

Magnetic Separation of Human Podocalyxin-like Protein 1 (hPCLP1)-Positive Cells from Peripheral Blood and Umbilical Cord Blood Using Anti-hPCLP1 Monoclonal Antibody and Protein A Expressed on Bacterial Magnetic Particles

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ABSTRACT. Hemangioblasts are common progenitors of hematopoietic and angiogenic cells, which have been demonstrated in the mouse to possess a unique cell surface marker, podocalyxin-like protein 1 (PCLP1) (Hara, T. *et al.*, *Immunity*, 11: 567–578, 1999). In this study, we prepared a novel monoclonal antibody against human PCLP1 (hPCLP1) and attempted to isolate human hematopoietic progenitor cells from umbilical cord blood and peripheral blood using nano-sized bacterial magnetic particles (BacMPs) coupled with the anti-hPCLP1 antibody. Flow cytometric analysis demonstrated that the purity of separated hPCLP1-positive cells from peripheral blood was approximately 95% whereas peripheral blood mononuclear cells contained only 0.1% PCLP1⁺ cells. Umbilical cord blood was demonstrated to be a better source for PCLP1⁺ cells than peripheral blood. These results suggest that the separation of human PCLP1⁺ cells using BacMPs with anti-hPCLP1 were extremely effective and may be useful as a means to prepare human hematopoietic progenitor cells.

Key words: hematopoietic progenitor cells/podocalyxin-like protein 1 (PCLP1)/bacterial magnetic particles (BacMPs)/cell separation

Introduction

Hematopoietic progenitor cells are widely useful for medical applications such as regenerative medicine and transplantation. Successful use of hematopoietic progenitor cells requires cell surface markers to correctly identify and purify them. As the CD34 antigen has been widely used as a hematopoietic progenitor cell marker, it has been established that CD34⁺ cells are employed for transplantation (Losordo *et al.*, 2007). Recently, CD133 was also shown to be a hematopoietic progenitor cell marker (Yin *et al.*, 1997)

and has been used to isolate hematopoietic progenitor cells using anti-CD133 antibody (Freund *et al.*, 2006; Gordon *et al.*, 2003). However, few markers are available except CD34 and CD133 for the separation of hematopoietic progenitor cells from peripheral blood, umbilical cord blood, or bone marrow. Therefore, the development of novel antibodies as new tools for separation of hematopoietic progenitor cells is required.

Podocalyxin or podocalyxin-like protein 1 (PCLP1, also called Myb-Ets progenitor antigen 21 [MEP21], and thrombomucin) was originally shown to be a major component of the cell coat or glycocalyx of the rat podocytes (Kerjaschki *et al.*, 1984). PCLP1 is a type 1 transmembrane protein, and normally expressed on hematopoietic progenitors, vascular endothelia, and kidney podocytes (Kerjaschki *et al.*, 1984; Kershow *et al.*, 1995, 1997; McNagny *et al.*, 1997). In the mouse, PCLP1 has been demonstrated to be a cell surface marker for hemangioblasts (Hara, 1999). PCLP1 belongs to a large family of cell surface sialomucins and are related to CD34 and endoglycan (Kerjaschki *et al.*, 1984;

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Somasiri *et al.*, 2004). The expression of podocalyxin has been shown to block hematopoietic and epithelial cell adhesion and cell-cell contact (Takeda *et al.*, 2000). In humans, PCLP1 expression decreases during hematopoietic cell maturation, and PCLP1 is likely a marker for a subset of these stem cells (Kerosuo *et al.*, 2004). However, the function of PCLP1 in human hematopoietic cells has yet to be clearly understood because PCLP1⁺ cells are a minor component of mononuclear cells and therefore the isolation of these cells is difficult. Effective purification strategies for PCLP1⁺ cells warrant the potential use of these cells for stem cell therapy.

Separating target cells from heterogeneous mixtures of various cell types such as blood samples is widely used in a variety of biological and medical applications. Among the current cell separation techniques, magnetic cell separation is a popular tool for target cell isolation from cell suspensions. Usage of immunomagnetic particles has been proven to be more convenient as a tool for rapid and simple isolation of specific cell types than fluorescence-activated cell sorting (FACS) (Cobbold *et al.*, 2005; Kuhara *et al.*, 2004). Nano-sized magnetic particles provide numerous advantages including assay sensitivity, efficiency, precision and conferring no toxicity to the cells (Kim *et al.*, 2006). However, manipulation of the magnetic nanoparticles is complicated because the particles have only weak magnetic force, which interferes with effective isolation.

The magnetic bacterium, *Magnetospirillum magneticum* AMB-1, contains intracellular particles of magnetite (Fe₃O₄), known as magnetosomes, which are aligned in chains. These bacterial magnetic particles (BacMPs) of 50–100 nm in diameter have a single magnetic domain of magnetite exhibiting strong ferrimagnetism and are developed with a stable lipid bilayer. The molecular mechanism of BacMPs synthesis is a multi-step process, including vesicle formation, iron transport, and magnetite crystallization (Spring and Schleifer, 1995; Schüller, 1999). Recent molecular studies have uncovered these steps of BacMPs synthesis (Okamura *et al.*, 2001; Matsunaga *et al.*, 2000; Nakamura *et al.*, 1995). Several proteins localized on or in the BacMPs membranes have been isolated and analyzed in AMB-1. Furthermore, functional proteins have been expressed on BacMP surfaces. BacMP-specific proteins were used as anchor proteins, which localized and oriented functional proteins on BacMPs. To date various proteins, including protein A, estrogen receptor, and dopamine receptor have been displayed onto BacMPs (Yoshino and Matsunaga, 2006; Yoshino *et al.*, 2004). Currently, BacMPs displaying protein A (protein A-BacMPs) have been used for cell separation (Kuhara *et al.*, 2004; Matsunaga *et al.*, 2006). In these experiments, flow-cytometric analysis showed that target cells were successfully isolated to more than 95% purity. The strong magnetism and good dispersion of BacMPs not only allowed us to obtain samples with high purity, but also to improve the separation efficiency of rare cells from

peripheral blood. This magnetic separation system using protein A-BacMPs is easily adaptable to the other target cells by substituting an antibody to a cell specific marker protein.

In this study, we developed an efficient separation system to isolate hPCLP1⁺ cells from mononuclear cells in umbilical cord blood and peripheral blood. At first, monoclonal antibodies were raised against the human PCLP1, and were used to identify and isolate hPCLP1⁺ cells. Using BacMPs displaying protein A and anti-hPCLP1 antibodies, we demonstrated that hPCLP1⁺ cells were effectively purified from blood samples.

Materials and Methods

PCLP-1 expression

The hPCLP-1 polypeptide was amplified using the primers: 5'-GGATCCTCCGCCGACCGCA-3' and 5'-CTCGAGGTGTGTG TCTTCCTCCTCATC-3'. PCR production was ligated in frame into the BamH I and XhoI sites of pcDNA3.1/Myc-His A (Invitrogen) which encodes a c-terminal Myc epitope and six His polypeptides. To include the MycHis tag, the reverse primer 5'-CTCGAGGTGT GTGCTTCCTCCTCATC-3', excluded the stop codon. CHO cells were transfected with pcDNA3.1/hPCLP1mH using Trans IT (PanVera, Madison, WI, USA) and maintained in F12 HAM containing 10% FCS (Sigma, St. Louis, MO, USA), G418 sulfate (Gibco, Grand Island, NY, USA) at final concentration of 700 µg/ml to established stable lines. The established cell line was verified by Western blotting with anti-Myc antibody.

Production of monoclonal antibodies to PCLP-1

Twenty-five µl of PBS and complete Freund's adjuvant (1:1) was injected into BALB/c mice, and transfectants expressing full length hPCLP1 were then injected into the mouse at 1, 4, and 7 days. Three days after the final injection, lymph-node cells removed from immunized mice and were fused with P3U1 myeloma cells at a ratio of 5 to 1 by polyethyleneglycol-400 procedure. Hybridoma supernatants were screened on transfectants expressing PCLP1 and cell line, HUVEC and MCF7, expressing endogenous PCLP1 by flow cytometric analysis. After cloning the hybridomas, the monoclonal antibodies were purified by protein G Sepharose column chromatography.

SDS-PAGE and Western Blotting

SDS-PAGE was performed according to the method of Laemmli with 10% polyacrylamide in the separation gel and 5% in the stacking gel (Laemmli, 1970). The electrophoresis buffer was 25 mM Tris with 20 mM glycine, pH 8.3. Sample was prepared by boiling for 5 min in Laemmli sample buffer with mercaptoethanol. The proteins were blotted to polyvinylidene difluoride membrane (Millipore, Bedford, MA, USA) by electrophoretic transfer. After

blocking with PBS containing 2% skim milk, the membrane was treated with anti-Myc monoclonal antibody for 1 hr at room temperature. Then, peroxidase-conjugated anti-mouse IgG (MBL, Nagoya, Japan) was added and incubated for 1 hr at room temperature. Finally, the enzyme activity was detected with enhanced chemiluminescence detection using ECL Western blotting detection reagents (Amersham Pharmacia Biotech).

Preparation of BacMP-antibody complexes

Expression plasmid, pUM13ZZ containing the synthetic protein A gene *EZZ* and the *mms13* fusion gene with the *mms16* promoter (Yoshino and Matsunaga, 2006) was transferred into a wild type AMB-1 by electroporation (Okamura *et al.*, 2003). Transformants were microaerobically cultured in magnetic spirillum growth medium (MSGM) containing 5 µg/mL ampicillin at 28°C. BacMPs expressing protein A (protein A-BacMPs) were purified from strain AMB-1 transformants, according to the method described by Tanaka and Matsunaga, 2000. The collected protein A-BacMPs were washed with HEPES at least 10 times with weak sonication and collection using a columnar neodymium-iron-boron (Nd-Fe-B) magnet. In order to prepare protein A-BacMPs binding with antibody, 500 µL of mouse anti-hPCLP1 IgG2a antibody (53D11, 1 mg/mL) was added to 500 µL of protein A-BacMPs suspension (2 mg/mL). Protein A binds strongly to the Fc region of mouse IgG2a. The complexes were separated by Nd-Fe-B magnet and unbound antibody was removed, then the complexes were washed three times with PBS. The BacMP-antibody complexes were stored at 4°C in PBS, 0.1% sodium azide until use.

Purification of mononuclear cells from umbilical cord blood

Human umbilical cord blood samples were provided by the Ina Central Hospital. All donors gave informed consent, and the study protocol was accepted by the ethical review board of the Ina Central Hospital and the Medical & Biological Laboratories Co., Ltd. Purification of mononuclear cells was performed according to the methods described by Ten *et al.* (Ten *et al.*, 1989). Umbilical blood collected into heparinized tubes containing 1000 U/mL heparin, mixed with hetasep (Stemcell Technologies), and incubated for 1 hr at room temperature. After erythrocytes were sedimented, the upper phase containing the leukocytes was carefully layered on one-third volume of Histopaque-1077 (Sigma) and centrifuged at 400×g for 30 min. The interface fractions containing mononuclear cells was transferred to a sterile tube, and washed twice with PBS containing 0.5% BSA and 2 mM EDTA. The mononuclear cells were incubated with FcR blocker (Miltenyi Biotec) at room temperature for 10 min to inhibit nonspecific reaction by Fc receptor in flowcytometric analysis. The cells were finally washed with PBS containing 0.5% BSA and 2 mM EDTA.

Purification of mononuclear cells from peripheral blood

Peripheral blood samples were provided by donors working in Medical & Biological Laboratories, Co. with informed consent. The study protocol was approved by the ethical review board of MBL. Purification of mononuclear cells from peripheral blood was performed. Peripheral blood was collected in heparinized tubes containing heparin, was mixed with twice the volume of PBS. Then it was layered on 1/2 volume of Histopaque-1077 (Sigma) and centrifuged at 400 g for 30 min to collect mononuclear cells at the plasma-Histopaque interface. The interface containing the mononuclear cell fraction was transferred to a sterile tube and washed twice with PBS containing 0.5% BSA and 2 mM EDTA. Mononuclear cells were incubated with FcR blocker (Miltenyi Biotec) at room temperature, for 10 min to prevent Fc receptor-mediated phagocytosis. Cells were washed with PBS containing 0.5% BSA and 2 mM EDTA.

Flow cytometry and cell sorting

For dual-color immunofluorescence analysis, we labeled anti-hPCLP1 antibody, 53D11, with fluorescein isothiocyanate (FITC) or biotin. Mononuclear cells (2×10^7 cells) were pre-incubated with anti-CD16 and anti-CD32 monoclonal antibody (Immunotech, Marseille, France) on ice for 10 min to reduce nonspecific reaction. Then, mononuclear cells (2×10^7 cells) were incubated with FITC conjugated anti-hPCLP1 monoclonal antibody and PE conjugated anti-CD34 monoclonal antibody (Immunotech). To sort of PCLP1⁺ cells, mononuclear cells (2×10^7 cells) were incubated with biotinylated anti-hPCLP1 monoclonal antibody and Streptavidin R-phycoerythrin (Invitrogen, Carlsbad, CA, USA). Flow cytometric analysis and sorting of PCLP1⁺ cells were performed using EPICS ALTRA (Beckman Coulter, Fullerton, CA). Sorted cells were re-analyzed by flow cytometer.

Magnetic separation of PCLP1 positive cells

A total of 2×10^7 mononuclear cells were used in the following experiments. Mononuclear cells (2×10^7 cells) were incubated with protein A-BacMPs binding with anti-hPCLP1 antibody at 4°C, for 10 min to separate specific cells magnetically, and subsequently reacted with FITC-labeled anti-mouse IgG antibody at 4°C for 10 min to analyze via flow cytometer. Cell suspension was transferred to the test tube (10 mm × 75 mm) and cells were magnetically collected by applying the magnetic field using a columnar neodymium-boron (Nd-B) magnet (diameter 22.5 mm, height 12.5 mm), which produced a uniform magnetic field (0.5 T at the surface). Magnetic separation was performed for 5 min. The magnetically separated cells were resuspended with PBS containing 0.5% BSA and 2 mM EDTA as the positive fraction. The positive fraction includes anti-hPCLP1 antibody-protein A-BacMP and stained with FITC-labeled anti mouse IgG. The supernatant, in the washing process, was analyzed as a negative fraction. Collected cell purity was analyzed with a FCM. A total of 10,000 events were analyzed for each sample.

Results

Production of antibodies against human PCLP1 expressed on the cell surface

The open reading frame encoding human PCLP1 was amplified from a human placenta cDNA library. CHO cells were transfected with an hPCLP1 cDNA expression vector and stably transfected cell lines were created. To confirm the hPCLP1 expression on CHO transfectants, cell lysates of the transfectants were examined by Western blotting. The myc-His-tagged hPCLP1 expression was detected using a monoclonal anti-Myc antibody. As shown in Fig. 1, three bands were detected between 80 kDa and 235 kDa. These results are generally similar to those previously reported for rat PCLP1 (Takeda *et al.*, 2000). The apparent molecular mass of hPCLP1 revealed by Western blotting appeared to be larger than that predicted for the hPCLP1 polypeptide because this protein is heavily glycosylated.

BALB/c mice were immunized with hPCLP1-expressing cells, after which hybridomas were produced by fusion of lymphocytes from immunized mice to mouse myeloma cells. After several rounds of screening by flow cytometric analysis, 13 hybridoma clones were obtained, each of which produced specific monoclonal antibodies reacting to hPCLP1 expressed on hPCLP1-transfectants. Among these hybridoma clones, 2 clones were found to produce antibodies that reacted to endogenous hPCLP1 on HUVEC, a human endothelial cell line. A monoclonal antibody, designated as 53D11, produced by one of these two clones was used for further experiments. The reactivity of 53D11 (mouse IgG2a antibody) to hPCLP1 on the transfectants was confirmed by flow cytometry (Fig. 2A). In addition, 53D11 was found to react to endogenous hPCLP1 expressed on MCF-7, a breast carcinoma cell line, which has been proved to express PCLP1 (Kerosuo *et al.*, 2004; Somasiri *et al.*, 2004) (Fig. 2B).

Flow cytometric analysis of hPCLP1 expression in mononuclear cells from umbilical cord blood (CBMC) and peripheral blood (PBMC)

The expression of hPCLP1 in human CBMC or PBMC was examined by immunofluorescence staining of mononuclear cells with FITC-conjugated anti-hPCLP1 monoclonal antibody (FITC-53D11) and PE-conjugated anti-CD34 monoclonal antibody. The results revealed that CBMC contained 1.1% of CD34⁺ cells and 1.2% of PCLP1⁺ cells (Fig. 3A), while PBMC contained 0.1% of CD34⁺ cells and 0.1% PCLP1⁺ cells (Fig. 3B), indicating that CBMC were rich in these hematopoietic progenitor cells including hemangioblasts as compared to PBMC. CD34⁺ PCLP1⁺ double positive cells were very rare, if any, in CBMC or PBMC. Analyzing 50,000 to 100,000 CBMC or PBMC, a few dots were uncertainly detected in the double positive fractions by

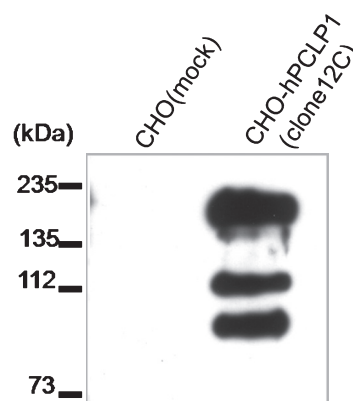


Fig. 1. Expression of Myc-His-tagged hPCLP1 in CHO cells. Stable transfectants of CHO cells were lysed, and resolved by 10% SDS-PAGE, after which hPCLP1 bands were detected by Western blotting using the anti-Myc monoclonal antibody.

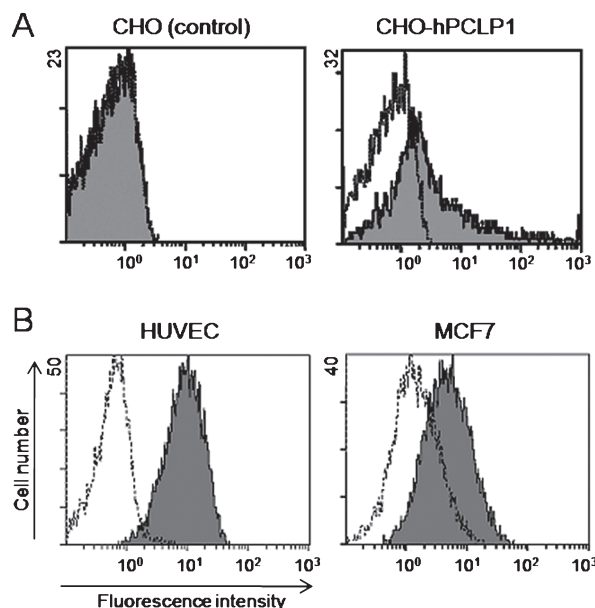


Fig. 2. Expression of hPCLP1 on CHO transfectants and two human cell lines, HUVEC and MCF7 as revealed by flow cytometry using 53D11. (A) 53D11 specifically bound to CHO hPCLP1-transfectant cells but not to control CHO cells were used as negative control. (B) 53D11 bound to endogenous PCLP1 on HUVEC cells and MCF7 cells. Binding of 53D11 was detected with FITC-conjugated anti-mouse IgG.

flowcytometry but sorting out these cells has yet to be accomplished.

Magnetic separation of PCLP1⁺ cells using protein A-BacMPs coupled with 53D11

PCLP1⁺ cells were isolated from mononuclear cells contained in blood samples by magnetic separation using

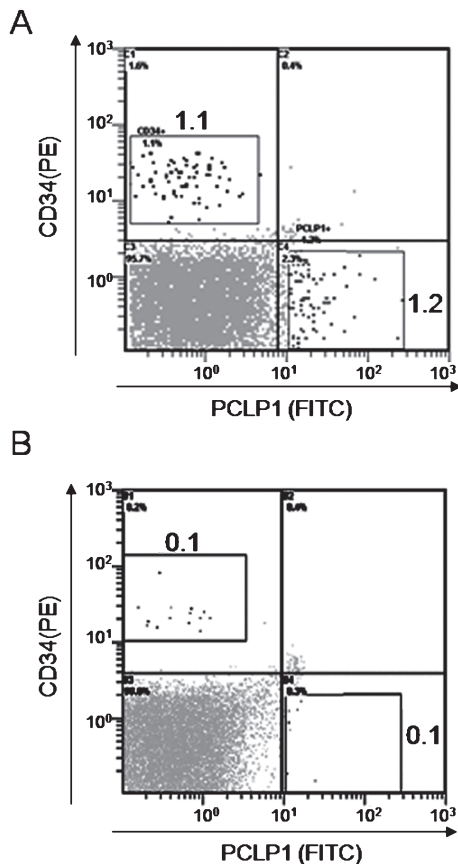


Fig. 3. PCLP1 expression in CBMC and PBMC. Double staining experiments of CBMC (A) or PBMC (B) using FITC-labeled anti-PCLP1 and PE-labeled anti-CD34 antibody. Percentages of PCLP1- or CD34-positive cells are indicated by numerals.

BacMPs, and compared to those isolated by the conventional FACS method. Mononuclear cells from cord blood were incubated with biotinylated anti-hPCLP1 monoclonal antibody (biotin-53D11), after which the cells were reacted with streptavidin-PE. Fluorescently labeled cell fractions were then separated by cell sorting and were subsequently examined by flow cytometry. On the other hand, PCLP1⁺ cells were separated using protein A-BacMPs bound with anti-hPCLP1 antibody (53D11). Cells separated by these two separation methods were subjected to flowcytometry so as to determine the purity of hPCLP1-positive cells. The results showed that the fractions separated by FACS were of $88.3 \pm 5.2\%$ purity and those separated by BacMPs were of $94.6 \pm 0.5\%$ purity (Fig. 4A). These results clearly indicated that hPCLP1⁺ cells could be efficiently separated from CBMC using protein A-BacMPs coupled with 53D11. Human PCLP1⁺ cells were also separated from PBMC by BacMPs, and were analyzed by flowcytometry (Fig. 4B). After magnetic separation, the separated fraction contained $95.4 \pm 3.9\%$ fluorescent cells. Each separation was performed

with 4 independent samples, and essentially reproducible results were obtained.

Finally, May-Grunwald-Giemsa stain of the separated cells by BacMPs was performed. The results revealed that the separated cells appeared to be homogeneous and had high nuclear/cytoplasm (N/C) ratio, indicating the typical staining pattern of stem cells (Fig. 5).

Discussion

Cell separation techniques of specific cells present as a minor population in biological samples are very important for biological and medical researches. These infrequent cells include various stem cells in tissues and circulating tumor cells in blood samples. Magnetic particle technology has been recognized as a simple, rapid and efficient separation means for these purposes. Effectiveness of the efficient separation is largely depends on the quality of the magnetic particles and the specificity of the antibody. In this study, we have developed a novel antibody to the hPCLP1 antigen expressed on the surface of human hematopoietic progenitor cells, and demonstrated that these cells were efficiently separated by using magnetic nanoparticles produced by bacteria with the hPCLP1 antibody.

In this study, we used human PCLP1 as a surface marker for hematopoietic progenitor cells. PCLP1 is a novel surface marker for endothelial-like cells in the aorta-gonad-mesonephros (AGM) region of mouse embryos, that contains long term repopulating hematopoietic stem cells (LTR-HSCs), and can be used as a source for preparing mouse hematopoietic and endothelial progenitor cells named hemangioblasts (Hara *et al.*, 1999). Macrophage colony-stimulating factor modulates the development of hematopoiesis by stimulating the differentiation of PCLP1⁺CD45⁻ cells to endothelial cells in the AGM region. In humans, previous studies suggested that PCLP1 may be a useful marker for a subset of hematopoietic stem cells (Kerosuo *et al.*, 2004). Here, we developed a monoclonal antibody against extracellular region of human PCLP1 (hPCLP1), termed 53D11. 53D11 robustly reacted to over-expressed hPCLP1 on CHP cells and to hPCLP1 endogenously expressed in HUVEC endothelial cells and MCF-7 breast carcinoma cells. To obtain antibodies that reacted to hPCLP1 expressing cells, we used hPCLP1-overexpressing cells as antigens so that the antibodies recognized the native form of hPCLP1. As expected, a monoclonal antibody, 53D11, was indeed reactive to cells that endogenously express hPCLP1 on their surfaces.

The remarkable developmental and replicative capacity of human stem cells promises an almost unlimited supply of cell types for transplantation therapies. There are various sources of stem cells like blastocysts, bone marrow, umbilical cord blood or peripheral blood. Stem cells from blastocysts, named embryonic stem (ES) cells, have attracted the

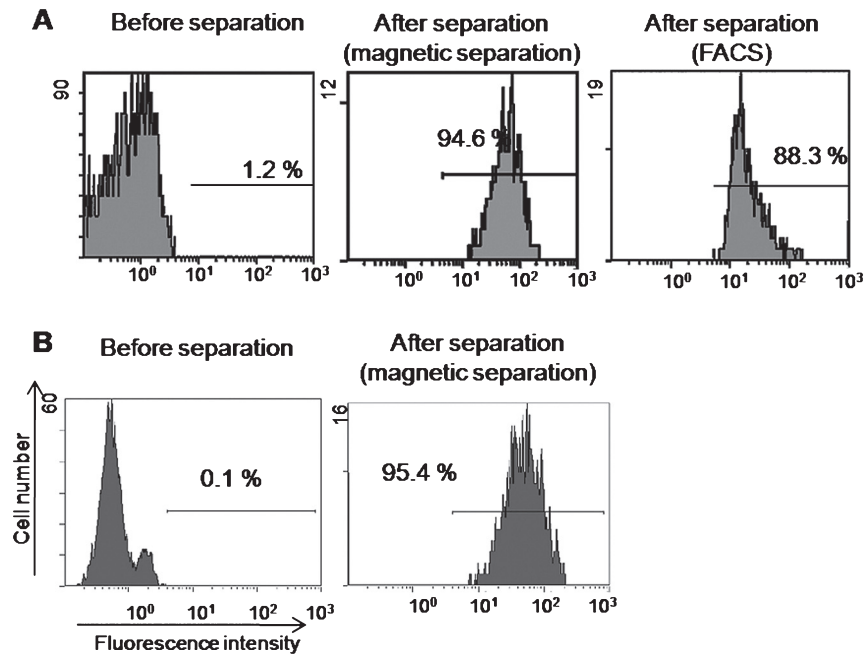


Fig. 4. Separation of PCLP1⁺ cells from CBMC and PBMC. (A) PCLP1⁺ cells were labeled with PE-conjugated 53D11 and separated from CBMC using cell sorter (FACS) or protein A-BacMPs coupled with 53D11(magnetic separation). Magnetically separated cells were detected by PE-conjugated anti-mouse IgG. (B) PCLP1⁺ cells were separated from PBMC using protein A-BacMPs coupled with 53D11 and detected by FITC-conjugated anti-mouse IgG.

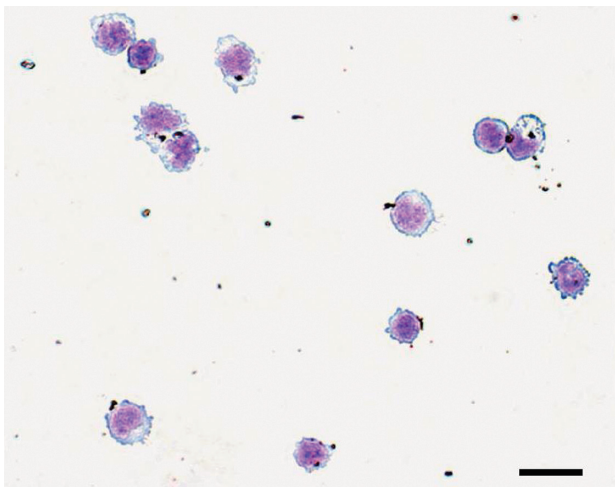


Fig. 5. Microscopic photographs of May-Grunwald-Giemsa-stained cells. PCLP1⁺ cells separated using protein A-BacMPs coupled with 53D11 from PBMC were subjected to air-dried cytocentrifuge, followed by staining with May-Grunwald-Giemsa. Scale bar : 20 μm.

most attention and are more pluripotent than other stem cells. However, these cells could not be used practically in cell therapy due to ethical concerns. In contrast, somatic stem cells are safer than ES cells and are currently used for cell transplantation, even with difficult and costly isolation and preparation.

Human cord blood has come to be widely used as a source of stem cells and its therapeutic application has grown steadily, as they are stored in increasing numbers of cord blood banks. Cord blood is a viable alternative to bone marrow for allogeneic hematopoietic progenitor cell transplantation (Barker *et al.*, 2001). In this study, we demonstrated the existence of hPCLP1 cells in CBMC (containing 1.1%) and separated PCLP1⁺ cells with a high purity from CBMC using anti-hPCLP1 antibody. These hPCLP1⁺ cells may be used as hematopoietic progenitor cells for therapeutic purposes in the future.

Another source of human stem cells is peripheral blood that is easily collected from adult tissues. Stem cells in PBMC are a very rare population, as compared with those in CBMC. In this study, PBMC contained 0.1% of hPCLP1⁺ cells, while CBMC contained 1.1% (Fig. 4). After magnetic separation using protein A-BacMPs, the positive fraction contained more than 95% of hPCLP1⁺ cells. These results suggest BacMPs are useful to magnetically separate hPCLP1⁺ cells from PBMC. We have performed *in vitro* colony assays with purified hPCLP1⁺ cells and found that these cells formed colonies including CFU-GM, CFU-mix and BFU-E when co-cultured with the murine OP9 stromal cell line (Riken Cell Bank, RCB1744) under specific conditions (M.S., T.O. and M.K. unpublished observations). We have initially detected relatively high levels of endotoxin (~4.4 EU/ml) in samples of BacMPs, but have removed endotoxin to the level as low as 0.17 EU/ml or lower that

does not affect biological assays using BacMPs (unpublished results).

In conclusion, a novel method for efficient separation of human PCLP1⁺ cells from CBMC and PBMC using protein A-BacMPs coupled with an anti-hPCLP1 antibody, 53D11 is reported. 53D11 specifically reacts with the intact PCLP1 cell surface protein. This is the first report showing the separation of human PCLP1⁺ cells from blood samples. These promising results illustrate that separation of PCLP1⁺ cells from blood could be a powerful and useful tool for various medical applications.

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Conflict of interest. This work was performed through a collaborating research agreement between Tokyo University of Agriculture and Technology and Medical & Biological Laboratories, Co. Ltd. M. K., M. S. and S. M. belong to MBL and T. O. belongs to ACTGen, Inc. Patents concerning hPCLP1 molecules revert to MBL and University of Tokyo.

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