF-1 on engraftment of NOD/SCID bone marrow (BM) by human CD34$^+$ cells. (**A**) (Panel a) Human cord blood CD34$^+$ cells (*6*) treated with two alternative antibodies to CXCR4 or with anti-CD34 as a control were transplanted into mice. Alternatively, anti–SDF-1 was coinjected with the cells and reinjected 24 hours later. After 2 weeks human progenitor cells were quantified in semi-solid media assays (*5*). The following cell types were counted: colony-forming unit–granulocyte/macrophage (CFU-GM) (white bars), blast-forming unit–erythroid (BFU-E) (dashed bars), and multilineage colony (CFU-GEMM) (striped bars). Data are average ± SE (*$P < 0.01$, as determined by paired Student's t test) of three experiments. (Panel b) Human bone marrow (black bars) or mobilized peripheral blood (stippled bars) CD34$^+$ cells were treated with the indicated antibodies and transplanted into NOD/SCID mice, and total human progenitors were quantified after 1 month as for panel a. (**B**) Antibodies to CXCR4 were injected at the indicated times after transplantation of NOD/SCID mice with cord blood CD34$^+$ cells. Control cells were incubated with anti-CD34. After 2 weeks, bone marrow was assayed by Southern blot for human DNA with a human-specific α satellite probe (*5*). (**C**) Cord blood CD34$^+$ cells were either not treated (CT) or treated for 24 hours with SDF-1 or PMA. (Panel a) CXCR4 surface expression of CD34$^+$ cells. (Panel b) Transwell migration assay (*13*) of untreated cells without SDF-1 (CT−) or with SDF-1 (CT+), and migration to SDF-1 of treated cells. (Panel c) The percent of human cells in NOD/SCID mice 1 month after transplantation was determined by FACS analysis with antibodies to human CD45. Data are average ± SE (*$P < 0.01$) of three experiments.



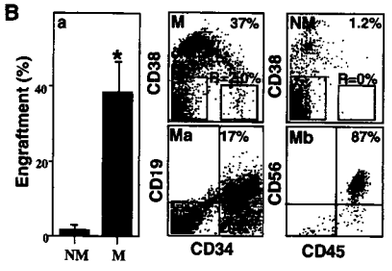


Fig. 2. SDF-1 induces the migration of SRCs. (**A**) (Panel a) Transwell migration assay with CD34$^+$ cord blood (C), bone marrow (B), or mobilized peripheral blood (MB) cells (*13, 14*). CT, migration without SDF-1. SDF-1 migrating (M) and nonmigrating (NM) cells were assayed for progenitors (panel b) or transplanted into NOD/SCID or β_2-microglobulin knockout NOD/SCID mice (3×10^4cells per mouse) (panel c) (*20, 23*). The percent of human cells was quantified as in Fig. 1C, panel c. Data are average ± SD of 11 (panel a) or 3 experiments (panel b), or average ± SE of three experiments (panel c) (*$P <$ 0.01). (Panels CB and BM) SDF-1 preferentially induces migration of CD34$^+$CD38$^{-/low}$CXCR4$^+$ cells. Surface expression of CD38 on cord blood (panel CB) and bone marrow (panel BM) CD34$^+$ cells was analyzed by flow cytometry on SDF-1 migrating (M) or nonmigrating (NM) cells. R gates CD34$^+$/CD38$^-$ cells. (**B**) Sorted cord blood CD34$^+$CD38$^{-/low}$ cells, (Panel a) SDF-1 migrating (M) or nonmigrating (NM) cells were transplanted into NOD/SCID mice (3×10^4cells per mouse). After 6 weeks, percent of engraftment was quantified as in Fig. 1C, panel c. Data are average ± SE (*$P < 0.01$) of three experiments. Phenotype analysis of engrafted M and NM cells. Numbers indicate percent of human cells. (Panels Ma and Mb) The presence of human lymphoid CD45$^+$CD19$^+$ pre-B cells (panel Ma) and progenitors for human CD45$^+$CD56$^+$ natural killer cells (panel Mb) is shown.

chemokine SDF-1 (*10*) binds to its receptor CXCR4, which is expressed on many cell types, including some CD34$^+$CD38$^-$ cells (*11, 12*). In vitro SDF-1 attracts certain CD34$^+$CXCR4$^+$ cells, and in vivo it is produced by bone marrow stromal cells as well as by epithelial cells in many organs (*11, 13, 14*). Mice that lack SDF-1 or do not express CXCR4 exhibit many defects, including the absence of both lymphoid and myeloid hematopoiesis in the fetal bone marrow (*10, 15*). Overexpression of human CD4 and CXCR4 receptors on murine CD4$^+$ T cells led to enhanced homing of these cells to the murine bone marrow (*16*).

To examine the in vivo role of SDF-1 and its receptor CXCR4 in migration and repopulation by human SRCs, we treated CD34$^+$-enriched cord blood cells either with two different antibodies to CXCR4 or with control antibodies to CD34 (anti-CD34) before transplantation of NOD/SCID mice (Fig. 1A, panel a). Only anti-CXCR4 reduced engraftment. Similar treatment of human CD34$^+$-enriched cells from mobilized peripheral blood or adult bone marrow also resulted in inhibition of engraftment (Fig. 1A, panel b). Antibodies to SDF-1 coinjected with human CD34$^+$ cord blood cells and readministered after 24 hours significantly reduced the level of engraftment (Fig. 1A, panel a). The first 24 hours were critical to the engraftment process. Antibodies administered intraperitoneally 30 min after transplantation blocked engraftment (Fig. 1B). Antibodies administered 24 hours later reduced engraftment less effectively and when administered 4 days after transplantation were completely ineffective (Fig. 1B) (*17*).

The effects of SDF-1 desensitization and CXCR4 down-regulation on the ability of human CD34$^+$ cells to migrate and engraft NOD/SCID mice were further studied. SDF-1 and phorbol esters [phorbol 12-myristate 13-acetate (PMA)] cause internalization and down-regulation of CXCR4 surface expression on human CD4$^+$ T cells (*18*). Cord blood CD34$^+$ cells were incubated overnight with high doses of SDF-1. Cells were subsequently washed and tested for CXCR4 expression and migration to SDF-1 in a transwell assay. Treatment of CD34$^+$ cells with SDF-1 or PMA reduced CXCR4 cell surface expression (Fig. 1C, panel a) and abolished the migration of CD34$^+$ cells in response to SDF-1 (Fig. 1C, panel b) (*19*). Prolonged treatment of CD34$^+$ cells with SDF-1 significantly blocked the engraftment of transplanted NOD/SCID mice (Fig. 1C, panel c). Thus, SDF-1 probably affects SRC engraftment by mediating chemotaxis to the bone marrow, linking migration to SDF-1 in vitro to human stem cell function in vivo.

The migration potential of human CD34$^+$ cells from cord blood, bone marrow, or mo-

bilized peripheral blood was tested in vitro in a transwell assay. Consistent with previous studies (*13*), 20 to 25% of cord blood and bone marrow $CD34^+$ cells migrated in response to a chemotactic gradient of SDF-1 in all donors tested (Fig. 2A, panel a). Migration of mobilized peripheral blood $CD34^+$ cells from multiple donors in response to SDF-1 was variable (between 8 to 60%), suggesting the involvement of SDF-1 in the mobilization process (Fig. 2A, panel a). The migrating and nonmigrating $CD34^+$ cell populations did not differ in the incidence of progenitor cells, as determined by in vitro colony assays (Fig. 2A, panel b); however, the engraftment potential of the migrating and nonmigrating $CD34^+$ cells was different. Equal numbers of migrating (M) and nonmigrating (NM) $CD34^+$ cells were washed and transplanted into NOD/SCID or NOD/SCID β_2 microglobulin knockout mice (*20*). Whereas mice transplanted with nonmigrating cells were poorly engrafted, mice transplanted with migrating cells were significantly better engrafted (Fig. 2A, panel c). The low concentrations of SDF-1 and the limited exposure time caused only a transient decrease of CXCR4 expression that did not prevent engraftment. These results are further evidence for the link between in vitro motility to SDF-1 and in vivo stem cell function.

Although only 20 to 25% of cord blood $CD34^+$ cells migrated toward SDF-1, this population contained a significantly higher percentage of primitive $CD34^+CD38^-$ cells than did nonmigrating cells (Fig. 2A, panel CB). In $CD34^+$ cells from bone marrow, the proportion of immature $CD34^+CD38^{-/low}$ cells migrating to SDF-1 was larger than in cord blood (Fig. 2A, panel BM). Nevertheless, most cord blood $CD34^+CD38^-$ cells (60%) did not migrate to SDF-1, demonstrating that $CD34^+CD38^-$ cells are a heterogeneous population composed mostly of nonmigrating cells. Sorted $CD34^+CD38^{-/low}$ cord blood cells from different donors were evaluated for their ability to migrate toward a chemotactic gradient of SDF-1 in vitro on the basis of surface CXCR4 expression and for their content of SRCs in vivo. Only 26% (±7%) of the $CD34^+CD38^{-/low}$ cells migrated to a gradient of SDF-1 in the transwell assay. Transplantation of migrating $CXCR4^+$ cells into NOD/SCID mice resulted in high levels of multilineage engraftment (Fig. 2B). This was reflected in the engraftment of primitive $CD34^+CD38^-$ cells (Fig. 2B, panel M) and lymphoid (Fig. 2B, panels Ma and Mb), and myeloid colony-forming cells. In contrast, little engraftment was observed with nonmigrating $CXCR4^{-/low}$ cells (Fig. 2B, panel NM). Thus, the $CD34^+CD38^{-/low}CXCR4^+$ migrating cell population representing less than one-third of all $CD34^+CD38^{-/low}$ cells engrafts the murine bone marrow with SRCs.

Kim and Broxmeyer have demonstrated that stem cell factor (SCF) attracts $CD34^+$ cells, increases their motility, and synergizes with SDF-1, increasing migration to both cytokines in vitro (*21*). Unexpectedly, prolonged (24- to 48-hour) stimulation of mobilized peripheral blood $CD34^+$ cells with SCF resulted in increased CXCR4 expression (Fig. 3A), enhanced migration toward SDF-1 (Fig. 3B), and enhanced engraftment potential dependent on the exposure time to SCF (Fig. 3C). Engraftment potential was similarly increased when only half the cell number was injected after 40 hours of SCF treatment, compared with 16 hours of exposure or untreated cells transplanted at time 0 (Fig. 3D). Thus, enhanced CXCR4-dependent migration to SDF-1 was accompanied by an increase in the SRC fraction. Incubation of SCF-stimulated, mobilized peripheral blood $CD34^+$ cells with anti-CXCR4 prevented engraftment (Fig. 3C). Sorted $CD34^+$-$CD38^{-/low}CXCR4^{-/low}$ cord blood cells that did not migrate toward SDF-1 were either transplanted or treated with SCF for 48 hours. Whereas nontreated cells had low engraftment efficiency (Fig. 4A), SCF treatment resulted in increased migration

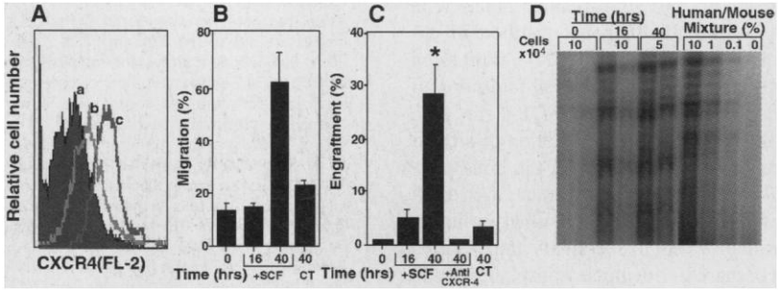


Fig. 3. SCF potentiates CXCR4 expression, cell migration, and SRC engraftment. (**A**) Mobilized peripheral blood $CD34^+$ cells stained with control antibody (curve a) or with anti-CXCR4 before (curve b) or after (curve c) 40 hours of treatment with SCF. (**B**) SDF-1 transwell migration of untreated (0), SCF-treated (16 and 40 hours), or control cells cultured for 40 hours without SCF (CT). Data are average ± SE of three experiments. (**C**) Percent of engraftment in NOD/SCID mice transplanted with 2×10^5 cells before (0) or after 16 or 40 hours of exposure to SCF and 40 hours of exposure to SCF followed by incubation with anti-CXCR4 (+ anti CXCR4). Control cells (CT) as in (B). Percent of engraftment was quantified as in Fig. 1C, panel c. Data are average ± SE (*$P <$ 0.01, SCF 40 hours versus 0 hours, SCF+anti-CXCR4, and CT 40 hours) of three mice per treatment, in a representative experiment. (**D**) Exposure times of mobilized peripheral blood $CD34^+$ to SCF as in (C). At time 0 and after 16 hours 1×10^5 cells per mouse were transplanted, and after 40 hours 0.5×10^5 cells per mouse were transplanted. Human engraftment was quantified after 1 month by Southern blot analysis.

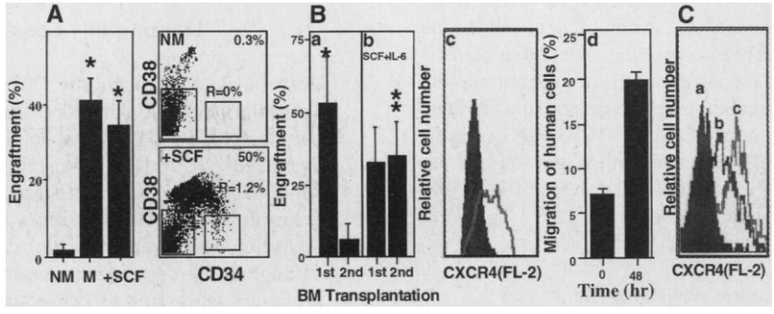


Fig. 4. Increase in SRCs and of stem cell self-renewal by up-regulation of CXCR4 expression. (**A**) Sorted $CD34^+CD38^{-/low}$ cord blood cells migrating toward SDF-1 were transplanted into NOD/SCID mice (3×10^4 cells per mouse) (M). Nonmigrating cells were either injected directly (NM) or treated with SCF for 48 hours and then injected (+SCF). After 6 weeks engraftment levels were quantified as in Fig. 1C, panel c. Data in the left panel are average ± SE (*$P < 0.01$) of four experiments. (**B**) Bone marrow cells from mice transplanted 4 to 6 weeks before with human cord blood $CD34^+$ cells in panels a and b were retransplanted untreated (2nd in panel a) or after SCF and IL-6 treatment for 48 hours (panel b) into secondary β_2-microglobulin knockout NOD/SCID mice. Data in panels a and b are the average ± SE of four experiments (panel a, *$P < 0.01$, 1st versus 2nd; **$P < 0.05$, 2nd in panel b versus 2nd in panel a). (Panel c) Human CXCR4 expression on cord blood cells from transplanted mice immediately labeled (solid) or after 48 hours treatment with SCF and IL-6 (open). (Panel d) SDF-1 migration of cord blood cells from the marrow of transplanted mice before and after treatment with SCF and IL-6 for 48 hours. Data in panel d are the average of triplicates in a representative experiment. (**C**) Cord blood $CD34^+$ cells were stained with control antibody (curve a) or antibody to CXCR4 after a 48-hour exposure to SCF (curve b) or SCF and IL-6 (curve c). Percent of engraftment in (A) and (B) was quantified as in Fig. 1C, panel c.

toward SDF-1 and efficient engraftment by converted $CD34^+CD38^{-/low}CXCR4^+$ cells, properties that were similar to those of the original migrating fraction (M) (Fig. 4A).

Self-renewal of stem cells can only be determined by their ability to also repopulate secondary transplanted recipients with high numbers of both myeloid and lymphoid cells. Consistent with previous studies, secondary transplanted mice that received untreated human cells showed little engraftment (Fig. 4B, panel a) (*22*). Human interleukin-6 (IL-6) synergizing with SCF induced high levels of CXCR4 expression on $CD34^+$ cord blood cells (Fig. 4C). Incubation of bone marrow cells from primary transplanted mice with SCF and IL-6 for 48 hours resulted in up-regulation of surface CXCR4 expression (Fig. 4B, panel c) and increased migration of human progenitor cells to SDF-1 in vitro (Fig. 4B, panel d). Transplantation of similar numbers of human cells from the bone marrow of primary transplanted mice after treatment with these cytokines resulted in higher engraftment levels in secondary transplanted mice compared with mice transplanted with untreated cells (Fig. 4B, panel b versus panel a). Thus, by up-regulating surface CXCR4 expression on primitive cells, the population of self-renewing $CD34^+CD38^{-/low}$ SRC stem cells could be increased.

Our data provide evidence that CXCR4-dependent migration to SDF-1 is essential for human stem cell function in NOD/SCID mice. We characterized SRCs further as $CD34^+CD38^{-/low}CXCR4^+$ stem cells and showed that $CD34^+CD38^{-/low}CXCR4^{-/low}$ cells can be converted into functional $CXCR4^+$ stem cells by cytokine treatment. This suggests that migration to SDF-1 is associated with localization of stem cells in the bone marrow, permitting differentiating cells with reduced migration levels to exit into the blood circulation. In conclusion, our findings define human $CD38^{-/low}CXCR4^+$ cells as stem cells endowed with migration and repopulation potential and provide insights into human stem cell biology.

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23. Human cells were obtained after informed consent according to procedures approved by the Weizmann Committee. In all experiments, samples of the same initial cell pool were compared. Differences in the results are due to the different $CD34^+$ cell sources (cord blood, bone marrow, and mobilized peripheral blood). $CD34^+$ enrichment, flow cytometry, and fluorescence-activated cell sorting (FACS) were performed as previously described (*5*, *6*). SDF-1 (125 ng/ml, R&D Systems) transmigration assays were done as previously described (*13*) with 2×10^5 $CD34^+$ cells. Percents in the results represent percent of initial 2×10^5 cells in the migrating and nonmigrating cell fractions. The sources for the reagents are as follows: PMA (100 ng/ml), was purchased from Sigma, stem cell factor (SCF) and IL-6 (50 ng/ml) from R&D Systems, and antibodies to CXCR4 from Pharmingen {12g5 monoclonal antibody [immunoglobulin G2a (IgG2a)]} or R&D Systems [MBA171 monoclonal antibody (IgG2a)] (10 μg per 2×10^5 cells). CXCR4 expression was always analyzed by double staining with anti-CD34. Polyclonal anti–SDF-1 (10 μg per mouse, R&D Systems) was injected intravenously with the cells (2×10^5 cells per mouse) and 24 hours later injected again intraperitoneally. Control cells were incubated with anti-CD34 [IgG1, Becton Dickinson, 10 μg per 2×10^5 cells]. Human lymphoid and myeloid cells were immunostained with anti-CD45 (Immuno Quality Products, Groningen, Netherlands), anti-CD19, and anti-CD56 (Coulter). Natural killer cells differentiated into mature $CD56^+$ cells after incubation with human SCF (100 ng/ml) and human IL-15 (100 ng/ml, R&D Systems) for 10 days. NOD/SCID, and NOD/SCID β_2-microglobulin knockout (*20*) mice were bred and maintained under defined flora in intraventilated cages and transplanted by injection into the tail vein after sublethal (375R) irradiation according to established protocols (*5*, *6*) approved by the Weizmann animal ethics committee. Southern (DNA) blot analysis with a human-specific α satellite probe and human-specific progenitor assays were done as previously described (*5*, *6*). Percent engraftment always indicates the percent of either human DNA or of human CD45 cells in the mouse bone marrow. The levels of engraftment were dependent on the injected cell dose, the duration of the experiment, and the source of human $CD34^+$ cells. Cells were cultured either in serum-free media as previously described (*6*) or in media supplemented with 10% fetal calf serum.

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CD3- and CD28-Dependent Induction of PDE7 Required for T Cell Activation

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Costimulation of both the CD3 and CD28 receptors is essential for T cell activation. Induction of adenosine 3′,5′-monophosphate (cAMP)–specific phosphodiesterase-7 (PDE7) was found to be a consequence of such costimulation. Increased PDE7 in T cells correlated with decreased cAMP, increased interleukin-2 expression, and increased proliferation. Selectively reducing PDE7 expression with a PDE7 antisense oligonucleotide inhibited T cell proliferation; inhibition was reversed by blocking the cAMP signaling pathways that operate through cAMP-dependent protein kinase (PKA). Thus, PDE7 induction and consequent suppression of PKA activity is required for T cell activation, and inhibition of PDE7 could be an approach to treating T cell–dependent disorders.

Activation of peripheral T cells in vivo by an antigen-presenting cell is a result of the engagement of both the T cell receptor–CD3 complex (TCR-CD3) and the CD28 costimulatory receptor. When both receptors are occupied by their appropriate ligands, T cells are stimulated to proliferate and produce interleukin-2 (IL-2), whereas occupation of the T cell receptor alone favors T cell anergy or apoptosis (*1*). Occupation of the CD28 receptor alone appears to have no obvious effect on T cells; nevertheless, CD28 costimulation is required for full activation of CD4 T helper cells, if not all T cells (*2*). Why is CD28 costimulation required for T cell activation? One possible reason has been suggested by

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