PLURIPOTENTIAL QUIESCENT STEM CELL POPULATION

Technical Field

The invention is related to the field of cell sorting and cell subpopulations. More precisely, the invention concerns a stem cell population which is pluripotential and relatively quiescent.

Background Art

Stem cells are thought to be manufactured in the adult bone marrow as relatively undifferentiated progenitors for a variety of blood cells including erythrocytes, lymphocytes, and polynuclear cells. Stem cells are also present in the peripheral blood and in umbilical cord blood. It is believed that at some point in their development and differentiation, individual cells become committed to a particular cellular goal. Thus, the population of stem cells found in the bone marrow of a subject at any one time will contain stem cells in various stages of differentiation and development.

In murine models, this hematopoietic system has been divided into three functionally distinct subpopulations: the youngest is an uncommitted pluripotential stem cell population capable of self-renewal and further differentiation; these differentiate further into a committed multipotential progenitor population; these, then, finally become committed unipotential progenitors. In the human system, all of

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these stem cell populations express the CD34 antigen; it appears CD34 expression decreases with differentiation.

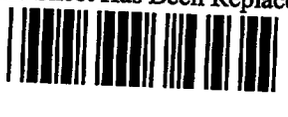
Attempts have been made to obtain a subset of the total stem cell population which is uncommitted, and thus provides the most versatile population for engraftment, and that has the best potential for genetic modification in gene therapy. U.S. Patent 5,061,620 to SyStemix utilizes cell surface markers to provide a defined subpopulation in human stem cells. Murine subpopulations have also been isolated by the removal of lineage-committed cells followed by positive selection for cells bearing the Thy-1^{lw}, the stem cell antigen (Sca-1) and the wheat germ agglutinin $(WGA)^+$ phenotype (Visser et al., J Exp Med (1984) 59:1576). Murine stem cells have also been separated based on size to obtain pluripotential cells relatively free of multipotent progenitors (Jones, et al., Nature (1990) 78:403a).

Each of the foregoing separation methods yields a subpopulation with particular characteristics; it is not certain that all of these characteristics are desirable for use in engraftment or in gene therapy.

It has now been found that a subset of human stem cells can be obtained by subjecting low density mononuclear bone marrow cells (or other sources of stem cells) to counterflow elutriation and then recovering $CD34^+$ cells from the fractions containing the cells of smallest size. This subpopulation has the characteristics expected for a very primitive stem cell, and could be particularly useful in transplantation and gene therapy.

An abstract describing the results of counterflow elutriation followed by $CD34^+$ segregation of human low-density mononuclear bone marrow cells was published in Blood (1991) 78:No. 1603 on 15 November 1991.

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Disclosure of the Invention

The invention provides a subpopulation of human stem cells that are quiescent and pluripotent. These cells are those of small dimension that have $CD34^+$ markers on their surfaces. This population is depleted in cells which express CD33, CD38, HLA/DR, CD19 and CD3. These cells also have a high density of MIP1α, Steel factor (stem cell factor, SCF), IL-6, IL-3, IL-1α, and G-CSF receptors.

Thus, in one aspect, the invention is directed to a population of human hematopoietic stem cells consisting of mononuclear low density hematopoietic cells which correspond in size to those in flow rate fractions of 25-29 ml/min in a rotor of size equivalent to Beckman JE 5.0, spun at 900 x g, and which are $CD34^+$. A subject population of $CD34^+$ human hematopoietic cells can comprise cells with a surface ligand makeup of greater than 7% ± 3% $CD2^+$; less than 3% ± 1% $CD19^+$; and less than 1% $CD33^+$. More particularly, in one embodiment, the population can comprise 30% ± 0% CD2, 0% ± 0% CD19, 0% ± 0% CD33, 9% ± 2% CD38, and 18% ± 4% HLA-DR cells. In short-term bone marrow cultures, these cells do not provide CFU-GM, BFU-E, or CFU-GEMM. More than 90% of this population contains receptors for SCF and MIP-1α, but the population does not proliferate in standard long-term bone marrow culture in the presence or absence of SCF. However, under appropriate conditions the cells are capable of proliferation *ex vivo*.

In other aspects, the invention is directed to methods to obtain the cell population of the invention and to methods of using this population.

Brief Description of the Drawings

Figures 1A-1D show the distribution of $CD34^+$ cells in various fractions from counterflow elutriation.

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Figure 2 is a graph showing CFU-GM from various $CD34^+$ fractions.

Figure 3 is a graph showing proliferation of various $CD34^+$ fractions.

Figure 4 depicts histograms plotting FITC fluorescence intensity (X-axis) vs. the cell number (Y-axis). The cells analyzed were derived from bone marrow samples from normal volunteers that were separated by elutriation into R/O, 33/37 and 25/29 fractions and from which $CD34^+$ cells were subsequently isolated. The dark solid lines represent the control samples, and the light dotted lines represent those cells stained with the growth factor. However, for fraction 33, IL-6, the dark solid lines represent those cells stained with the growth factor, and the light dotted lines represent the control samples.

Modes of Carrying Out the Invention

The invention provides a population of cells that could be particularly useful in gene therapy and in transplantation. The cells appear to be extremely primitive. They are collectively characterized by a typical surface ligand expression pattern and are collectively positive or negative for these markers. Specifically, this population is depleted in cells which express CD33, CD38, HLA/DR, CD19 and CD3, but have a high density of macrophage inhibitory protein MIP1α, stem cell factor (SCF), IL-6, IL-3, IL-1α, and GM-CSF receptors.

The population is conveniently prepared by an initial size separation of the low density (LD) mononuclear fraction of cells of bone marrow or peripheral blood origin, so that cells recovered are agranular and small and have 0-1 nucleoli. This population is equivalent in size to that obtained in counterflow elutriation as described below in the

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fraction representing a flow rate of 25 to 29 ml/minute (FR 25/29) when the separation is conducted with a Beckman JE 5.0 rotor at 900 x g. The examples below illustrate the recovery of cells of this size using a specific counterflow elutriation (CE) procedure. However, any method for separating cells according to size can be used to collect an equivalent fraction (i.e., density gradient sedimentation could be substituted).

This subpopulation of appropriate size distribution is then subjected to further fractionation in two steps, one of which is optional. An optional step comprises treating the population with a solid support to which soybean agglutinin has been bound as an affinity ligand. The nonadherent cells are recovered. Typically, the contact is conducted at room temperature for about 1 hour on a nonvibrating surface. Appropriate solid supports coupled to soybean agglutinin may be obtained from Applied Immune Sciences, Santa Clara, CA.

The nonadherent fraction of the cells contacted with the soybean agglutinin-derivatized support or the initial FR 25/29 cells from size separation are then contacted with a solid phase substrate to which has been bound a ligand specifically reactive with the CD34 marker. Supports derivatized with, for example, antibodies or fragments thereof specific for CD34 may be obtained by methods known in the art and are commercially available from, for example, Applied Immune Sciences, Santa Clara, CA. The supports are treated with the small cellular fraction under conditions wherein cells containing CD34 markers adhere to the support. Typically, incubation is conducted at room temperature for from 1 hour to several hours on a nonvibrating surface. Nonadherent cells are removed, the surface washed if desired, and adherent cells are then recovered.

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The recovered cells constitute the quiescent pluripotential cell population of the invention.

The two or three steps involved in the fractionation of low density mononuclear bone marrow cells to obtain the desired cell population are most conveniently conducted in the order described above; however, it is within the scope of the invention that the order can be altered. It is most convenient, however, to minimize the number of cells subjected to contact with affinity ligand-derivatized solid supports in view of the expense of preparing such supports and of the ligands themselves.

The population of $CD34^+$ cells obtained is characterized by its small size. A typical size distribution is shown in Figure 1, further described in Example 1 below. As shown, $CD34^+$ cells are distributed over a large range of granularities (X-axis, SSC) and sizes (Y-axis, FSC) in the original bone marrow (Figure 1A). However, the FR 25/29 fraction contains $CD34^+$ cells of smaller size and less granulation (Figure 1C). The $CD34^+$ cells of FR 33/37 (collected at flow rates of 33-37 ml/min) and R/O (rotor off) fractions are larger (Figures 1D and 1B).

The cells of the FR 25/29 $CD34^+$ population also contain high densities of MIP1α and SCF surface receptors, which are understood to characterize primitive cells, and to provide mechanisms for modulating proliferation. Accordingly, the cells of the invention could be cultured under conditions wherein analogues that block or suppress MIP1α receptors are attached to those receptors. Alternatively, analogues that activate SCF receptors can be attached to the SCF receptors. Thus, the addition of SCF may encourage growth, as can the addition of other growth factors for which there are receptors on these cells.

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Other characteristics of the cell population of the invention include their quiescent nature both in short-term and long-term bone marrow cultures, in the absence of activating modulation of SCF receptors or suppressing modulation of MIP1α receptors, under standard conditions. Thus, without such culture adjustments, these cells do not provide positive results in CFU-GM, BFU-E or CFU-GEMM assays. They are also characterized by typical surface marker patterns, including depletion in CD33, CD38, HLA/DR, CD19 and CD3. By depletion is meant that the density of markers in this population of cells is at least 3-fold less, preferably 5-fold less, and most preferably 10-fold less than the total $CD34^+$ cell population of the low-density mononuclear hematopoietic cells from which they are derived.

On the other hand, they have enhanced expression of MIPα and SCF receptors, as stated above, as well as receptors for IL-6, IL-3 and GM-CSF. By enhanced expression is meant that the density of markers in this population of cells is at least 3-fold more, preferably 5-fold more, and most preferably 10-fold more than the total $CD34^+$ cell population of the low-density mononuclear hematopoietic cells from which they are derived.

The population of cells having the characteristics described above is useful in effecting cell transplantation into subjects in need of such cell transplants. The cells may be used per se, for example, in protocols involving autologous transplants to replenish the stem cells of a subject suffering depletion through treatments such as chemotherapy or radiation therapy. The cells may also be genetically modified through, for example, homologous recombination or retroviral infection to remedy gene defects or to provide recombinant expression of desired proteins and

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administered to a subject in genetically modified form. Techniques for administration of such cells are commonly practiced using conventional methods known in the art.

EXAMPLES

The following examples are intended to illustrate, but not to limit, the invention.

Example 1

Preparation of Uncommitted Stem Cells

Bone marrow samples were aspirated in small volumes from the posterior iliac crest of 22 healthy adult volunteer donors. Informed consent was obtained following the guidelines approved by the Committee on the Use of Human Subjects at the University of Minnesota.

Low density bone marrow mononuclear cells were isolated using Histopaque-1077 (Sigma Chemical Co., St. Louis, MO) and density gradient centrifugation (400 x g for 45 minutes), washed twice with RPMI-1640 (Gibco, Grand Island, NY) and resuspended in 10 ml of elutriation media.

For counterflow elutriation (CE), the washed low density mononuclear cells (2.7-7.8 x 10^8) were injected via a sampling site coupler into the inlet stream of a Beckman J6M/E centrifuge (Spinco Division, Beckman Instruments, Palo Alto, CA) equipped with a JE-5.0 rotor and standard chamber. One peristaltic pump (Masterflex, Cole Palmer Instruments, Chicago, IL) provided continuous flow of elutriation medium, which was 0.9% normal saline solution with 100 mg/dl D-glucose, 0.3 mM disodium ethylenediaminetetraacetic acid (EDTA) and 50 mg/dl bovine serum albumin (BSA, Boehringer Mannheim, FRG), pH adjusted to 7.20. The medium was sterilized prior to use.

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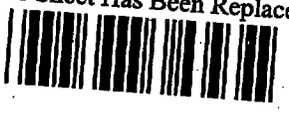
Cells were delivered at a total flow rate of 15 ml/min (FR 15), rotor speed of 900 x g and a temperature of 25°C. After 100 ml of eluate were collected, the flow rate was increased to 25 ml/min (FR 25). With the rotor speed held constant, the flow rates were sequentially increased to 29 ml/min (FR 29), 33 ml/min (FR 33), and 37 ml/min (FR 37), collecting 200 ml with each increment. The cells that remained in the chamber were captured by turning the rotor off (R/O fraction) and flushing the chamber with 100 ml of elutriation media. Each cell fraction was washed and centrifuged at 300 x g for 10 minutes.

FRs 25 and 29 (FR 25/29) and FRs 33 and 37 (FR 33/37) were then combined. Viability was determined by trypan blue dye exclusion and cell recoveries were determined with a ZBI Coulter counter (Coulter Electronics, Hialeah, FL).

Each FR 25/29, FR 33/37 and R/O fraction was washed with elutriation medium and resuspended in 1 mM Dulbecco's Phosphate Buffer (DPBS) with 0.1 mM EDTA and 0.5% Gammimune (Miles Laboratory, Eickert, IN) for a 30 minute incubation. Cells from each fraction were loaded onto AIS T-25 MicroCELLector devices (Applied Immune Sciences, Santa Clara, CA) covalently coated with soybean agglutinin, the cells at a density of 2.0×10^7 target cells/flask, and incubated for one hour at room temperature on a nonvibrating surface. The nonadherent cells were collected and the flasks were washed twice to remove all nonadherent cells from the flask surface.

1.5×10^7 cells from each fraction were loaded onto individual AIS T-25 MicroCELLector devices covalently coated with ICH3 (anti-CD34) and incubated for one hour at room temperature on a nonvibrating surface. The nonadherent cells were collected and the flasks were washed twice to remove all nonadherent cells. The

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adherent cells were collected by adding DPBS + 10% fetal bovine serum (FBS, Hyclone) and hitting the flask firmly. The resulting populations were designated FR 25/29 $CD34^+$, FR 33/37 $CD34^+$, and R/O $CD34^+$.

The distribution of cells in the CE-sized fractions, before and after treatment with soybean agglutinin and selection for CD34, is set forth in Table 1.

Table 1

Percentage of Bone Marrow Mononuclear Cells in Fraction

FR	Percentage
R/O (total)	61
33/37 (total)	26
25/29 (total)	11
R/O $CD34^+$	1.00
33/37 $CD34^+$	0.20
25/29 $CD34^+$	0.06

As shown in Table 1, about 1% of the original number of the unfractionated cells were recovered in all of the three $CD34^+$ fractions. About 1/9th of the low density mononucleated cells were recovered in FR 25/29, about 1/4th in FR 33/37, and about half in the R/O fraction. Soybean agglutinin (SBA) depletion results in the loss of 70-90% of cells treated for all fractions, and about 3-5% of the SBA$^-$ cells were recovered by the CD34 positive selection. About 25-55% of the $CD34^+$ cells in the LD mononuclear bone marrow fraction are recovered by this methodology.

The morphology of the $CD34^+$ cells varies between the three fractions. The $CD34^+$ cells recovered from FR 25/29 were small, lymphoid appearing cells with scant agranular cytoplasm and 0-1 nucleoli. $CD34^+$ cells

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from FR 33/37 were slightly larger, lymphoblast-like cells with scant agranular cytoplasm and 1-5 nucleoli. $CD34^+$ cells from the R/O fraction were significantly larger myelo/erythroblasts with few nucleoli and frequently a significant amount of cytoplasm containing granules. These results were confirmed by horizontal and vertical light scatter as shown in Figures 1A-1D. The X-axis measures horizontal side scatter (SSC) and is a measure of cell granularity. The Y-axis measures forward light scatter (FSC) and is a measure of cell size. Figure 1A shows the original population distribution, Figure 1B shows the R/O fraction, Figure 1C, FR 25/29 and Figure 1D, FR 33/37.

For the cells collected at flow rates of 25 to 29 ml/min (FR 25/29) the cellular size was between 8 to 8.5 μm. For the rotor fraction collected at flow rates of 33 to 37 ml/min, the population had a cellular size of between 9 to 9.5 μm. For the cells that remained in the chamber, captured by turning the rotor off (R/O fraction), the cellular size was >15 μm. These cellular sizes were established by standard methodologies.

Example 2

Phenotypic Markers of Fractionated Cells

Upon phenotype analysis, ≥85% of the cells were recognized by the monoclonal antibody 8G12 (Becton Dickinson), an antibody that specifically binds to the CD34 antigen. Unfractionated (UNF) cells and each cell fraction were individually preincubated with 0.5% Gammimune and then washed with phosphate-buffered saline (PBS, Gibco) containing 0.1% sodium azide (Sigma Chemical Co.). Phycoerythrin-conjugated anti-CD2, CD19, CD33, CD38 or anti-HLA-DR (Becton Dickinson, Sunnyvale, CA) was added to the cell pellet and incubated for 30 minutes at 4°C. The cells were washed twice with PBS-azide and

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resuspended in 1% paraformaldehyde (Electron Microscopy Science) for analysis within one week. The cells were analyzed on a FACS Star Plus (Becton Dickinson) interfaced to a VACS computer using Becton Dickinson Concert 40 software (Becton Dickinson, Sunnyvale, CA). Positive binding was defined as fluorescence on antibody-reacted cells beyond that on control cells.

The results are shown in Table 2:

Table 2

FRACTION	SURFACE ANTIGEN ANALYSIS*				
	%CD2$^+$	%CD19$^+$	%CD33$^+$	%CD38$^+$	HLA-DR$^+$
UNF	7± 3	7± 3	7± 5	2± 2	9± 1
FR 25/29	7± 3	3± 1	0± 0	4± 1	2± 1
FR 25/29 CD34$^+$	30± 0	0± 0	0± 0	9± 2	18± 4
FR 33/37	7± 2	8± 1	1± 0	17± 3	5± 2
FR 33/37 CD34$^+$	0± 2	13± 7	3± 2	33± 10	39± 10
R/O	0± 2	4± 1	21± 4	39± 14	15± 3
R/O CD34$^+$	0± 2	4± 1	62± 11	83± 2	80± 3

* Mean and standard deviation for 7 experiments.

While the majority of the CD34$^+$ cells in the R/O fraction expressed CD38 and HLA-DR, and to a slightly lesser extent CD33, few if any of the cells expressed CD2 or CD19. Most of the CD34$^+$ cells in FR 25/29 did not

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express CD19, CD33, CD38 or HLA-DR, but did express about 30% CD2 (range 21-40% in 4 experiments). The frequency of $CD34^+$ cells from FR 33/37 expressing CD38 and HLA-DR falls in between the other fractions, with about 40% of the cells expressing CD38 and HLA-DR. Of the $CD19^+/CD34^+$ cells, the majority are recovered in FR 33/37.

Example 3

Assay for Progenitors

A portion of each fraction was cultured with 1.32% methylcellulose (1,500 CP, Sigma), 30% FBS (Hyclone Laboratories Inc., Logan, UT), 5 x 10-4 mol/L 2-mercaptoethanol, 5% phytohemagglutinin-leucocyte conditioned medium (PHA-LCB), 1% BSA, 10-6 mol/L of methylprednisolone sodium succinate ester, and 1 U/ml of recombinant human erythropoietin (Amgen, Thousand Oaks, CA). Cultures were seeded in triplicate at 1 x 10^3 and 1 x 10^5 cells/ml in 1 ml culture dishes (Nunc) and maintained in humidified atmosphere at 37°C in 5% CO_2.

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The plates were scored for BFU-E and CFU-GM at day 14 using an inverted microscope. These results are shown in Table 3.

Table 3

FRACTION	FREQUENCY*		
	CFU-GM/10^5	BFU-E/10^5	ENRICHMENT
UNF	185± 23	113± 10	---
FR 25/29	0± 0	0± 0	0.0
FR 25/29 $CD34^+$	6± 1	14± 1	0.1
FR 33/37	4± 2	16± 6	0.1
FR 33/37 $CD34^+$	248± 27	142± 46	1.3
R/O	170± 13	410± 98	1.9
R/O $CD34^+$	3220± 420	5300± 512	28.0

* Mean and standard deviation for 7 experiments.

There was no marked enrichment of colony-forming cells (CFU-GM, CFU-GEMM or BFU-E) in any fraction after elutriation, but the R/O $CD34^+$ subpopulation was 20-59 fold enriched in these colony-forming cells. FR 25/29 $CD34^+$ produced no CFU-GM, BFU-E or CFU-GEMM.

Example 4

Long-Term Bone Marrow Culture

Normal allogeneic stromal layers were cultured in T-25 flasks (Corning Glass Works, Corning, NY) for 14-28 days and then irradiated at 1000 cGy with a MARK I Cesium irradiator (Shepard and Associates, Glendale, CA). Five to seven days after irradiation, cells from each

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$CD34^+$ fraction (10^5 to 10^6), in 5 mL McCoy's 5A medium with 12.5% FCS, 12.5% horse serum (Hyclone Laboratories), 2 mM L-glutamine, 0.07% sodium bicarbonate solution, 1% (v/v) MEM nonessential amino acid solution, 1% (v/v) MEM vitamin solution, 1% (v/v) sodium pyruvate solution, 1000 units/mL penicillin, 100 units/mL streptomycin, and 10^{-7} M hydrocortisone (A-Hydrocort, Abbott Laboratories, Chicago, IL), were seeded into each flask. Cultures were maintained in humidified atmosphere at 37°C in 5% CO_2. At weekly intervals the cultures were fed by removing half of the supernatant and replacing it with fresh media. Nonadherent cells were counted and assayed for the presence of CFU-GM and BFU-E in methylcellulose assay on weeks 0, 2, 5, 6, 7 and 8.

The results for CFU-GM are shown in Figure 2. There were increases and/or sustenance in the total number of cells in the nonadherent layer at several time points, for cultures initiated with either FR 33/37 or R/O $CD34^+$ cells. Similarly, clonogenic cells were recovered from both FR 33/37 and R/O $CD34^+$ cells as late as week 8. However, in 4 experiments, few nucleated cells and no CFU-GM, BFU-E or CFU-GEMM were ever recovered from the nonadherent layer of long-term bone marrow cultures seeded with FR 25/29 $CD34^+$ cells (Figs. 2 and 3). These data indicate the very primitive nature of this $CD34^+$ cell population, and serve to contrast the population from other subpopulations of $CD34^+$ cells which do proliferate in long-term bone marrow culture.

Example 5

Assay for Cytokine Receptors

Cells from each $CD34^+$ fraction were assayed for the presence of receptors for Steel factor (SCF), granulocyte-macrophage colony stimulating factor

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(GM-CSF), granulocyte colony stimulating factor (G-CSF), interleukin-1 (IL-1), IL-2, IL-3, IL-6, macrophage inhibitory protein MIP1α. Samples were washed twice in Hepes-buffered saline (150 mM NaCl, 25 mM hepes, ph 7.4) and then incubated with 100 ng biotinylated recombinant human SCF (rhSCF) (R&D Systems, Minneapolis, MN) for 60 min on ice. Afterwards, cells were washed twice in 2 mL RDF1 wash buffer (R&D Systems) and then incubated with 100 ng avidincarboxyfluorescein (R&D Systems) for 30 min on ice in the dark. The cells were washed with 2 mL RDF1 buffer and resuspended in 300 uL of Hepes-buffered saline for flow cytometric analysis.

Stained cell samples were analyzed for fluorescent intensity on either the EPICS Profile II or Epics 752 flow cytometer (Coulter Electronics, Hialeah, FL). Positive samples were compared to samples stained with avidin-carboxyfluorescein alone. Binding affinity for the biotinylated cytokine was confirmed by complete inhibition or binding in the presence of a 10 molar-fold excess of unlabelled cytokine. The results are shown in Table 4.

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Table 4

FRACTION	CYTOKINE RECEPTOR ANALYSIS*					
	%SCF$^+$	%IL-6$^+$	%IL-3$^+$	%GM-CSF$^+$	%G-CSF$^+$	%IL-1
FR 25/29 CD34$^+$	74± 10	64± 9	53± 11	62± 8	39± 18	85± 2
FR 33/37 CD34$^+$	66± 13	39± 39	18± 8	33± 28	12± 6	64± 35
R/O CD34$^+$	64± 18	30± 26	7± 2	20± 26	13± 2	67± 29

* Mean and standard deviation of 7 experiments.

Virtually all (>90%) the $CD34^+$ cells expressed receptors for SCF, but the intensity of fluorescence was greatest for FR 25/29 $CD34^+$ cells. Bright and dim populations of cells were routinely observed in this fraction. Only single populations of cells were noted for FR 33/37 and R/O $CD34^+$ cells with the R/O $CD34^+$ subpopulation having only dim cells (Figure 4).

As shown in Fig. 4, bone marrow samples from normal volunteers were separated by elutriation into the R/O, 33/37 and 25/29 fractions. $CD34^+$ cells were subsequently isolated. The purified $CD34^+$ cells were then stained with the indicated biotinylated growth factor and counterstained with avidin conjugated fluorescein isothiocyanate (FITC). As a control, the cells were stained solely with the avidin-FITC conjugate. In Fig. 4, histograms are shown plotting FITC fluorescence intensity (X-axis) vs. the cell number (Y-axis). The columns of samples are from the indicated size fractions. The rows are from the indicated growth factors. The dark solid lines represent the control samples, and the light dotted lines represent those cells stained with the growth factor. However, for fraction 33, IL-6, the dark solid lines represent those cells stained with the growth factor, and the light dotted lines represent the control samples. The data from Fig. 4 is summarized in Table 5.

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Table 5

Level of Growth Factor Binding

	R/O	33/37	25/29
G-CSF	-	-	-
GM-CSF	-	-	++
IL-1α	+	+	+++
IL-3	-	-	++
IL-6	-	-	++
SCF	+	+	+++
MIP1α	+	+	+++

Blocking experiments with unlabeled growth factors (data not shown) proved the specificity of the growth factor binding to the cells.

Virtually all the $CD34^+$ cells had receptors for MIP1α, but FR 25/29 $CD34^+$ cells exhibited the greatest number of receptors. The R/O $CD34^+$ cells exhibited the least number of receptors.

Receptors for IL-1, IL-3, IL-6, GM-CSF and G-CSF were found on all subpopulations of $CD34^+$ cells. Comparing the 3 fractions, receptors for IL-3, IL-6 and GM-CSF were highest on FR 25/29 $CD34^+$ cells.

By analysis of this receptor profile appropriate culture protocols for these cells are devised.

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CLAIMS

1. A population of human hematopoietic stem cells consisting essentially of low density, mononuclear hematopoietic cells which are $CD34^+$, and which correspond in size to fractions obtained at a flow rate of 25-29 ml/min in counterflow elutriation (CE) using a rotor equivalent to Beckman JE 5.0 spun at 900 x g.

2. The population of claim 1 which is depleted in CD33, CD38, HLA/DR, CD19 and CD3 surface ligands; wherein more than 90% of said cells contain receptors for SCF, and wherein said population does not proliferate in long-term bone marrow culture in the presence of SCF; wherein said cells do not proliferate in response to combinations of stem cell factor (SCF), IL-3, IL-1, GM-CSF and erythropoietin, and do not form colonies in methylcellulose in response to these factors; wherein said cells contain a high density of MIP1α receptors; and, wherein said cells contain a high density of IL-6, IL-3, IL-1α or GM-CSF receptors.

3. A method to effect cellular transplantation in a subject, which method comprises administering to a subject in need of such transplantation an effective amount of the cells of claim 1.

4. The method of claim 3 further comprising a step of genetically altering said cells prior to the administering step.

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5. A method to prepare a population consisting essentially of quiescent stem cells, which method comprises steps of:

subjecting low-density mononuclear hematopoietic cells to size separation to obtain a first fraction of cells, the first fraction of cells corresponding in size to fractions obtained at a flow rate of 25-29 ml/min in counterflow elutriation (CE) using a Beckman JE 5.0 rotor spun at 900 x g;

optionally contacting said first fraction with a solid support coupled to soybean agglutinin; and recovering a second fraction of cells which are nonadhering to said solid support;

contacting said first fraction or optional second fraction with a solid support to which an affinity ligand for CD34 is bound;

recovering a third fraction adherent to said solid support, said third fraction constituting said quiescent stem cell population.

6. The method of claim 5 wherein said hematopoietic cells are obtained from bone marrow.

7. The method of claim 5 wherein said hematopoietic cells are obtained from peripheral blood.

8. A population of cells prepared by the method of claim 5.

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9. A subject population of $CD34^+$ human hematopoietic cells, wherein said population comprises cells with a surface ligand makeup of greater than 7% $CD2^+$; less than 3% $CD19^+$; and less than 1% $CD33^+$.

10. The population of claim 9 wherein the population comprises 30% ± 0% CD2, 0% ± 0% CD19, 0% ± 0% CD33, 9% ± 2% CD38, and 18% ± 4% HLA-DR.

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