



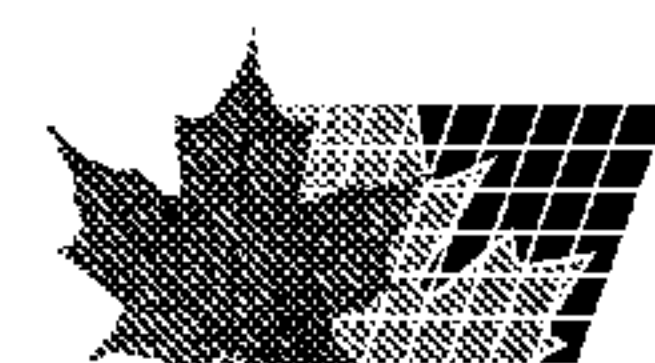
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(54) **Titre : CELLULES SOUCHES HEMATOPOIETIQUES TRAITEES PAR FUCOSYLATION IN VITRO ET METHODES
D'UTILISATION**
(54) **Title: HEMATOPOIETIC STEM CELLS TREATED BY IN VITRO FUCOSYLATION AND METHODS OF USE**

(57) **Abrégé/Abstract:**

A method of in vitro fucosylation of selectin ligands on cord blood-derived hematopoietic stem cells for bone marrow transplantation is disclosed. In this method, an effective amount of an α 1,3-fucosyltransferase, e.g., α 1,3-fucosyltransferase VI, is used in vitro to treat cord blood-derived hematopoietic stem cells to convert non-functional PSGL-1 or other ligands on the cell surface into functional forms that bind selectins, especially P-selectin or E-selectin. The treated cells have enhanced effectiveness in reconstituting bone marrow in patients in need of such therapy.



ABSTRACT

A method of *in vitro* fucosylation of selectin ligands on cord blood-derived hematopoietic stem cells for bone marrow transplantation is disclosed. In this method, an effective amount of an α 1,3-fucosyltransferase, e.g., α 1,3-fucosyltransferase VI, is used *in vitro* to treat cord blood-derived hematopoietic stem cells to convert non-functional PSGL-1 or other ligands on the cell surface into functional forms that bind selectins, especially P-selectin or E-selectin. The treated cells have enhanced effectiveness in reconstituting bone marrow in patients in need of such therapy.

**Hematopoietic Stem Cells Treated by
In Vitro Fucosylation and Methods of Use**

**Statement Regarding Federally Sponsored
Research or Development**

[0002] Some aspects of this invention were made in the course of Grant 5P5OHL54502 awarded by the National Institutes of Health and therefore the Government has certain rights in some aspects of this invention.

Background of the Invention

[0003] This invention generally relates to methods of treating hematopoietic stem cells (HSCs) for improving their therapeutic usefulness and more particularly, but not limited to, treating hematopoietic stem cells derived from cord blood, and hematopoietic stem cells thus treated.

[0004] During inflammation, P-selectin and E-selectin cooperatively mediate leukocyte rolling and adhesion on vascular surfaces (McEver, R.P. Selectins: lectins that initiate cell adhesion under flow. *Curr Opin Cell Biol.* 2002 Oct;14:581-856). In the process of bone marrow transplantation, P-selectin and E-selectin also mediate the homing of intravenously injected HSCs to bone marrow. (Frenette, P.S., Subbarao, S., Mazo, I.B., Von Andrian, U.H., Wagner, D.D. Endothelial selectins and vascular cell adhesion molecule-1 promote hematopoietic progenitor homing to bone marrow. *Proc.Natl.Acad.Sci.USA.* 1998;95:14423-14428). In most tissues, P-selectin and E-selectin are expressed on endothelial cells after stimulation of agonists, but they are expressed constitutively on bone marrow endothelial cells. Selectins use α 2,3-sialylated and α 1,3-fucosylated glycans such as sialyl Lewis^x (sLe^x) on glycoproteins or glycolipids as ligands. P-selectin binds to the N-terminal region of P-selectin glycoprotein ligand-1 (PSGL-1), which contains tyrosine sulfates and an O-glycan capped with sLe^x. E-selectin binds to one or more different sites on PSGL-1. To interact with E-selectin, PSGL-1 does not require tyrosine sulfation, but expression of sLe^x on O-glycans enhances binding. E-selectin also interacts with other ligands on HSCs. An isoform of CD44 on HSCs has been

shown to bind to E-selectin *in vitro* (Dimitroff, C.J., Lee, J.Y., Rafii, S., Fuhlbrigge, R.C., Sackstein, R. CD44 is a major E-selectin ligand on human hematopoietic progenitor cells. *J. Cell Biol.* Jun 11 2001;153:1277-1286). Another potential ligand for E-selectin on HSCs is E-selectin ligand-1 (ESL-1) (Wild, M.K., Huang, M.C., Schulze-Horsel, U., van Der Merwe, P.A., Vestweber, D. Affinity, kinetics, and thermodynamics of E-selectin binding to E-selectin ligand-1. *J Biol Chem.* 2001 Aug 24;276:31602-31612). Each of these glycoprotein ligands is thought to carry sLe^x structures.

[0005] Hematopoietic stem cells harvested from one individual can be transplanted to the bone marrow of another individual following an intravenous infusion. The approach has been widely used in treatment of various hematological disorders such as leukemia (Thomas, E.D. History, current results, and research in marrow transplantation. *Perspectives Biol. Med.* 38:230-237.1995). Clinically, human HSCs are obtained from three different sources: bone marrow, adult peripheral blood after mobilization, and cord blood obtained from umbilical cords after delivery. Although there are more than 5 million unrelated bone marrow volunteer donors registered worldwide, finding a fully human leukocyte antigen (HLA)-matched unrelated donor remains a problem for many patients because of HLA polymorphism. Compared with bone marrow and adult peripheral blood, cord blood has several potential advantages, in particular the wide and rapid availability of cells and less stringent requirements for HLA identity between donor and recipient because of the lower risk of acute and chronic graft-versus-host disease (GVHD) (Rocha, V., et. al., Comparison of outcomes of unrelated bone marrow and umbilical cord blood transplants in children with acute leukemia. *Blood.* 97:2962-71.2001). Potential advantages of transplantation using cord blood HSCs rather than HSCs from bone marrow or adult peripheral blood include: (1) a large potential donor pool; (2) rapid availability, since the cord blood has been prescreened and tested; (3) greater racial diversity can be attained in the banks by focusing collection efforts on hospitals where children of under represented ethnic backgrounds are born; (4) reduced risk or discomfort for the donor; (5) rare contamination by viruses; and (6) lower risk of graft-versus-host disease (wherein the donor's cells attack the patient's organs and tissues), even for recipients with a less-than-perfect tissue match. Thus, cord blood-derived HSCs have been increasingly used for bone marrow transplantation in recent years.

[0006] In the transplantation setting, the intravenously infused HSCs specifically extravasate in the bone marrow to engraft and proliferate, a process that is defined as HSC homing. Homing has been studied extensively both *in vivo* and *in vitro* and is believed to rely on adhesion molecule interactions between HSCs and endothelium of bone marrow. Selectins are a group of adhesion molecules containing a N-terminal carbohydrate-recognition domain related

to those in Ca^{++} -dependent (C-type) animal lectins. P-selectin, expressed on activated platelets and endothelial cells, and E-selectin, expressed on activated endothelial cells, bind to glycoconjugate ligands on leukocytes and HSCs. The best-characterized glycoprotein ligand is PSGL-1, a mucin with many sialylated and fucosylated O-linked oligosaccharides. PSGL-1 is expressed on leukocytes and HSCs. Studies with PSGL-1-deficient mice have shown that PSGL-1 mediates leukocyte tethering to and rolling on P-selectin and supports tethering to E-selectin in flow. PSGL-1 also binds to L-selectin, which initiates leukocyte-leukocyte interactions that amplify leukocyte rolling on inflamed endothelial cell surfaces. In human PSGL-1, the P-selectin and L-selectin binding site comprises a peptide sequence containing three tyrosine sulfate residues near a threonine to which is attached a specific branched, fucosylated core-2 O-glycan (McEver, R.P., Cummings, R.D. Role of PSGL-1 binding to selectins in leukocyte recruitment. *J Clin Invest.* 100:S97-103. 1997; R.P. McEver: Selectins: Ligands that initiate cell adhesion under flow. *Curr. Op. in Cell Biol.* 14: 581-586, 2002). The fucose moiety is essential for P-selectin binding as measured by *in vitro* assays using synthetic glycosulfopeptides. The fucosylation is catalyzed by a family of α 1,3-fucosyltransferases. Among them, α 1,3-fucosyltransferase IV (FT-IV) and α 1, 3-fucosyltransferase VII (FT-VII) are primarily expressed in human leukocytes. These enzymes catalyze the transfer of a fucose residue from a donor, e.g., GDP-fucose, to an acceptor in α 1,3-linkage to GlcNAc in Gal-GlcNAc-sequences. Both FT-IV and FT-VII make the fucose addition which is necessary to form the sLe^x structure (NeuAc α 2,3Gal β 1,4[Fuc α 1,3]GlcNAc β 1-R). The sLe^x on a core-2 O-glycan attached to a specific threonine in the N-terminal amino acid sequence of human PSGL-1 is critical for binding to P-selectin.

[0007] HSCs have the potential to differentiate into different lineages of hematopoietic cells such as red blood cells, myeloid cells, lymphocytes and platelets. Human HSCs express a surface glycoprotein, CD34, which is routinely used for HSC identification and separation. Such human CD34⁺ cells (cells which express CD34 antigen) represent a heterogeneous population of progenitors with various degrees of hematopoietic maturation. The absence of ("−") or reduced ("low") expression of another surface protein, CD38, on human CD34⁺ cells is considered to be a surrogate marker of a primitive subpopulation of CD34⁺ cells. Thus, the cells of the CD34⁺CD38^{low/-} sub-population, which comprise approximately 10-20% of the total CD34⁺ cells from bone marrow or adult peripheral blood, are highly enriched for multiprogenitor and stem cell activity, including engraftment ability. Notably, approximately 30% of cord blood HSCs are CD34⁺CD38^{low/-}. However, unlike CD34⁺CD38^{low/-} adult peripheral blood stem cells, cord blood CD34⁺CD38^{low/-} HSCs are known to have reduced homing to murine bone marrow, which is primarily dependent on interactions of human HSCs with murine P-selectin on the

microvascular endothelium (Hidalgo, A., Weiss, L.A., and Frenette, P.S. Functional selectin ligands mediating human CD34⁺ cell interaction with bone marrow endothelium are enhanced postnatally. Adhesion pathways mediating hematopoietic progenitor cell homing to bone marrow. J. Clin. Invest. 110:559-569. 2002). Flow cytometry analyses indicate that this homing defect results from non-functional PSGL-1 expressed on these CD34⁺CD38^{low/-} cord-blood derived HSCs. Thus, the impaired ability of the CD34⁺CD38^{low/-} HSCs to bind to P-selectin explains in at least in part the delayed platelet and myeloid engraftment associated with cord blood HSC transplantation. The use of cord blood HSCs for transplantation has been primarily restricted to children (which require fewer cells for transplantation) due to the limited quantities and defective homing ability of HSCs isolated from umbilical cords.

[0008] An invention which corrects the homing defect of HSCs would significantly increase the potential of umbilical cord blood as a source of hematopoietic stem cells and would thereby lead to lower risks for acute and chronic graft-versus-host disease and improved success of bone marrow reconstitution.

Summary of the Invention

[0009] The present invention in one embodiment contemplates a method of treating HSCs comprising the steps of providing a quantity or population of HSCs, at least some of which lack or have reduced expression of surface protein CD38, and treating the quantity or population of HSCs *in vitro* with an α 1,3 fucosyltransferase and a fucose donor, wherein the treated HSCs have enhanced binding to P-selectin and E-selectin. Furthermore, the HSCs are typically characterized as comprising P-selectin glycoprotein ligand-1 (PSGL-1) and/or other selectin ligands which does not effectively bind to P-selectin or E-selectin. More particularly, the PSGL-1 or other selectin ligands which occurs on the CD34⁺ CD38^{low/-} HSCs lack, or have fewer, fucosylated glycans, particularly O-glycans, and may for example, have PSGL-1 which have core-2 O-glycans which comprise NeuAc α 2,3Gal β 1,4GlcNAc and which lack fucose in α 1,3 linkage to the GlcNAc. The HSCs, in their untreated state prior to fucosylation as described herein, have reduced bone marrow homing ability. In one embodiment of the invention, the HSCs are derived from human umbilical cord blood, though they may be derived from bone marrow or peripheral blood, as long as they are characterized as having enhanced bone marrow homing ability after the fucosylation treatment. In the method contemplated herein, the α 1,3 fucosyltransferase may be, for example, an α 1,3 fucosyltransferase IV, an α 1,3 fucosyltransferase VI, or an α 1,3 fucosyltransferase VII, or a combination thereof. The fucose donor may be, for example, GDP-fucose.

[0010] The invention further contemplates in one embodiment a composition of treated human HSCs which comprise cord blood-derived CD34⁺ HSCs lacking or having reduced expression of surface protein CD38 (CD38^{low/-}), wherein the HSCs are able to bind to P-selectin or E-selectin. The HSCs may be disposed in a pharmaceutically acceptable carrier, or diluent, or vehicle for storage or administration to a patient. The invention is further directed to a treatment method, comprising administering an effective amount of the HSCs to a subject having a hematological disorder or other disease requiring or benefiting from a transplantation of HSCs for treatment.

[0011] As noted above, after the fucosylation treatment described herein, the treated CD34⁺ HSCs (including CD34⁺CD38^{low/-} HSCs) have enhanced binding to P-selectin or E-selectin, as compared to untreated CD34⁺ HSCs. Enhanced binding to P-selectin (or E-selectin) is defined as at least 10% of the treated HSCs having fluorescence in a P-selectin (or E-selectin, respectively) binding assay which is greater than a predetermined fluorescence threshold (as defined below). In another embodiment, at least 25% of the treated HSCs exceed the predetermined fluorescence threshold. In another embodiment, at least 50% of the treated HSCs exceed the predetermined fluorescence threshold. In another embodiment, at least 75% of the treated HSCs exceed the predetermined fluorescence threshold. In another embodiment, at least 90% of the treated HSCs exceed the predetermined fluorescence threshold. In another embodiment, at least 95% of the treated HSCs exceed the predetermined fluorescence threshold.

[0012] The present invention further contemplates a blood product produced by the method including the steps of providing a quantity or population of HSCs, at least a portion of which are CD34⁺ and which lack or have reduced expression of protein CD38, and treating the quantity of HSCs *in vitro* with an α 1,3 fucosyltransferase and a fucose donor, wherein the majority of the treated HSCs have enhanced binding to P-selectin (or E-selectin) as described herein. The quantity of HSCs are preferably derived from umbilical cord blood but may be obtained from bone marrow or adult peripheral blood. The quantity or population of HSCs could comprise a portion, or unfractionated sample, of blood or bone marrow.

Brief Description of the Figures

[0013] Figure 1. A. CD34 antibody staining of mononuclear cells (MNCs) isolated from human cord blood. B. CD34 antibody staining of cells after CD34-enrichment. C. Isotope control IgG staining of CD34⁺ cells. Axes are fluorescence intensity as measured by flow cytometry.

[0014] Figure 2. A. CD34⁺ cells isolated from cord blood express PSGL-1. B. CD34⁺ cells consist of about 30% CD34⁺CD38^{low/-} cells (primitive progenitors) and about 65% CD34⁺CD38⁺ cells. Axes are fluorescence intensity as measured by flow cytometry.

[0015] Figure 3. A. CD34⁺ cells are gated as P-selectin binding cells (R2) or non-P-selectin binding cells (R1). B. 24% ± 5% of CD34⁺ cells from R1 region have no or reduced expression of CD38. The result is representative of four independent flow cytometry analyses and shows that significant numbers of non-P-selectin binding HSCs are CD34⁺ and CD38^{low/-}.

[0016] Figure 4. Viability of cells after *in vitro* fucosylation as measured by propidium iodide (PI) staining. A. Cells without treatment. B. Sham-treated cells. C. FT-VI-treated cells. Axes are fluorescence intensity as measured by flow cytometry.

[0017] Figure 5. A. 15% of the CD34⁺ cells obtained from cord blood express low or no fucosylated epitopes as stained with sLe^x-specific monoclonal antibody HECA 452. B. *In vitro* α1,3-fucosylation with FT-VI and GDP-fucose dramatically increases sLe^x epitopes on cord blood-derived CD34⁺ cells. Axes are fluorescence intensity as measured by flow cytometry.

[0018] Figure 6. Titration of soluble P-selectin binding to CD34⁺ HSCs by flow cytometry for determining a saturating amount of P-selectin.

[0019] Figure 7. Binding of a saturable concentration of soluble P-selectin to cord blood-derived CD34⁺ cells. A. About 27% of untreated cord blood-derived CD34⁺ cells do not bind to or have low level binding to P-selectin. B. *In vitro* α1,3-fucosylation converts CD34⁺ cells which are negative or low for P-selectin binding into cells which are positive and high for P-selectin binding. C. and D. P-selectin binds to PSGL-1 on cord blood-derived CD34⁺ cells as verified by blocking monoclonal antibodies to P-selectin (G1) and PSGL-1 (PL1). EDTA also inhibits binding, consistent with the requirement for Ca²⁺ to support P-selectin binding to PSGL-1. Axes are fluorescence intensity as measured by flow cytometry.

[0020] Figure 8. Rolling of CD34⁺ cells on human serum albumin (HSA) or on human P-selectin under shear force. Treatment of cord blood-derived CD34⁺ cells with GDP-fucose and FT-VI significantly augments cell rolling on P-selectin in shear flow.

[0021] Figure 9. Binding of a saturable concentration of soluble E-selectin to cord blood-derived CD34⁺ cells. A. About 24% of untreated cord blood-derived CD34⁺ cells do not bind to or have low level binding to E-selectin. B. *In vitro* α1,3-fucosylation converts CD34⁺ cells which are negative or low for E-selectin binding into cells which are positive and high for E-selectin binding. C. and D. E-selectin binds to cord blood-derived CD34⁺ cells as verified by blocking monoclonal antibodies to E-selectin (9A9). EDTA also inhibits binding. Axes are fluorescence intensity as measured by flow cytometry. The result is representative of three independent measurements.

[0022] Figure 10. *In vitro* fucosylation significantly augments CD34⁺ cells rolling on human soluble E-selectin under shear forces. A and B. Treatment of CB CD34⁺ cells with GDP-fucose and FT-VI significantly enhances the number of cells rolling on E-selectin under different shear forces. The rolling is E-selectin dependent as the cells did not roll on human serum albumin (HSA) and rolling was specifically blocked by ES1, a mAb to human E-selectin, but not by PL1, a mAb which binds to the P-selectin binding site of PSGL-1. C and D. The fucosylated CD34⁺ cells are more resistant to shear forces and roll slower than untreated CD34⁺ cells. The data represent the mean $\pm$ SD of four independent experiments.

[0023] Figure 11. Fucosylated CB HSCs exhibit enhanced engraftment in bone marrow of sublethally irradiated NOD/SCID mice. Bone marrow (BM) or peripheral blood (PB) from mice 6 weeks after transplantation with 8×10^6 sham-treated or FTVI-treated CB cells were analyzed for engraftment of human-derived hematopoietic cells. (A) Flow cytometry analysis of BM and PB cells stained with a mAb to the human pan-leukocyte marker CD45 demonstrated a doubling of human-derived cells in mice transplanted with fucosylated CB cells. (B) Compared with mice transplanted with CB cells without fucosylation, BM cells from mice transplanted with fucosylated CB cells contain significantly more human colony-forming progenitors, which include BFU-E, CFU-GM, and CFU-GEMM, as demonstrated by human hematopoietic progenitor assays. Bone marrow of control mice injected with saline only produced no colonies, confirming the specificity of the assay.

Detailed Description of the Invention

[0024] The present invention in one embodiment contemplates a method of treating HSCs comprising providing a quantity or population of HSCs which lack or have reduced expression (less than the normal level of expression of CD38) of surface protein CD38, and treating the quantity or population of HSCs *in vitro* with an α 1,3 fucosyltransferase and a fucose donor, wherein the HSCs so treated have enhanced binding to P-selectin or E-selectin over the untreated HSCs. Furthermore, the untreated HSCs are typically characterized as predominantly comprising PSGL-1 or other selectin ligands which do not adequately bind to P-selectin or E-selectin. The PSGL-1 or other selectin ligands which occur on the CD38^{low/-} HSCs lack or have reduced numbers of fucosylated glycans, such as O-glycans, and may for example, have PSGL-1 which have core-2 O-glycans which comprise NeuAc α 2,3Gal β 1,4GlcNAc but which lack a fucose in α 1,3 linkage to the GlcNAc. The CD38^{low/-} HSCs, in their untreated state prior to fucosylation, have reduced bone marrow homing ability. Preferably, the HSCs are derived from human umbilical cord blood (CB), although they may be derived from bone marrow or peripheral blood, as long as they are characterized as

needing, or benefiting from, further fucosylation to enhance their bone marrow homing ability. In the method contemplated herein, the α 1,3 fucosyltransferase may be for example α 1,3 fucosyltransferase IV, α 1,3 fucosyltransferase VI, or α 1,3 fucosyltransferase VII. The fucose donor may be for example GDP-fucose.

[0025] The invention contemplates in one embodiment a composition of treated human HSCs which comprise cord blood-derived HSCs lacking or having reduced expression of surface protein CD38 (CD38^{low/-}), wherein the treated HSCs comprise PSGL-1 or other selectin ligands that are properly fucosylated (e.g., comprises sialyl Lewis^x) and which are able to bind to P-selectin (or E-selectin). The treated HSCs may be disposed in a pharmaceutically acceptable carrier or vehicle for storage or administration to a patient. The invention is further directed to a treatment method, comprising administering an effective amount of the treated HSCs to a subject having a hematological disorder or other disease requiring transplantation of HSCs for treatment.

[0026] In one embodiment, the composition of treated HSCs comprises a population of human HSCs derived from umbilical cord blood, at least a portion of which are characterized as CD34⁺CD38^{low/-} HSCs having enhanced binding to P-selectin (or E-selectin). Enhanced binding to P-selectin (or E-selectin) is defined as at least 10% of the treated HSCs having fluorescence in a P-selectin binding assay (or E-selectin binding assay, respectively) which is greater than a predetermined fluorescence threshold. In another embodiment, at least 25% of the treated HSCs exceed the predetermined fluorescence threshold. In another embodiment, at least 50% of the treated HSCs exceed the predetermined fluorescence threshold. In another embodiment, at least 75% of the treated HSCs exceed the predetermined fluorescence threshold. In another embodiment, at least 90% of the treated HSCs exceed the predetermined fluorescence threshold. In another embodiment, at least 95% of the treated HSCs exceed the predetermined fluorescence threshold. The composition of human HSCs preferably is disposed in a pharmaceutically-acceptable carrier or vehicle for storage or for administration to a subject.

[0027] The predetermined fluorescence threshold in one embodiment is determined by first obtaining a sample of cells containing at least 100 CD34⁺CD38^{low/-} HSCs from a mononuclear fraction of ordinary umbilical cord blood (cord blood from healthy full term babies). This control (baseline) sample of HSCs is assayed using the P-selectin binding assay (or E-selectin binding assay) described elsewhere herein, or by any other P-selectin fluorescence binding assay (or E-selectin binding assay, respectively) known in the art. P-selectin (or E-selectin) binding fluorescence levels are measured for the CD34⁺CD38^{low/-} HSCs in the control (baseline) sample. In one embodiment, a fluorescence value is selected which exceeds the P-selectin (or E-selectin) binding fluorescence levels of at least 95% of the CD34⁺CD38^{low/-} HSCs in the control

sample. The selected fluorescence value is designated as the predetermined fluorescence threshold against which is compared the P-selectin (or E-selectin) binding fluorescence of the treated (i.e., fucosylated) HSCs.

[0028] The present invention further contemplates a blood product produced by the method of providing a quantity or population of HSCs, at least a portion of which are CD34⁺ and which lack or have reduced expression of protein CD38, and treating the quantity of HSCs *in vitro* with an α 1,3 fucosyltransferase and a fucose donor, wherein the majority of the treated HSCs bind to P-selectin (or E-selectin). The quantity of HSCs may be derived from umbilical cord blood, but may be obtained from bone marrow or adult peripheral blood.

[0029] In general, the present invention contemplates a method wherein non-functional or suboptimally functional PSGL-1 or other selectin ligands expressed on cord blood HSCs modified by *in vitro* α 1,3-fucosylation technology to correct the homing defect, which improves their use in bone marrow transplantation.

[0030] As noted above, CD34⁺ cord blood HSCs may be defined as either CD38⁺ (positive for CD38) or CD38^{low/-} (reduced or no expression of CD38). CD38^{low/-} cord blood HSCs can be identified using fluorescence techniques as described below. Cord blood HSCs are treated with a CD34-binding antibody having a fluorophore linked thereto, and with a CD38-binding antibody having a different fluorophore linked thereto. CD34⁺ cells are defined as those HSCs which exhibit fluorescence from the anti-CD34 antibody fluorophore upon irradiation. CD38^{low/-} HSCs are defined as the 30% of CD34⁺ HSCs which have the lowest fluorescence as measured from the anti-CD38 binding antibody, or as the CD34⁺ HSCs which have anti-CD38 binding antibody fluorescence levels of 50 units or less (as measured by a fluorescence flow cytometer as described elsewhere herein). In one embodiment, the anti-CD34 binding antibody fluorophore is FITC (fluorescein isothiocyanate) while the anti-CD38 binding antibody fluorophore is phycoerythrin (PE).

[0031] As explained previously, CD34⁺ cells express PSGL-1 or other selectin ligands, yet a significant amount of primitive CD34⁺ cells which are low in or lack CD38, (e.g., which comprise about 30% of the total CD34⁺ cord blood cells), do not bind to P-selectin (or E-selectin) or bind only low amounts of P-selectin (or E-selectin, respectively). PSGL-1 is a homodimeric mucin expressed on almost all leukocytes including CD34⁺ cells. To be functional, i.e., able to bind to P-selectin or E-selectin, PSGL-1 requires several post-translational modifications leading to formation of an sLe^x group thereon, including α 1,3-fucosylation. Insufficient α 1,3-fucosylation, for example, results in impaired ability of naive T cells to interact with vascular selectins. In the present invention it has been discovered that the inability of cord blood derived HSCs to bind to P-selectin or E-selectin is due to inadequate α 1,3-fucosylation of PSGL-1 or other selectin

ligands. Therefore, the basis of the present invention is that the treatment of CD34⁺ cells *in vitro* with an α 1,3-fucosyltransferase (e.g., FT-VI), which also catalyzes the synthesis of the sLe^x structure, will increase fucosylation of PSGL-1 or other selectin ligands and thereby correct the homing defect of the HSCs.

[0032] Fucosyltransferases which are able to transfer fucose in α 1,3 linkage to GlcNAc are well known in the art. Several are available commercially, for example from Calbiochem. Further, at least five different types of α 1,3 fucosyltransferases (FTIII-VII) are encoded by the human genome. These include: the Lewis enzyme (FTIII), which can transfer fucose either α (1,3) or α (1,4) to Gal β 4GlcNAc or Gal β 3GlcNAc respectively (Kukowska-Latallo et al., Genes Dev. 4:1288, 1990); FTIV, which forms α (1,3) linkages, which does not prefer sialylated precursors (Goelz, et al., Cell 63; 1349, 1989; Lowe, et al., J. Biol. Chem. 266; 17467, 1991); FTV (Weston, et al., J. Biol. Chem. 267:4152, 1992a) and FTVI (Weston, et al., J. Biol. Chem. 267:24575, 1992b) which form α (1,3) linkages, which can fucosylate either sialylated or nonsialylated precursors, and FTVII, (Sasaki, et al., J. Biol. Chem. 269:14730, 1994); Natsuka, et al., J. Biol. Chem. 269:16789, 1994) which can fucosylate only sialylated precursors.

[0033] FTIII is encoded by GDB:135717; FTIV by GDB:131563; FTV by GDB:131644; FTVI by GDB:135180; and FTVII by GDB:373982. A sixth α 1,3 fucosyltransferase (FTIV) is encoded by GDB:9958145 (Genome Database Accession ID numbers are available from the GDB^(TM) Human Genome Database Toronto (Ontario, Canada): The Hospital for Sick Children, Baltimore (Maryland, USA): Johns Hopkins University, 1990-. Available from Internet: URL <http://www.gdb.org/>). The present invention further contemplates using other, non-human α 1,3 fucosyltransferases available and known to those of ordinary skill in the art, for example as shown in U.S. Patent Nos: 6,399,337 and 6,461,835.

[0034] As noted previously, human HSCs can be obtained for treatment with α 1,3 fucosyltransferase, for example, by separation from the other cells in a source of umbilical cord blood, peripheral blood, or bone marrow. Various techniques may be employed to separately obtain the CD34⁺CD38^{low/-} stem cells alone, or in combination with CD34⁺CD38⁺ HSCs. Monoclonal antibodies are particularly useful for identifying markers (surface membrane proteins) associated with particular cell lineages and/or stages of differentiation. The antibodies may be attached to a solid support to allow for crude separation. The separation techniques employed should maximize the retention of viability of the fraction to be collected. The particular technique employed will depend upon efficiency of separation, cytotoxicity of the methodology, ease and speed of performance, and necessity for sophisticated equipment and/or technical skill.

[0035] Procedures for separation may include magnetic separation, using antibody-coated magnetic beads, and "panning" with antibody attached to a solid matrix, e.g., plate, or other convenient technique. Techniques providing accurate separation include fluorescence activated cell sorters, which can have varying degrees of sophistication, e.g., a plurality of color channels, low angle and obtuse light scattering detecting channels, and impedance channels.

[0036] Conveniently, the antibodies may be conjugated with markers, such as magnetic beads, which allow for direct separation; biotin, which can be removed with avidin or streptavidin bound to a support; fluorochromes, which can be used with a fluorescence activated cell sorter (FACS), or the like, to allow for ease of separation of the particular cell type. Any technique may be employed which is not unduly detrimental to the viability of the remaining cells.

[0037] In one embodiment, the HSCs lacking the mature cell markers, may be substantially enriched, wherein the cells may then be separated by the FACS or other methodology having high specificity. Multi-color analyses may be employed with the FACS which is particularly convenient. The cells may be separated on the basis of the level of staining for the particular antigens. Fluorochromes, which may find use in a multi-color analysis, include phycobiliproteins, e.g., phycoerythrin and allophycocyanins, fluorescein, and Texas red, for example. Alternatively, HSCs can be treated with fucosyltransferases before separation of the desired HSCs from the unfractionated blood or bone marrow sample, for example, using total mononuclear cells from cord blood, peripheral blood, or bone marrow.

[0038] In one embodiment, the $CD34^+HSC$, including $CD34^+CD38^{low/-}$ cells may be treated by adding free fucosyltransferase to the cell composition, wherein the final blood product containing the fucosylated $CD34^+CD38^{low/-}$ also contains the fucosyltransferase which was used to treat the cells. In another embodiment, the HSCs may be treated using fucosyltransferases which are bound to a support, such as magnetic beads, or any other support known by those of ordinary skill in the art, which can be separated from the cell composition after the treatment process is complete.

[0039] METHODS AND RESULTS

[0040] Umbilical cord blood samples were obtained from normal full-term vaginal deliveries in accordance with a protocol approved by the Institutional Review Board of the Oklahoma Medical Research Foundation (OMRF). 70 to 100 ml of cord blood was collected per delivery. Sodium citrate was used as anticoagulant. Any appropriate method known in the art for collecting cord blood is suitable, such as the method shown in U.S. Patent No. 6,440,010. The $CD34^+$ cells in the supernatant of the blood sample were enriched with a CD34-isolation mini-MACS kit (Miltenyi Biotec, Bergisch Gladbach,

Germany). Cord blood was first mixed with an equal volume of 6% dextran 70 in 0.9% sodium chloride (McGaw, Inc., Irvine, CA). After sedimentation of two to three hours, the cells in the supernatant were removed, and washed once in Hanks' balanced salt solution (HBSS, Cellgro) containing 2 mM EDTA and 0.5% human serum albumin (HSA). Contaminating red blood cells were lysed in FACS Lysing solution (BD Biosciences, San Jose, CA). Low-density mononuclear cells (MNCs) were separated after centrifugation at 250 *g* over Ficoll-Hypaque (*d* = 1.077 g/ml). CD34⁺ cells were purified from the MNC fraction using the CD34-isolation mini-MACS kit following the manufacturer's instructions. The purity of the isolated CD34⁺ cells was about 96% as examined by flow cytometry (Figure 1). The following experiments were then carried out.

[0041] *Verification by flow cytometry that CD34⁺ cells isolated from cord blood express PSGL-1 and the CD34⁺ cells are heterogeneous.*

[0042] For this purpose, triple-colored staining was used. The cells enriched by the mini-MACS sorting were incubated with anti-CD34 monoclonal antibody (mAb, clone AC136 from Miltenyi Biotec) conjugated with FITC, anti-CD38 mAb conjugated with PE (BD Pharmingen, San Diego, CA), and anti-PSGL-1 monoclonal antibody conjugated with Cy5 (BD Pharmingen, San Diego, CA) after blocking the Fc receptor with human IgG. After washing, the cells were analyzed by flow cytometry on a FACScan (Becton Dickinson). Data were collected using the CellQuest program. Light scatter-gated events were plotted on a log scale of fluorescence intensity. Virtually all CD34⁺ cells express PSGL-1 (Fig. 2A), and about 30% of the CD34⁺ cells have low or no expression of CD38 (Figure 2B), representing the sub-population of primitive progenitor cells. Further, about 25% of the HSCs that do not bind to P-selectin are CD34⁺ and CD38^{low/-} (Fig. 3). These results confirm existing data.

[0043] *In vitro α 1-3-fucosylation of PSGL-1 on purified CD34⁺ cells.*

[0044] To introduce fucose on core 2 O-glycans attached to PSGL-1 or other selectin ligands on CD34⁺ cells, 2-4 X 10⁶ cells were treated with 1 mM guanosine diphosphate (GDP)-fucose (Calbiochem), 20 mU/mL α 1-3-fucosyltransferase VI (FT-VI) (Calbiochem), and 10 mM MnCl₂ in 0.5 mL HBSS/1% HSA for 40 minutes at 37°C, in an atmosphere containing 5% CO₂. This treatment produces optimal fucosylation of PSGL-1 on CD34⁺ cells as measured by maximum P-selectin binding, yet results in minimum toxicity to CD34⁺ cells as tested by propidium iodide staining (Figure 4).

[0045] *Measurement of fucosylated epitopes on CD34⁺ cells and verification by flow cytometry that in vitro α 1,3-fucosylation creates fucosylated epitopes on CD34⁺ cells.*

[0046] Sialyl Lewis^x is a fucosylation epitope. By incubating with an anti-sLe^x mAb HECA 452 (rat IgM, hybridoma from American Type Culture Collection [ATCC]), we examined the sLe^x epitopes on the CD34⁺ cells. The bound mAb was detected with FITC-conjugated goat F(ab)[']₂ fragments to rat IgM (Caltag). As indicated by Figure 5A, 26% of the CD34⁺ cells obtained from cord blood express low or no fucosylated epitopes. These data demonstrate that a subset of CD34⁺ cells are not properly fucosylated. To investigate if *in vitro* α 1,3-fucosylation can create fucosylated epitopes on the CD34⁺ cells, we stained the cells with HECA 452 after treatment of the CD34⁺ cells with FT-VI and GDP-fucose in the presence of Mn²⁺ using the method described above. We found that the *in vitro* α 1,3-fucosylation dramatically increased sLe^x epitopes on cord blood-derived CD34⁺ cells as indicated by HECA 452 staining (Figure 5B).

[0047] P-selectin Binding - Results

[0048] *Verification of the binding profiles of soluble P-selectin on cord blood-derived CD34⁺ cells.*

[0049] For the P-selectin binding assay, cord blood-derived CD34⁺ cells, after Fc receptor blocking, were incubated with anti-CD34-PE and with P-selectin isolated from human platelets. P-selectin binding was detected with FITC-labeled S12, a non-blocking mAb to human P-selectin. Incubations of the cells were performed at 4°C for 20 min. A saturating amount of P-selectin was used in the experiments after a serial titration (Figure 6). In control experiments, P-selectin incubations of the cells were carried out in the presence of G1, a blocking mAb to P-selectin, PL1, a blocking mAb to PSGL-1, or 10 mM EDTA, which eliminates Ca²⁺-dependent selectin-ligand interactions. Flow cytometry analyses showed that about 27% of the CD34⁺ cells (primarily comprising the CD38^{low/-} cells) did not bind to P-selectin, which is consistent with previously published data (Figure 7A) (Hidalgo, A., Weiss, L.A., and Frenette, P.S. Functional selectin ligands mediating human CD34⁺ cell interaction with bone marrow endothelium are enhanced postnatally. Adhesion pathways mediating hematopoietic progenitor cell homing to bone marrow. J. Clin. Invest. 110:559-569. 2002). Figure 7C showed that P-selectin bound specifically to PSGL-1 on the CD34⁺ cells because the G1 and PL1 antibodies and EDTA abolished binding.

[0050] *Demonstration by flow cytometry that in vitro α 1,3-fucosylation of the surface of CD34⁺ cells increases binding to P-selectin.*

[0051] The cord blood-derived CD34⁺ cells were first treated with GDP-fucose and FT-VI as described above, and then stained with both anti-CD34-PE and P-selectin. The P-selectin binding was detected with FITC-labeled mAb S12. Treatment with exogenous FT-VI

significantly increased binding of CD34⁺ cells to human P-selectin (Figure 7B). The augmented binding to P-selectin was due to the increased functional PSGL-1 on the CD34⁺ cells after the α 1,3-fucosylation because binding was blocked by antibodies G1 and PL1 and by EDTA (Figure 7D). To find optimal conditions for *in vitro* α 1,3-fucosylation, various incubation times and concentrations of FT-VI, GDP-fucose, and Mn were examined (data not shown). A condition (shown above) was chosen for all the experiments that produced optimal fucosylation of PSGL-1 on CD34⁺ cells as measured by maximum P-selectin binding (Figure 7B), yet resulted in minimum toxicity to CD34⁺ cells as tested by propidium iodide staining (Fig. 4).

[0052] *Demonstration that in vitro α 1,3-fucosylation increases CD34⁺ cell adhesion to P-selectin in physiological shear flow.*

[0053] Cord blood-derived CD34⁺ cells were divided into two groups for further processing. One group was incubated with GDP-fucose and FT-VI as described above, and another was treated with FT-VI without GDP-fucose (sham-treated control). The P-selectin-binding ability of the two groups of cells was compared using an *in vitro* flow chamber rolling assay system as described below. P-selectin isolated from human platelets was immobilized in a parallel-plate flow chamber. A P-selectin site density of 145 sites/ μ m² was used as measured by binding of ¹²⁵I-labeled anti-P-selectin mAb S12. Sham-treated or FTVI-treated CD34⁺ cells (10⁶/ml in Hanks' balanced salt solution and 0.5% human albumin) were perfused over P-selectin at a wall shear stress of 1 dyn/cm². The accumulated number of rolling cells was measured with a videomicroscopy system coupled to an image analysis system. The CD34⁺ cells rolled in a Ca⁺⁺-dependent manner by human P-selectin-PSGL-1 interactions because EDTA and antibodies G1 and PL1 abolished the rolling, and no rolling was observed on plates coated only with human serum albumin (Figure 8). Compared to sham-treated CD34⁺ cells, about 3-fold more FT-VI-treated CD34⁺ cells rolled on P-selectin.

[0054] E-selectin Binding - Results

[0055] *Binding profiles of soluble E-selectin to CB-derived HSCs.*

[0056] Murine soluble E-selectin/human IgM chimera (E-selectin/IgM) was used for the fluid phase E-selectin binding assay. CD45/human IgM chimera was used as negative control. The cells were incubated with the E-selectin/IgM after Fc receptor blocking. E-selectin binding was then detected with FITC-labeled goat anti-human IgM polyclonal antibodies. The cells were also stained with PE-labeled anti-CD34 mAb (BD Pharmingen, San Diego, CA). Incubations were performed at 4°C for 20 min. A saturated amount of E-selectin was used in the experiments after a serial titration. In control experiments, stainings were carried out in the

presence of 9A9, a blocking mAb to E-selectin, or 10 mM EDTA, which eliminates Ca^{2+} -dependent selectin-ligand interactions. Flow cytometry analyses showed that about 25% of the CD34^+ HSCs did not bind to E-selectin (Fig. 9A). Fig 9C showed that the interaction of CD34^+ HSCs with E-selectin was specific because mAb 9A9 and EDTA abolished it.

[0057] *In vitro* $\alpha 1,3$ -fucosylation increases CD34^+ HSC binding to E-selectin as measured by flow cytometry.

[0058] The CB-derived CD34^+ HSCs were divided into two groups. One group ($2-4 \times 10^6$ cells) was incubated with 1 mM GDP-fucose, 20 mU/ml FTVI (Calbiochem), and 10mM MnCl_2 in 0.5 ml HBSS/1% HSA for 40 minutes at 37°C , in an incubator containing 5% CO_2 . Another group was incubated with FT-VI without GDP-fucose (sham-treated control). The cells were then stained with both anti- CD34 and E-selectin/IgM. After the exogenous $\alpha 1,3$ -fucosyltransferase treatment, the binding of CD34^+ HSCs to E-selectin increased from 75% to 95% (Fig. 9A and B). The augmented binding to E-selectin was specific as verified by mAb 9A9 and EDTA (Fig. 9D). The residual binding after Ab 9A9 and EDTA blocking seen in Fig. 9C and D was non-specific because cells stained with negative control CD45/IgM had a similar profile (data not shown).

[0059] *In vitro* $\alpha 1,3$ -fucosylation increases HSC adhesion to E-selectin under physiological shear forces

[0060] The HSCs were divide into two groups and fucosylated as described above. The E-selectin-binding ability of the two groups of cells was compared using an *in vitro* flow chamber rolling system. Briefly, soluble human E-selectin was immobilized in a parallel-plate flow chamber. An E-selectin site density of 200 sites/ μm^2 was used as measured by binding of ^{125}I -labeled anti-human E-selectin mAb ES1. Sham-treated or FT-VI-treated HSCs ($10^6/\text{ml}$ in HBSS and 0.5% HSA) were perfused over E-selectin under different shear forces. The accumulated number and shear resistance of the rolling cells were measured with a videomicroscopy system coupled to an image analysis system. At shear forces examined, about 2-3 times more FT-VI-treated HSCs rolled on E-selectin compared to the sham-treated HSCs (Fig. 10A and B). The FT-VI-treated cells also rolled with lower velocity and were more resistant to detachment by shear forces (Fig. 10C and D). The interaction of HSCs with E-selectin was specific, as mAb ES1 abolished rolling and rolling was not observed on plates coated only with HSA (Fig. 10B). PL1, which blocks binding of P-selectin to PSGL-1, did not affect HSC rolling on E-selectin (Fig. 10B), confirming that E-selectin mediates rolling by binding to other sites on PSGL-1 or to other cell-surface ligands.

[0061] These results indicate that *in vitro* α 1,3-fucosylation enhances physiologically-relevant rolling adhesion of CD34⁺ cells to P-selectin and E-selectin under flow.

[0062] *In Vivo* Example

[0063] *Fucosylated* HSCs exhibit enhanced engraftment in bone marrow *in vivo*.

[0064] Methods

[0065] By *in vitro* analyses, it has been demonstrated herein that CB HSCs treated with GDP-fucose and FTVI exhibited a significant increase in fluid-phase binding to P-selectin and E-selectin and rolled much better on P-selectin and E-selectin coated surfaces under different wall shear forces, compared with CB HSCs without fucosylation. The fucosylated CB HSCs are further shown herein to have improved homing to and engraftment in bone marrow *in vivo*. Nonobese diabetic severe combined immunodeficiency (NOD/SCID) mice have been well established as xenogeneic recipients for *in vivo* studies of human HSCs. We have compared human hematopoietic engraftment in NOD/SCID mice transplanted with CB HSCs with or without fucosylation.

[0066] Male and female pathogen-free (NOD/SCID) mice (The Jackson Laboratory), 4-5 weeks of ages, were used as recipients. The mice were irradiated (230 rad) 2 or 3 hours before intravenous injections of FTVI-treated (fucosylated) or sham-treated (treated with FTVI but without GDP-fucose) CB HSCs (8×10^6 /mouse in 300 μ l saline) respectively. Control mice each received 300 μ l saline without CB HSCs.

[0067] Six weeks after transplantation, the mice were bled and sacrificed. Bone marrow cells were isolated from both femora and filtered through a 100-mm mesh filter to remove debris. After lysis of red blood cells, the bone marrow nucleated cells from each mouse were resuspended in HBSS at a concentration of 1×10^6 /ml. The engraftment was analyzed by both flow cytometry and human hematopoietic progenitor assays. For flow cytometry, bone marrow nucleated cells were incubated with a Cy5-conjugated anti-human CD45 mAb (BD Pharmingen, San Diego, CA).

[0068] For human hematopoietic progenitor assays, 1×10^5 bone marrow nucleated cells per 35-mm culture dish were plated into MethoCult H4433 media (Stem Cell Technologies, Vancouver, Canada) in duplicate and incubated at 37°C, 5% CO₂. Total colonies, burst-forming units-erythroid (BFU-E), colony-forming units-granulocyte/macrophage (CFU-GM), and colony-forming units-granulocyte/megakaryocyte/macrophage (CFU-GEMM) were counted on day 14 of culture and analyzed. The human origin of the colonies was confirmed by flow cytometry analysis of cells collected from different colonies stained with mAbs to human CD45 for myeloid cells and glycophorin A for erythroid cells, respectively.

[0069] Results

[0070] The irradiated NOS/SCID mice that received fucosylated CB HSCs had 2-3 fold more CD45 positive human-derived hematopoietic cells in bone marrow and peripheral blood than mice that received sham-treated CB HSCs, as analyzed by flow cytometry (Figure 11A). The significantly improved engraftment of human hematopoietic progenitors in bone marrow of mice transplanted with fucosylated cells was multilineage as demonstrated by the increases of BFU-Es, CFU-GMs, and CFU-GEMMs (Figure 11B). Of note, the sizes of the colonies derived from CB HSCs were not significantly different in either recipient group (data not shown), indicating that fucosylation did not change the growth potential of the CB progenitors. Thus, the *in vivo* study demonstrates that the FTVI-treated CB HSCs have much higher potential to home to and engraft in bone marrow of NOD/SCID mice than the sham-treated cells do. These results show that the HSCs of the present invention will have enhanced bone marrow engraftment in humans.

[0071] Utility

[0072] The fucosylated HSCs described herein may be used in a variety of ways. For example, since the cells are naive (primitive), they can be used to fully reconstitute the bone marrow of an irradiated subject and/or an individual subjected to chemotherapy.

[0073] Among the conditions which can be treated by administration of hemopoietic stem cells according to the present invention are leukemias and lymphomas such as chronic myelocytic (myelogenous) leukemia (CML), juvenile chronic myelogenous leukemia (JCML), acute myelocytic leukemia (AML), acute lymphocytic leukemia (ALL), malignant lymphoma, multiple myeloma, aplastic anemia gravis, myelodysplastic syndrome (MDS), and autoimmune diseases, for example.

[0074] Other diseases that may be treated with the treated HSCs of the present invention are: Gunther's disease, Hunter syndrome, Hurler syndrome, neuroblastoma, non-Hodgkin's lymphoma, Wiskott-Aldrich syndrome, X-linked lympho-proliferative syndrome, and solid tissue tumors, such as breast cancer.

[0075] In these treatments, populations of these treated HSCs can be given to a patient whose marrow has been destroyed by ablative therapy.

[0076] The cells of the present invention can be administered by intravenous injection, for example, or by any other appropriate method known by those of ordinary skill in the art. In methods for treating a host afflicted with a disease or condition, a therapeutically effective amount of HSCs is that amount sufficient to reduce or eliminate the symptoms or effects of the

disease or condition. The therapeutically effective amount administered to a host will be determined on an individual basis and will be based, at least in part, on consideration of the individual's size, the severity of symptoms to be treated, and the results sought. Thus, a therapeutically effective amount can be determined by one of ordinary skill in the art of employing such practice in using no more than routine experimentation. For detailed information on HSC transplantations, "*Hemopoietic Stem Cell Transplantation, Its Foundation and Clinical Practice*" [Modern Medicine, Special Issue, 53, 2, 1998] can be consulted.

[0077] In preparing the dosage of fucosylated stem cells to be administered, a variety of pharmaceutically acceptable carriers can be utilized. The carrier, diluent or vehicle may contain a buffering agent to obtain a physiologically acceptable pH, such as phosphate-buffered saline, and/or other substances which are physiologically acceptable and/or are safe for use. In general, the material for intravenous injection in humans should conform to regulations established by the Food and Drug Administration, which are available to those in the field. Pharmaceutically-acceptable carriers may be combined, for example, in a 1 volume: 1 volume ratio, with the treated HSC composition. The carrier may be for example, M199 or RPMI 1640 medium. Furthermore, in preparing said dosage form, various infusions in common use today can also be employed. In one embodiment, the dose amount conventionally used in the transplantation of HSCs can be employed. The dosage may be, for example, about $.01-10 \times 10^8$ treated MNCs/kg of weight (which includes treated CD38^{low/-} HSCs or other treated HSCs as defined elsewhere herein) of the patient, or more, or less where appropriate.

[0078] As described herein, the present invention contemplates a method of treating HSCs, comprising providing a quantity of HSCs, at least a portion of the HSCs lacking or having reduced expression of surface protein CD38, and treating the quantity of HSCs *in vitro* with an $\alpha 1,3$ -fucosyltransferase and a fucose donor forming treated HSCs, wherein the treated HSCs have enhanced binding to P-selectin or E-selectin. In one embodiment, the portion of HSCs lacking or having reduced expression of surface protein CD38 has reduced bone marrow homing ability. The HSCs may be derived from human umbilical cord blood, and may be an unfractionated quantity of human umbilical cord blood. Alternatively, the HSCs may be derived from peripheral blood, and may be an unfractionated quantity of peripheral blood. Alternatively, the HSCs may be derived from bone marrow, and may be an unfractionated quantity of bone marrow. The portion of HSCs lacking or having reduced expression of surface protein CD38 comprises PSGL-1 or other structures which have unfucosylated glycans or unfucosylated O-glycans. In the present method, the portion of HSCs lacking or having reduced expression of surface protein CD38 may comprise PSGL-1 having core-2 O-glycans comprising NeuAc $\alpha 2,3$

Gal β 1,4 GlcNAc and which are absent a fucose in α 1,3 linkage to the GlcNAc or which comprise other glycans which lack proper fucosylation. In one embodiment, at least 50% of the treated HSCs have P-selectin binding fluorescence which exceeds a predetermined fluorescence threshold in a P-selectin binding assay or E-selectin binding fluorescence which exceeds a predetermined fluorescence threshold in an E-selectin binding assay (as described elsewhere herein). In the present method, the α 1,3 fucosyltransferase may be α 1,3 fucosyltransferase IV, α 1,3 fucosyltransferase VI, or α 1,3 fucosyltransferase VII. Further, the fucose donor may be GDP-fucose.

[0079] The present invention further contemplates a composition of HSCs which comprises CD34⁺ HSCs derived from umbilical cord blood and lacking or having reduced expression of surface protein CD38, wherein at least 10% of the CD34⁺ HSCs bind to P-selectin (or E-selectin), and a pharmaceutically-acceptable carrier. In the composition, in alternative embodiments, at least 25%, 50%, 75%, 90%, or 95% of the CD34⁺ HSCs bind to P-selectin (or E-selectin).

[0080] The present invention also contemplates treating a subject with a hematological disease or other condition requiring a transplantation of HSCs by administering a quantity of the composition of treated HSCs described herein to the subject having a hematological disease or other condition requiring a transplantation of HSCs. The hematological disease may be, for example, acute lymphocytic leukemia, acute myelogenous leukemia, myelodysplasia, chronic myelogenous leukemia, juvenile chronic myelogenous leukemia, or sickle cell anemia.

[0081] Furthermore, the present invention contemplates a blood product comprising a population of human HSCs comprising cells characterized as CD34⁺CD38^{low/-}, wherein at least 10% of the CD34⁺CD38^{low/-} HSCs bind to P-selectin or E-selectin. In the blood product, in alternative embodiments, at least 25%, 50%, 75%, 90%, or 95% (or any percentage inclusive) of the CD34⁺CD38^{low/-} HSCs bind to P-selectin or E-selectin. In the blood product, the human HSCs may be derived from human umbilical cord blood, adult peripheral blood, or bone marrow. The blood product may also comprise a pharmaceutically acceptable carrier or vehicle, and may also comprise a free fucosyltransferase or a fucosyltransferase bound to a support.

[0082] The present invention also contemplates a blood product produced by the method comprising providing a quantity of HSCs, at least a portion of the HSCs lacking or having reduced expression of surface protein CD38, and treating the quantity of HSCs *in vitro* with an α 1,3-fucosyltransferase and a fucose donor to produce treated HSCs, wherein at least 10% of the treated HSCs bind to P-selectin or E-selectin. In an alternative embodiment at least 25%, 50%, 75%, 90%, or 95% (or any percentage inclusive) of the treated HSCs of the blood

product bind to P-selectin or E-selectin. In the blood product, the quantity of HSCs may be derived from human umbilical cord blood, peripheral blood, or bone marrow.

[0083] While the invention has been described above in connection with various embodiments so that aspects thereof may be more fully understood and appreciated, it is not intended to limit the invention to these particular embodiments. On the contrary, it is intended to cover all alternatives, modifications and equivalents as may be included within the scope of the invention as defined by the appended claims. Thus the previous examples will serve to illustrate the practice of this invention, it being understood that the particulars shown are by way of example and for purposes of illustrative discussion of the present invention only and are presented in the cause of providing what is believed to be the most useful and readily understood description of procedures as well as of the principles and conceptual aspects of the invention.

[0085] The scope of the claims should not be limited by the preferred embodiment and examples, but should be given the broadest interpretation consistent with the description as a whole.

CLAIMS:

1. A method of enhancing the binding to P-selectin or E-selectin of umbilical cord blood hematopoietic stem cells (HSCs) which have impaired binding to P-selectin or E-selectin, comprising:
5 treating a quantity of CD34⁺ CD38^{low/-} HSCs, at least a portion of which have impaired binding to P-selectin or E-selectin and are characterized by P-selectin glycoprotein ligand-1 (PSGL-1) lacking α 1,3 fucose, *in vitro* with an α 1,3-fucosyltransferase and a
10 fucose donor forming fucosylated HSCs, wherein the fucosylated HSCs have enhanced binding to P-selectin or E-selectin.
2. The method of claim 1, wherein a portion of the untreated quantity of HSCs has reduced bone marrow homing ability.
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3. The method of claim 1, wherein a portion of the untreated quantity of HSCs express PSGL-1 which has unfucosylated glycans or unfucosylated O-glycans.
- 20 4. The method of claim 1, wherein in a portion of the untreated quantity of HSCs expresses PSGL-1 having core-2 O-glycans comprising NeuAc α 2,3Gal β 1,4GlcNAc and which are absent a fucose in α 1,3 linkage to the GlcNAc or which comprise other glycans which lack proper fucosylation.
- 25 5. The method of claim 1, wherein in the step of treating the quantity of HSCs, at least 50% of the treated HSCs have P-selectin binding fluorescence which exceeds a predetermined fluorescence threshold in a P-selectin binding assay or which have E-selectin binding fluorescence which exceeds a predetermined fluorescence threshold in an E-selectin binding assay.
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6. The method of claim 1, wherein in the step of treating the quantity of HSCs, the α 1,3 fucosyltransferase is α 1,3 fucosyltransferase IV; α 1,3 fucosyltransferase VI; or α 1,3 fucosyltransferase VII.

7. The method of claim 1, wherein in the step of treating the quantity of HSCs, the fucose donor is GDP-fucose.

5 8. The method of any one of claims 1-7, wherein the untreated HSCs are derived from human umbilical cord blood.

9. The method of claim 8, wherein the human umbilical cord blood is an unfractionated quantity of human umbilical cord blood.

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10. A method of enhancing the binding to P-selectin or E-selectin of umbilical cord blood hematopoietic stem cells (HSCs) which have impaired binding to P-selectin or E-selectin, comprising:

15 treating a quantity of CD34⁺ CD38^{low/-} HSCs comprising PSGL-1 having core-2 O-glycans comprising NeuAc α 2,3Gal β 1,4GlcNAc which lack fucose in α 1,3 linkage to the GlcNAc and which have impaired binding to P-selectin or E-selectin *in vitro* with an α 1,3-fucosyltransferase and a fucose donor to produce fucosylated HSCs wherein at least 10% of the fucosylated HSCs bind to P-selectin or E-selectin.

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11. The method of claim 10, wherein the quantity of HSCs are disposed within a pharmaceutically acceptable carrier, diluent or vehicle.

25 12. The method of claim 10 or 11, comprising using a free fucosyltransferase or a fucosyltransferase bound to a support to treat the HSCs.

13. The method of any one of claims 10-12, wherein the quantity of HSCs is derived from human umbilical cord blood.

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14. The method of any one of claims 10-13, wherein at least 25% of the fucosylated HSCs bind to P-selectin or E-selectin.

15. The method of any one of claims 10-14, wherein at least 50% of the fucosylated HSCs bind to P-selectin or E-selectin.

5 16. The method of any one of claims 10-15, wherein at least 75% of the fucosylated HSCs bind to P-selectin or E-selectin.

17. The method of any one of claims 10-16, wherein at least 90% of the fucosylated HSCs bind to P-selectin or E-selectin.

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18. The method of any one of claims 10-17, wherein at least 95% of the fucosylated HSCs bind to P-selectin or E-selectin.

19. A method of enhancing the binding to P-selectin or E-selectin of bone marrow or peripheral blood hematopoietic stem cells (HSCs) which have impaired binding to P-selectin or E-selectin, comprising:

15 treating a quantity of bone marrow CD34⁺ CD38^{low/-} HSCs or peripheral blood CD34⁺ CD38^{low/-} HSCs, at least a portion of which have impaired binding to P-selectin or E-selectin and are characterized by P-selectin glycoprotein ligand-1 (PSGL-1) lacking α 1,3 fucose in vitro with an α 1,3-fucosyltransferase and a fucose donor forming fucosylated HSCs, wherein the fucosylated HSCs have enhanced binding to P-selectin or E-selectin.

20 20. The method of claim 19, wherein at least a portion of the quantity of HSCs has reduced bone marrow homing ability.

21. The method of claim 19 or 20, wherein a portion of the quantity of HSCs express PSGL-1 which has unfucosylated glycans or unfucosylated O-glycans.

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22. The method of claim 19 or 20, wherein a portion of the quantity of HSCs expresses PSGL-1 having core-2 O-glycans comprising

NeuAc α 2,3Gal β 1,4GlcNAc and which are absent a fucose in α 1,3 linkage to the GlcNAc or which comprise other glycans which lack proper fucosylation.

23. The method of claim 19 or 20, wherein at least 50% of the treated
5 fucosylated HSCs have P-selectin binding fluorescence which exceeds a predetermined fluorescence threshold in a P-selectin binding assay or which have E-selectin binding fluorescence which exceeds a predetermined fluorescence threshold in an E-selectin binding assay.

10 24. The method of claim 19 or 20, wherein the α 1,3 fucosyltransferase is α 1,3 fucosyltransferase IV; α 1,3 fucosyltransferase VI; or α 1,3 fucosyltransferase VII; and is free or is bound to a support.

15 25. The method of claim 19 or 20, wherein the fucose donor is GDP-fucose.

26. The method of any one of claims 19-25, wherein at least 10%, 25%, 50%, 75%, 90%, or 95% of the fucosylated HSCs bind to P-selectin or E-selectin.

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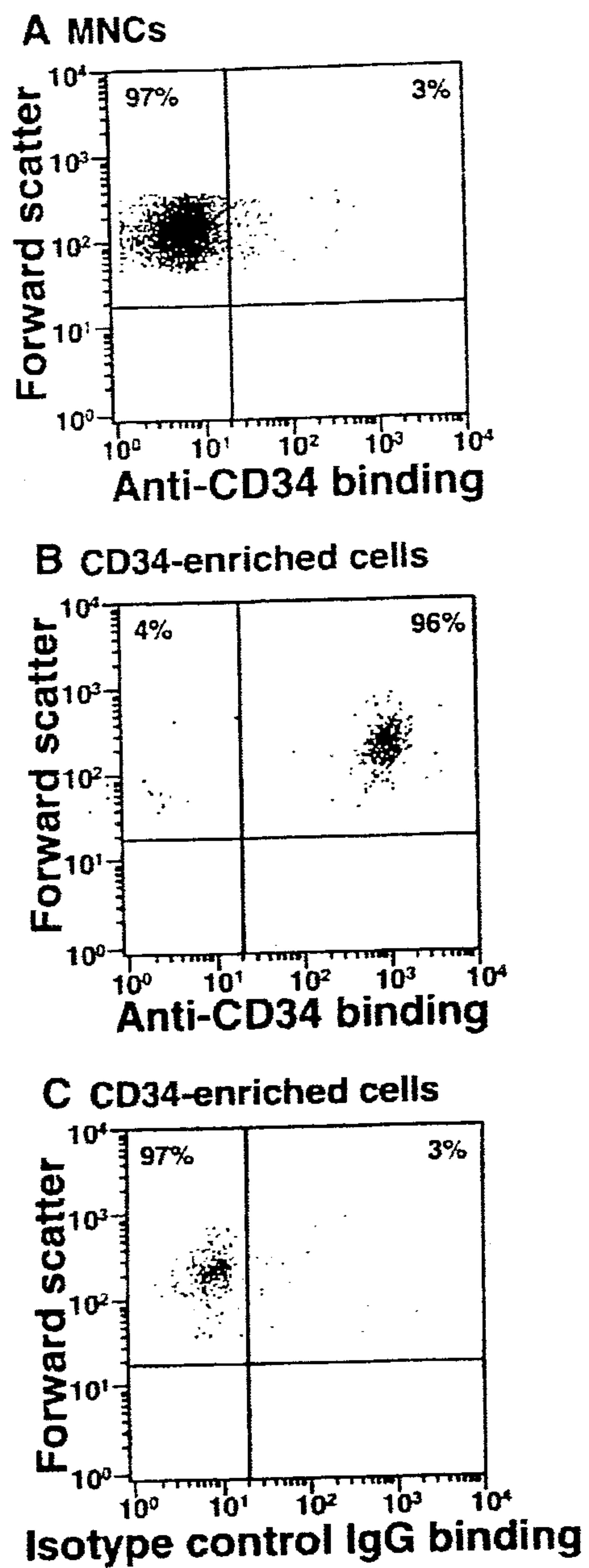


Figure 1

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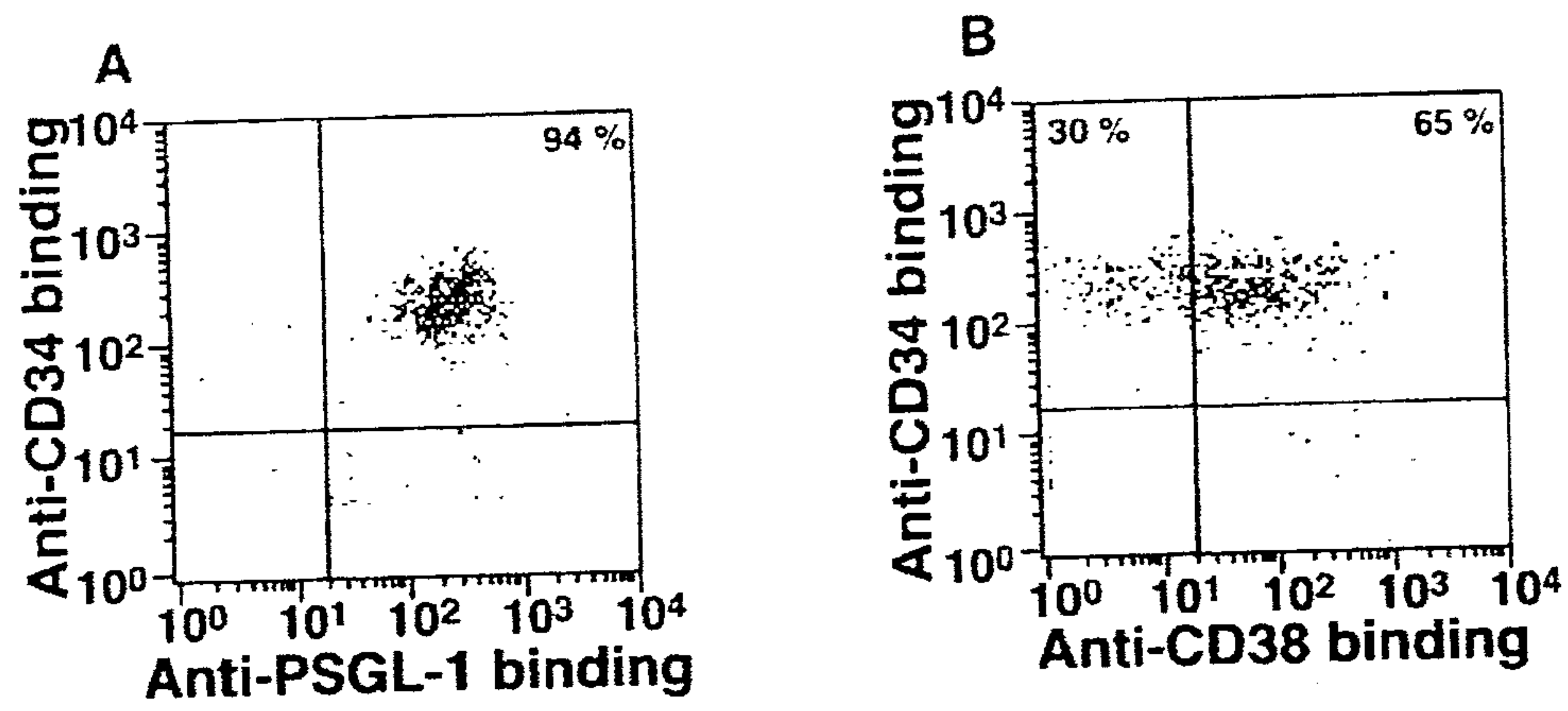


Figure 2

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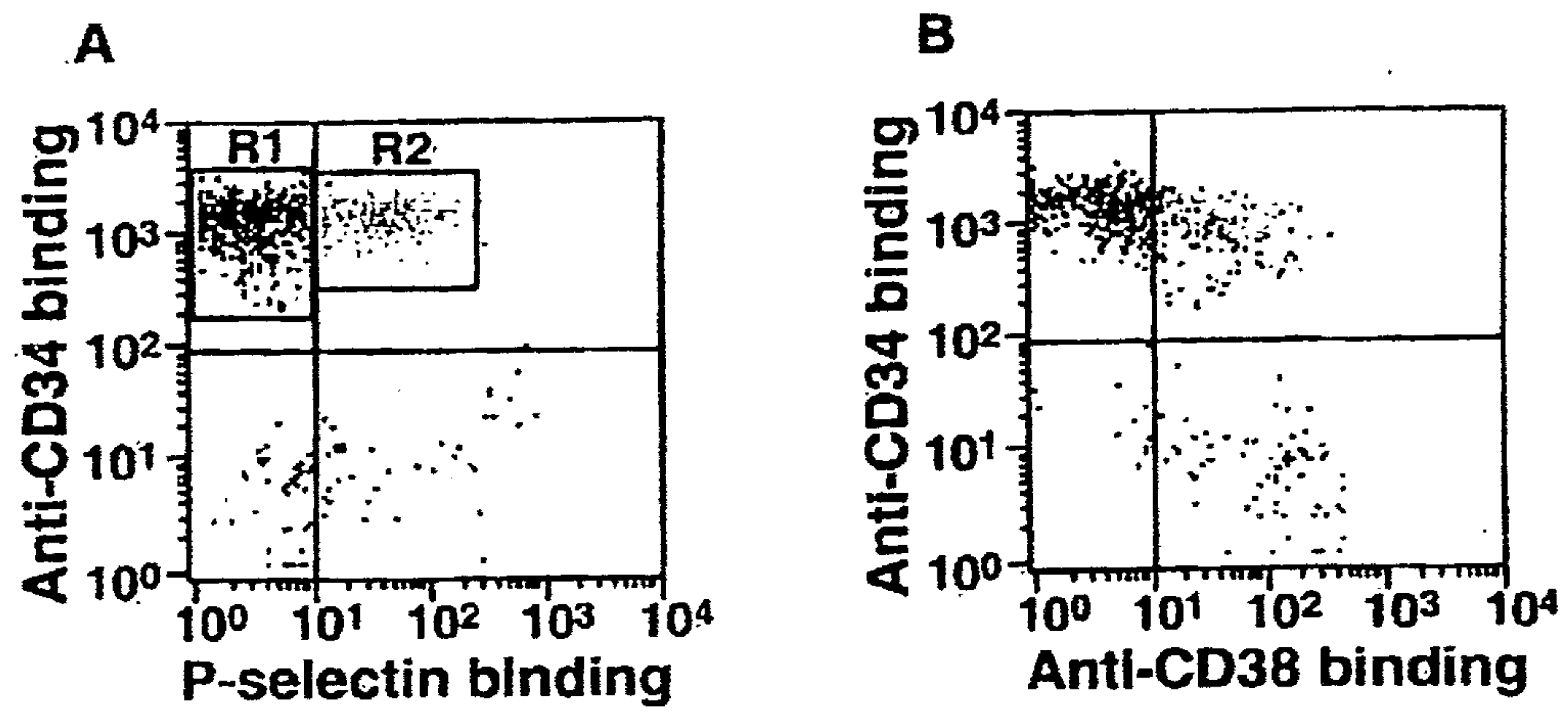


Figure 3

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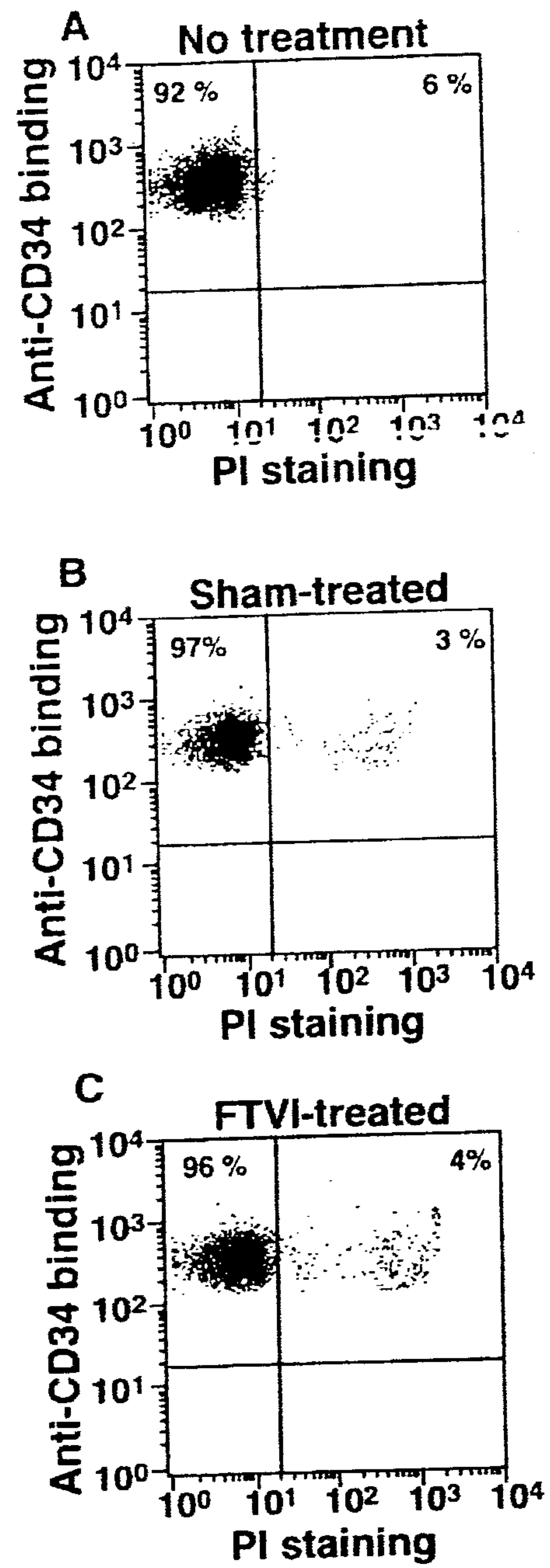


Figure 4

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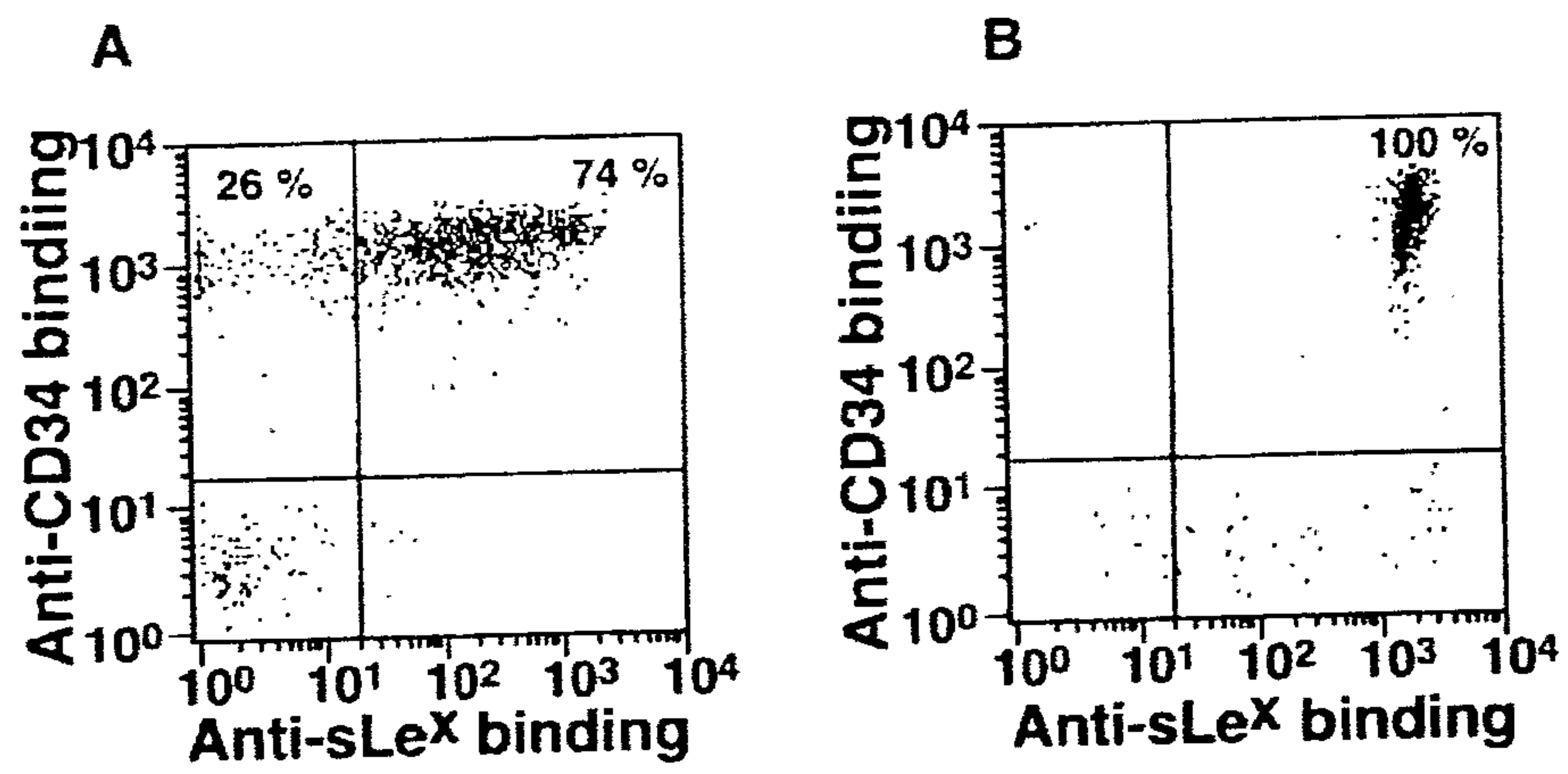
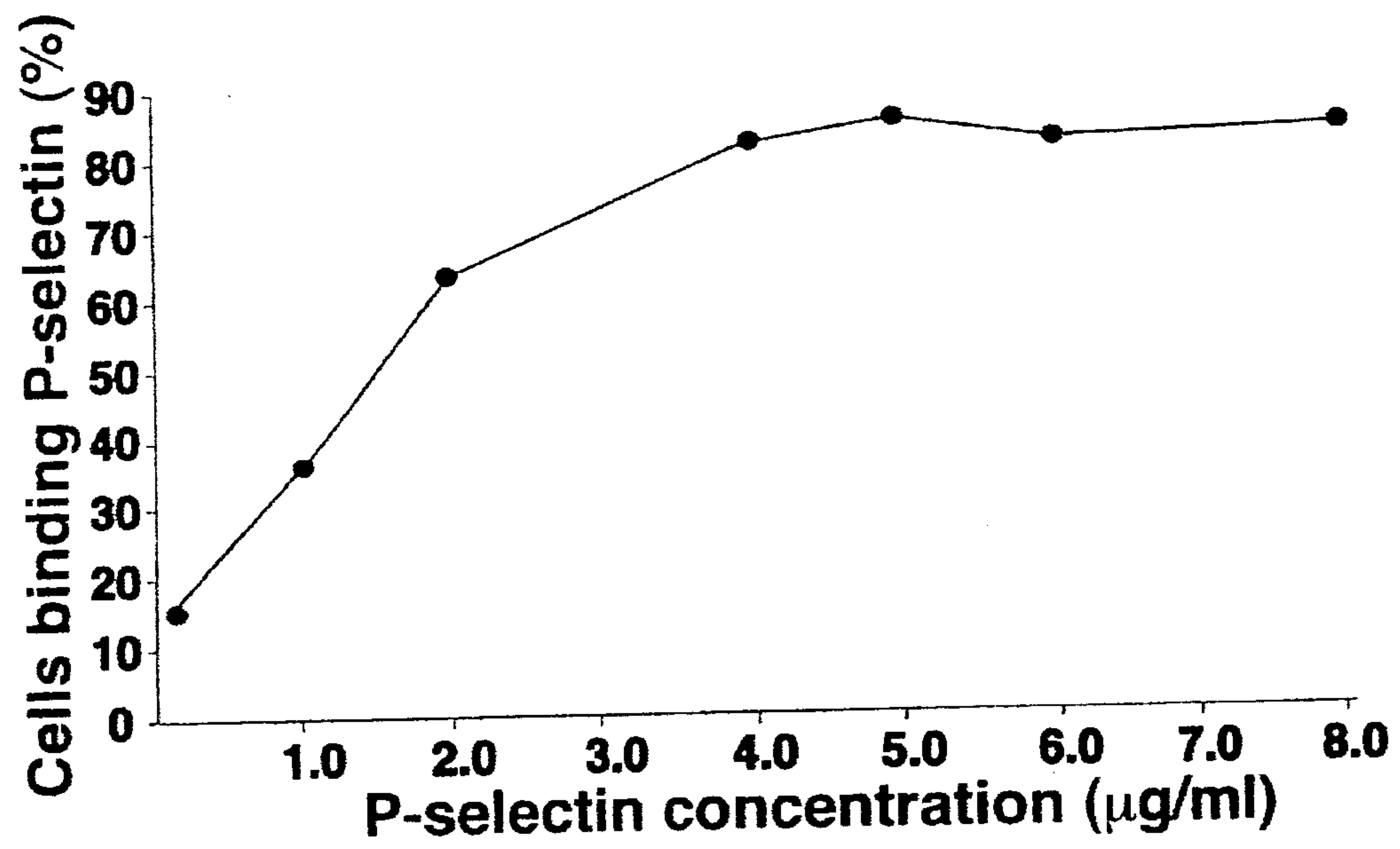


Figure 5

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**Figure 6**

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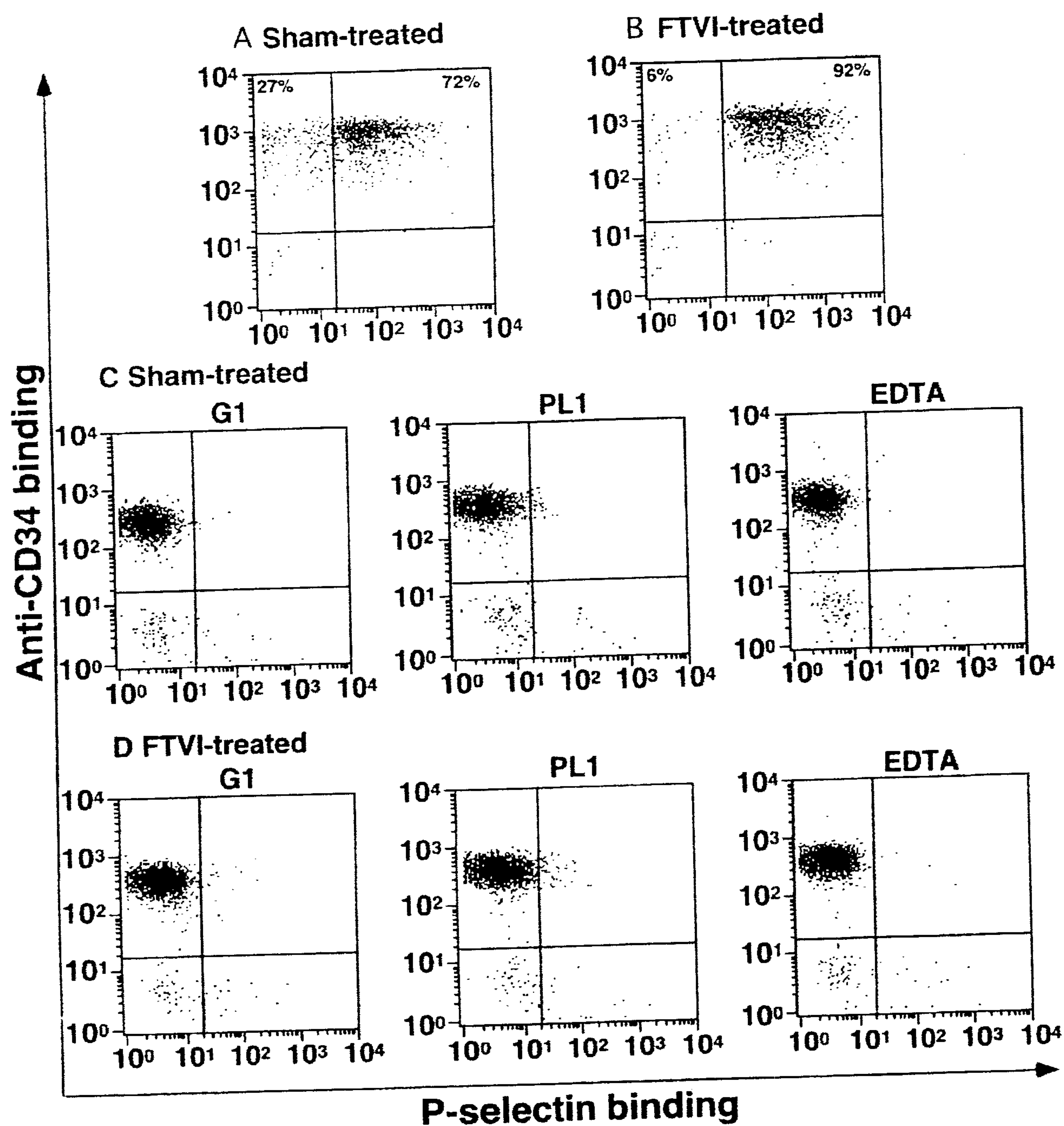
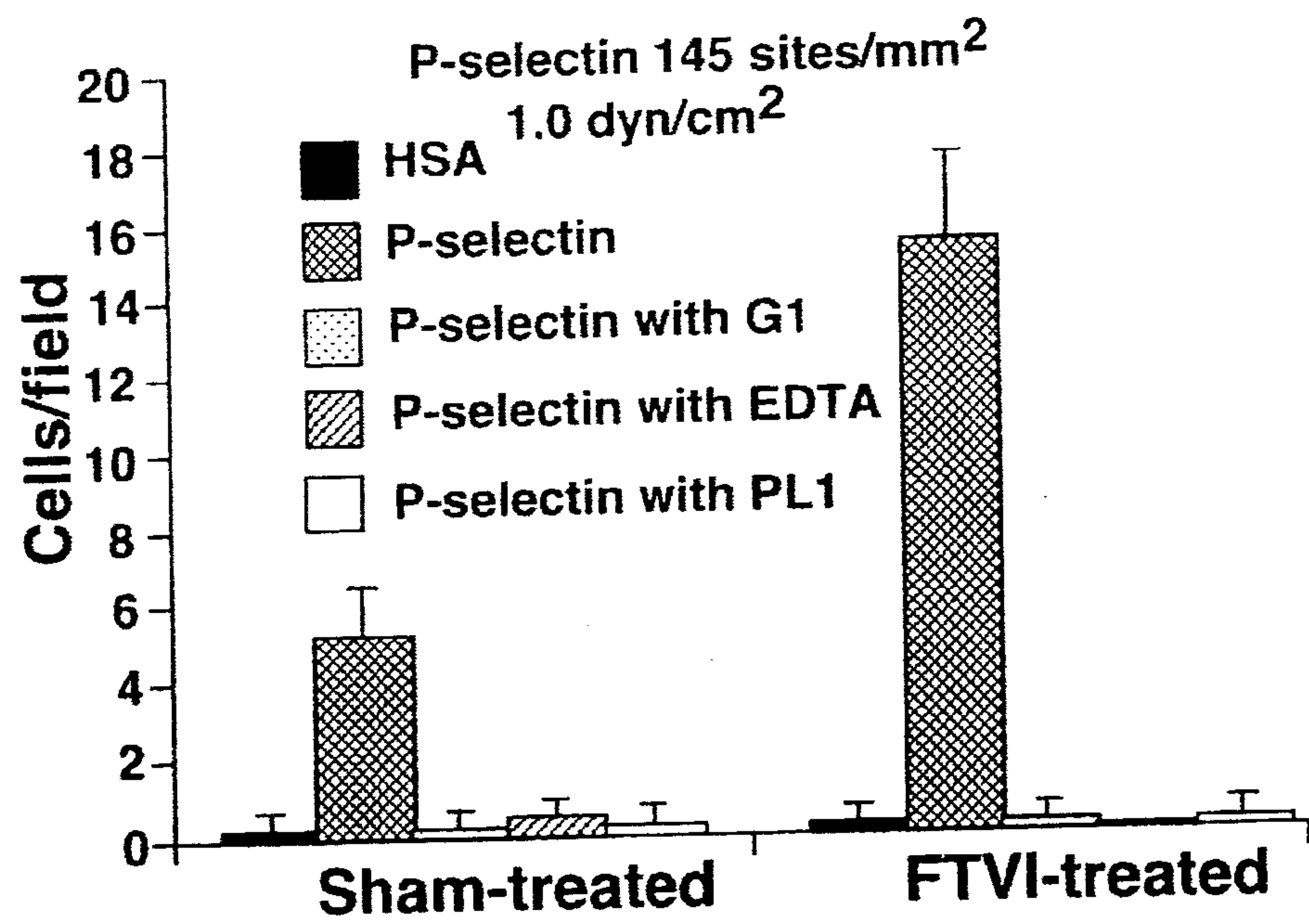


Figure 7

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**Figure 8**

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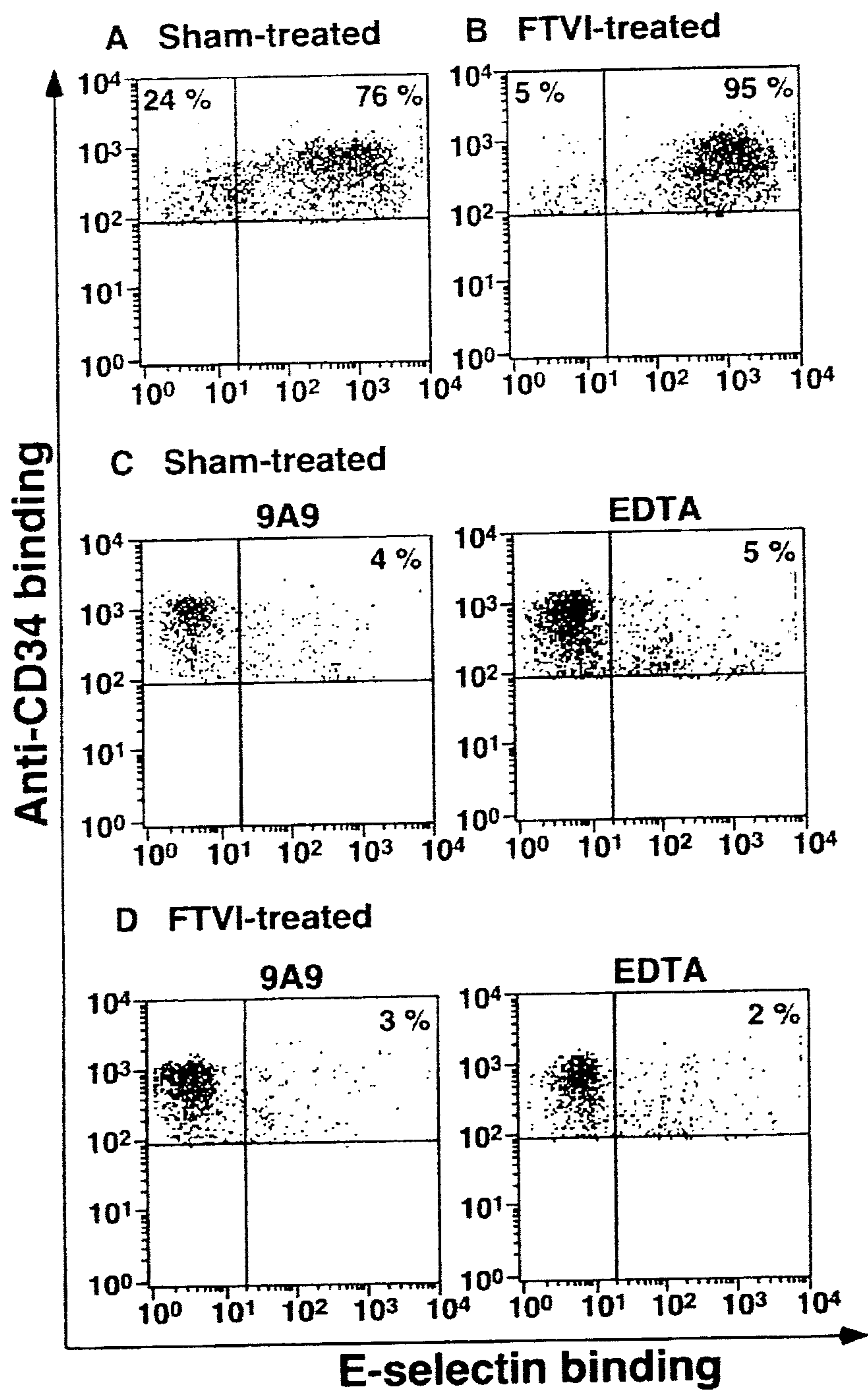


Figure 9

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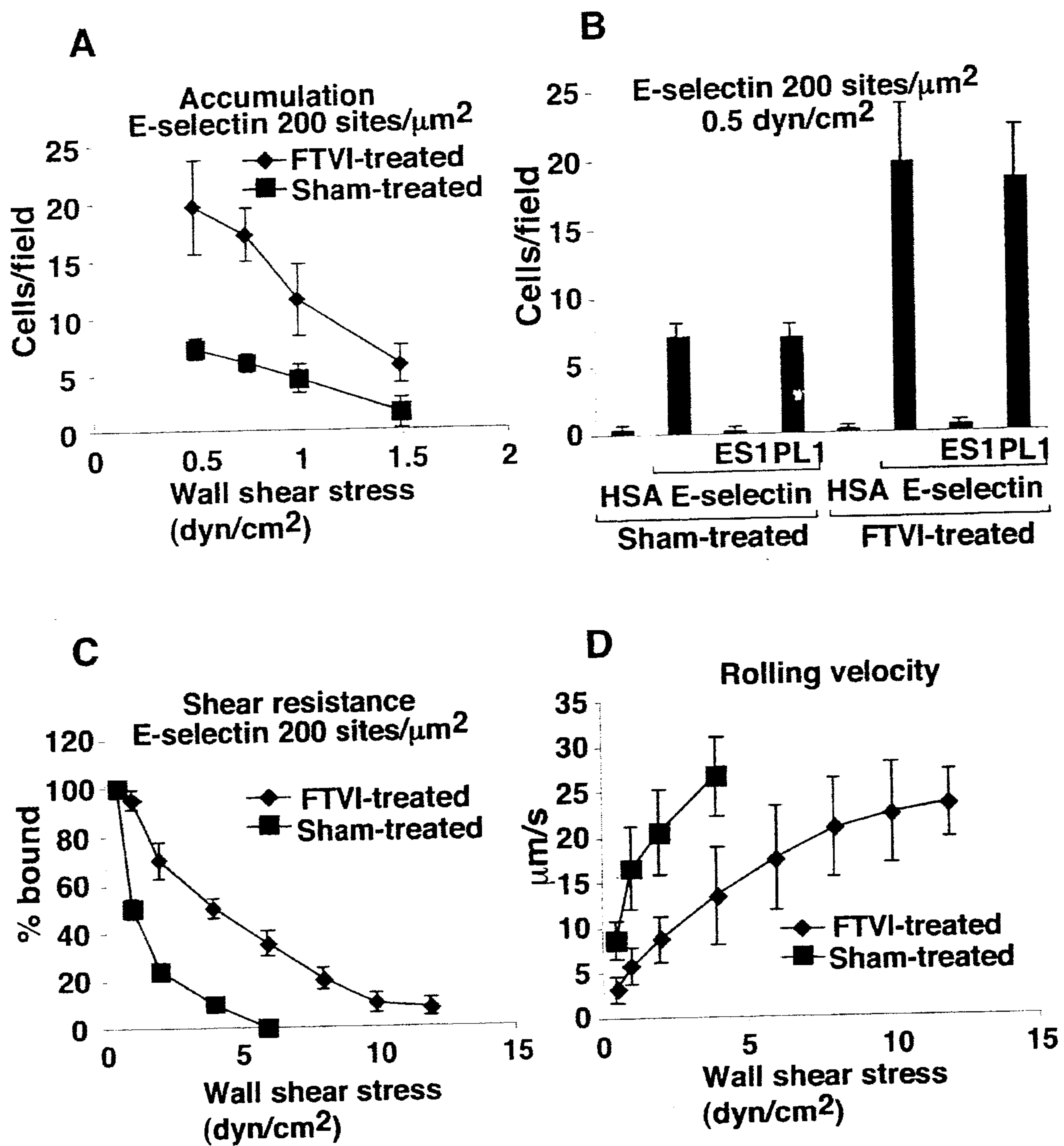


Figure 10

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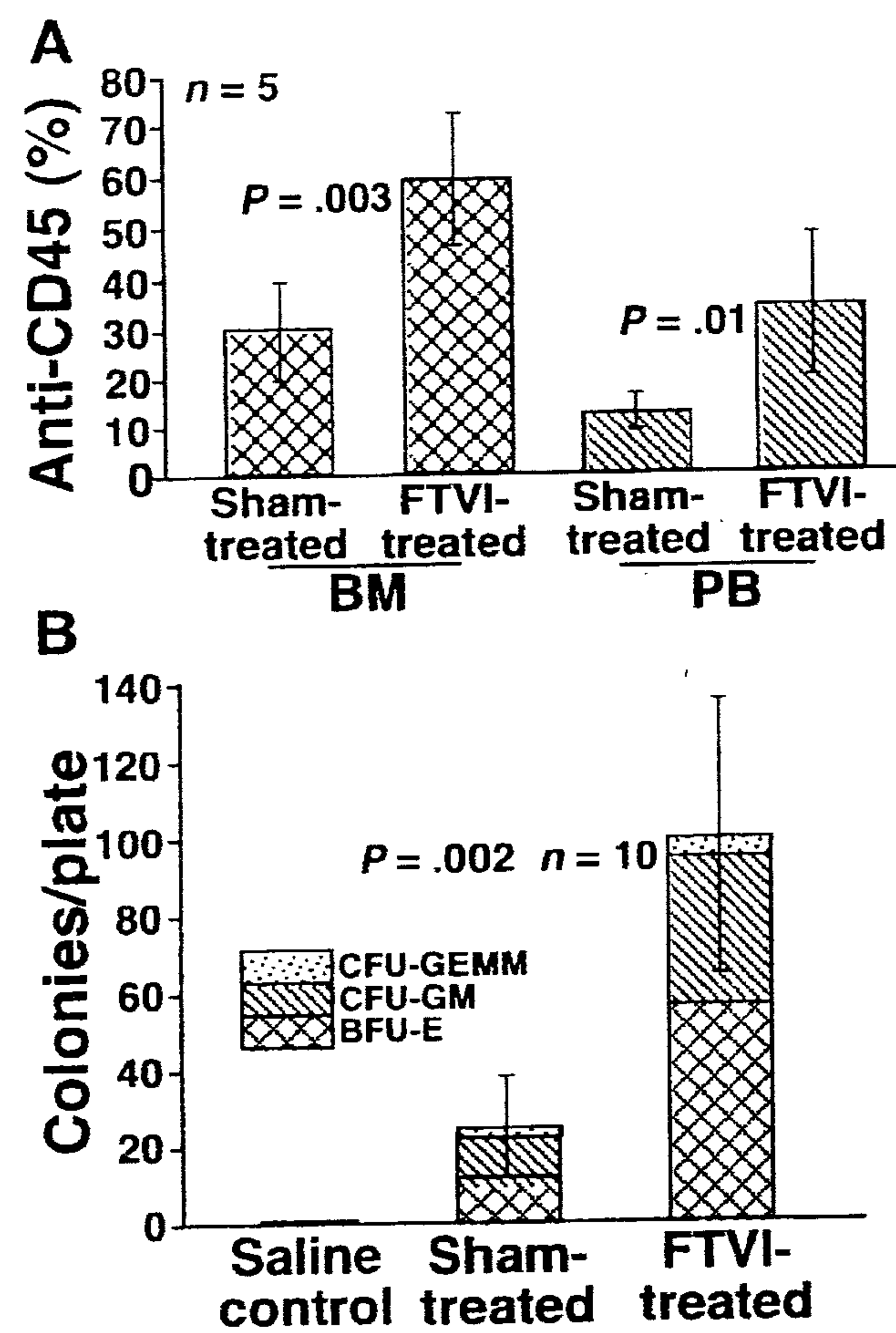


Figure 11