Original Article

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Differentiation ability of multipotent hematopoietic stem/progenitor cells detected by a porcine specific anti-CD117 monoclonal antibody

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Summary **CD117 is a cytokine receptor expressed on the surface of hematopoietic stem cells with a likely role in cell survival, proliferation and differentiation. In order to study the differentiation activity of porcine CD117 hematopoietic cells *in vitro* and *in vivo* we prepared an anti-swine CD117 Mab (2A1) with high specificity for flow-cytometrical analysis. The 2A1 Mab did not recognize mouse or human mast cells suggesting that 2A1 is species-specific. Swine bone marrow (BM) CD117$^+$ cells differentiated *in vitro* mainly into erythroid and monocyte lineages in the methylcellulose-based colony assay. When the swine BM CD117$^+$ cells were transplanted *in vivo* into immunodeficient NOG (NOD/SCID/IL-2gc-null) mice, a significant amount of swine CD45$^+$ leukocytes, including CD3 positive T cells, were developed in the mice. These results revealed that the swine BM CD117$^+$ cells possess hematopoietic stem/progenitor activity and when monitored in immunodeficient mice or *in vitro* they can develop into lymphoid, erythroid, and myeloid cells efficiently with the new monoclonal antibody.**

Keywords: Swine, CD117, hematopoietic cell, monoclonal antibody, xeno-transplantation

1. Introduction

In transplantation studies, stem cells from various tissues such as from the heart or bone marrow have become an important alternative source to using donor organs or whole tissue for engraftment (*1-5*). The development of new reagents and methods are needed for the identification of stem cell markers and for monitoring their purification, transplantation and differentiation in foreign environments such as with the xeno-transplantation of stem cells between different animal species, because most of the tools today are cross-reactive monoclonal antibodies prepared against human antigens and not species specific (*6*). The swine has been an useful animal model for human medical science research and transplantation studies (*7*). For example, organ transplantation was extensively studied with major histocompatibility complex (MHC)-defined swine lines, alpha 1, 3-galactosyltransferase gene-knockout and/or human decay-accelerating factor transgenic swine (*8*). However, in recent years, experimental organ transplantation in swine is also gradually being replaced by stem cell transplantation involving hematopoietic and other tissue stem cells (*9-11*). Concomitant with the development of the swine stem cell transplantation research there is a continued need to produce stem cell marker-specific monoclonal antibodies (Mabs) (*6*).

Recently, a hematopoietic stem cell (HSC)-transplanted severe-immunodeficient mouse system (known as humanized mice) was extensively investigated for its efficacy of engraftment of foreign hematopoietic cells (*12*). Although this and other

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mouse systems have limitations, they have been used efficiently to clarify the development pathway of hematopoietic cells, the efficacy of vaccination or drugs (*13*), and an improved humanized hematopoietic mouse system, human leukocyte antigen (HLA)-expressing mice, was recently reported (*14,15*). However, other animal stem cell models such as those using non-human primates or the swine also may be useful for evaluating xeno-reconstituted systems.

CD117 is a cytokine receptor tyrosine kinase type III expressed on the surface of wide variety of tissue stem cells including HSC, mesenchymal stem cells and multipotent progenitors (*16*). This molecule is also known as mast/stem cell growth factor receptor, proto-oncogene c-Kit or tyrosine-protein kinase kit and when activated by binding to a stem cell factor may play a role in regulating cell survival, proliferation and differentiation. As a cell surface marker, CD117 can be detected by using specific antibodies to identify and monitor the hematopoietic/mesenchymal stem/progenitor cell types and their stage of differentiation and activity in mice, humans and non-human primates. We have studied the xeno-transplantation of hematopoietic stem/progenitor cells of human, common marmoset and mouse and found that the cellularity of the reconstituted immune system in the transplantation environment was different among these animals, suggesting a significant diversity of hematopoietic cell characteristics (*17-19*). Thus, the stem cells of swine may have unique and different developmental potential compared to the mouse and non-human primates. Alternatively, they may have similar characteristics to the human and potentially could be used in experimental transplantation models for studying the pathogenesis and treatments of some human diseases. In this regard, Le Guern *et al.* (*20*) reported on the effect of long-term engraftment of swine stem cell factor (SCF)-positive cells in immunodeficient mice. However, because there were no species-specific Mabs readily available against swine CD117, researchers have tended to use the stem cell factor as a detectable stem cell differentiation marker and not CD117 (*21*).

In this study, we prepared a porcine-specific CD117 Mab and constructed a xeno-transplantation system for the engraftment of swine $CD117^+$ hematopoietic progenitor cells into immunodeficient NOG (NOD/SCID/IL-2gc-null) mice. In addition, we studied the differentiation of the swine $CD117^+$ hematopoietic progenitor cells after transplantation into the mice recipients by flow-cytometrical analysis.

2. Materials and Methods

2.1. *Animals and cells*

Newborn triple hybrid swine ((Landrace x Large While) x Duroc) were purchased from a commercial hog firm in Gifu prefecture, Japan and used for collection of bone-marrow (BM) cells. NOD/Shi-scid, IL-2gc-null (NOD/SCID/gc-null; NOG) mice were provided by Central Institute for Experimental Animals (CIEA, Kawasaki, Japan) and kept under specific pathogen-free conditions. Experiments using mice were approved by the Institutional Committee for Animal Care and Use and performed at Tokai University following the University guidelines.

Newborn swine or adult Duroc pigs were sacrificed under anesthesia (0.02 mg/kg Medetomidine Domitor; Nihon Zenyaku Kogyo Co. Koriyama, Japan, 0.2 mg/kg Midazolam; Dormicum injection 10mg, Astellas, Tokyo, Japan, 0.04 mg/kg Butophanol; Vetophale, Meiji Seika Pharma Co. Tokyo, Japan, intramuscular administration). After the birth of the piglets, the umbilical cord blood (CB) cells were collected from the umbilical cords of the expelled uterus using a 5ml syringe with 21Gx1 1/2 needle. The cells were centrifuged on Lymphocepal (IBL Co. Takasaki, Japan) at 2,000 rpm for 30 min. Mononuclear cells (MNCs) were collected and the remaining red blood cells (RBCs) were lysed with low osmotic buffer (20 mM Tris-HCl, pH7.4, 0.15 M NH4Cl). Femurs of the BMs were taken from the newborn swine and the cells were released from the tissues. RBCs were lysed and the cell suspension, prepared as mentioned above, was suspended in RPMI1640 medium (Nissui, Tokyo, Japan) containing 10% (v/v) heat-inactivated fetal calf serum (FCS; SAFC Biosciences, Tokyo, Japan) and used for the cytometric analyses.

2.2. *Preparation of CD117 transfectant*

The cDNA sequence of the swine CD117 gene used in this study was based on Sus scrofa KIT mRNA sequence (AB250963). RNA was extracted from cells by Isogen (Nippon Gene Co. LTD., Tokyo, Japan) and reverse-transcribed to cDNA by using the Superscript system (Invitrogen, Carlsbad, CA). A 2966 bp portion of the cDNA sequence that corresponds to an extracellular domain of the protein was amplified by PCR using cDNA as a template and AccuPrime Pfx DNA Polymerase (Invitrogen, Tokyo, Japan). The set of forward and reverse primers for PCR amplification of the CD117 gene used were as follows:

F, 5'-TAGAATTCGGTCTCACCGGTCGCCACCATG AGAGGCGCTCGCCGCGCCTGGGATT-3',
R, 5'-ATGATATCGGTCTCGGATCCTCAGACGTCT TCGTGGACAAGCA-3'.

Modified S/MAR-based episomal vectors (*22*) expressing swine CD117 cDNA were transfected into HEK293 or a mouse lymphoma cell line, A20, by electroporation using Gene Pulser (Neon® invitrogen,

Oregon, USA) according to the manufacturer's instructions.

2.3. *Monoclonal antibody preparation*

We initially immunized BALB/c mice with mitomycin C (MMC, Kyowahakko-Kirin, Tokyo Japan)-treated swine cord blood (CB) MNCs (1×10^6 cells/animal). For booster treatments, MMC-treated A20 transfectant was used biweekly for 3-6 times with 4×10^5 cells/animal with each immunization. MMC (final, 0.04 mg/mL) was added to the culture and incubated at 37°C for 30 min in 5% CO_2. The serum antibody titers of immunized mice were checked by flow cytometry analyses using CD117 cDNA-transfected HEK293 cells as a source of antigen. After 4 days of the final boost, mice were sacrificed and splenocytes were fused with the mouse myeloma cell line, P3-X63-Ag8-U1, according to a standard procedure. Positive clones were identified by using flow cytometry or an Imaging Analyzer (Array Scan, Thermo scientific, MA, USA). Briefly, CD117 transfected HEK293 cells were plated into the wells of 96 well plates. Culture supernatants were added to each well, incubated for 15 min and washed twice. APC-labeled (APC: allophycocyanin) anti-mouse IgG polyclonal antibody (Poly4053; Bio Legend, San Diego, US) was added and incubated for 15 min. Plates were washed and stained with Hoechst (Invitrogen, Oregon, USA) for 30 min at room temperature and analyzed using the Imaging Analyzer. Positive cells were picked according to the fluorescent intensity of APC and the co-expressed mVenus fluorescent protein. The positive clones were then isolated, expanded and stocked.

2.4. *Preparation of primary murine and human mast cells*

Murine mast cells were prepared from the femurs of four- to six-week-old C57BL/6J mice. BM cells were cultured in RPMI1640 (Sigma-Adrich, St.Louis, US) supplemented with 10% (v/v) heat-inactivated FCS (SAFC Biosciences, Tokyo, Japan), and 4 ng/ml recombinant interleukin (IL)-3 (PeproTech, Rocky Hill, US). BM mast cells were used for experiments after they were cultured for six to eight weeks. We obtained approval of the ethical review boards to prepare primary human mast cells from CB-derived $CD34^+$ MNCs (RIKEN BioResource Center, Tsukuba, Japan). The $CD34^+$ cells were cultured in serum-free Iscove methylcellulose medium (Stem Cell Technologies Inc., Vancouver, BC, Canada) and Iscove modified Dulbecco medium containing SCF at 200 ng/mL, IL-6 at 50 ng/mL and IL-3 at 1 ng/mL as previously described (*23*). On day 42 of culture, methylcellulose was dissolved in phosphate-buffered saline (PBS) and the cells were resuspended and cultured in Iscove modified Dulbecco medium containing SCF at 100 ng/mL and IL-6 at 50 ng/mL with 2% FCS.

2.5. *Flow cytometry*

Cells were incubated with appropriately diluted, fluorescence-labeled primary Mab for 15 min at 4°C and washed with 1% (w/v) bovine serum albumin-containing PBS. In some cases, cells were re-incubated with labeled secondary antibody. The Mabs used were as follows: anti-mouse TER119-FITC (eBioscience, San Diego, CA), anti-swine CD45-FITC (clone # K252-1E4, AbD Serotec, Kidlington, UK), anti-swine CD3 (clone # 8E6, Monoclonal Antibody Center, WA, USA), anti-mouse CD117 (clone # 2B8, eBioscience) and anti-human CD117 (clone # YB5.B8, BD Bioscience), goat anti-mouse IgG1-RPE (Southern Biotech, Uden, Netherland) respectively. Cells were washed and further incubated with FITC-conjugated mouse anti-human IgE Mab (clone # BE5, eBioscience) on ice for 20 min. Stained cells were analyzed on FACS Calibur (Becton Dickinson, New Jersey, USA).

2.6. *Colony assay*

Newborn swine BM cells were washed and an aliquot was stained with 2A1 Mab as described above. After the staining with the Mab, cells were rewashed and sorted by employing a magnetic cell sorting system (AutoMACS, Miltenyi Biotec, Bergisch Gladbach, Germany) to separate $CD117^+$ cells from $CD117^-$ cells. The isolated cells were replated at 5×10^4/dish in 1 mL of methylcellulose-containing medium (StemCell technologies, Vancouver, Canada) in a 35 mm dish with mouse or human SCF (10 ng/mL), IL-3 (10 ng/mL), erythropoietin (2 U/mL) and granulocyte colony-stimulating factor (G-CSF) (10 ng/mL) and cultured at 37°C in a 5% CO_2 atmosphere. After 14 days of culture, the different types and numbers of hematopoietic colonies (colony-forming units (CFU)) were counted according to standard criteria. Samples from each animal were processed and assayed in triplicate with two different animals used for each of two separate experiments.

2.7. *Transplantation of swine hematopoietic cells into NOG mice*

$CD117^+$ cells were purified from newborn swine BMs by cell sorter as described above in section 2.6. Purity was more than 98% based on quantitation by flow cytometry. Nine-week-old NOG mice were irradiated with 2.5Gy X-ray prior to transplantation and swine cells were injected into mice intravenously (Suppl. Table 1, *http://www.biosciencetrends.com/docindex.php?year=2014&kanno=6*). Peripheral blood was collected *via* orbit under inhalation anesthesia at two and

four weeks after transplantation. MNCs were prepared and analyzed by flow cytomety.

2.8. *Transplantation of swine hematopoietic cells into NOG mice*

Student's *t*-test was performed, and data were expressed as mean ± S.D.

3. Results

3.1. *CD117 monoclonal antibody preparation*

The protocol used for CD117 monoclonal antibody preparation is shown in Suppl. Figure 1 (*http://www.biosciencetrends.com/docindex.php?year=2014&kanno=6*). First, swine CD117 cDNA was inserted into N576, an expression vector containing a monomer Venus yellow-green fluorescent protein (mVenus)-reporter gene, and the modified vector was transfected into the mouse lymphoma cell line A20 by electropolation. The expression of the mVenus gene was observed in more than 50% of the transfected cells 18-24 hr after transfection (Suppl. Figure 1A). Second, BALB/c mice were injected intraperitoneally initially with mitomycin C (MMC)-treated swine cord blood (CB) mononuclear cells (MNCs) and then with MMC treated N576-A20 cells as the booster immunizations (Suppl. Figure 1B). Third, the titer of the antiserum was checked against the CD117 expressed by the N576-HEK293 cells. The crossreactivity between N576-HEK293 and the antisera was confirmed by flow cytometry (Suppl. Figure 1C).

We selected a specific 2A1 subclone (Figure 1 and Suppl. Figure 2, *http://www.biosciencetrends.com/docindex.php?year=2014&kanno=6*) for further use because this clone secreted a Mab that reacted with the transfectants expressing CD117 and the mVenus fluorescent protein with similar or identical staining patterns (Figures 1B and 1C). All of the 2A1 subclones showed similar staining patterns, suggesting that they were stable expression products.

As CD117 is expressed on mast cells, we examined if the 2A1 Mab could also interact with the human and mouse CD117 molecules using cell lines derived from human or mouse primary mast cells. As shown in Figure 1D, neither human nor mouse mast cells were stained with 2A1. These results suggest that the 2A1 Mab is specific to swine CD117.

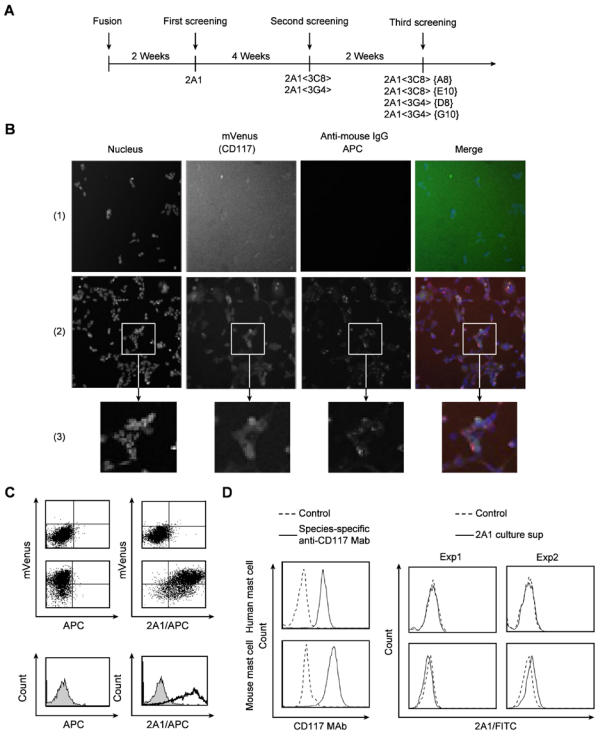

Figure 1. CD117-specific monoclonal antibody 2A1 specifically stains swine CD117. (A) Protocol for the cloning of swine CD117-specific Mab. Three screenings were performed after hybridoma fusion by limiting dilution of 2A1 hybridomas. **(B)** Imaging Analyzer image of the HEK293 parent cells **(1)** and CD117-HEK293 cells **(2)**. CD117 expression was monitored by mVenus expression. Screening of the hybridoma supernatants was performed by staining with the secondary antibody, anti-mouse IgG-APC. The encircled CD117-HEK293 cells in **(2)** are shown at a higher magnification (5×) in **(3)**. **(C)** The positive hybridoma supernatant submitted for the reactivity to the transfectant cells and analyzed by flow cytometry. The percentage of the cell numbers in the respective gates is shown in the quadrant panels. Control means the anti-mouse IgG-APC reactivity without the supernatants. **(D)** The reactivity of 2A1 Mab and species-specific anti-CD117 Mabs with human and mouse mast cells. As shown in the left panels, the human and mouse mast cells reacted with their respective species-specific anti-CD117 Mabs (anti-human CD117 or anti-mouse CD117 antibodies, respectively). Right panels; mast cells were stained with 2A1 and the dotted and solid curves are overlays of the control and 2A1 culture supernatants, respectively. Representative data of five independent assays are shown.

3.2. *CD117 expression in swine bone marrow (BM) cells*

To better clarify the existence of the swine CD117$^+$ cells in the lymphoid tissue, we examined their presence in the adult and newborn BMs, as hematopoietic stem/progenitor cells are abundant in the BM. Figure 2 shows that the newborn BM cells included a high level of 2A1-positive cells (ca.10%) compared to the ten fold lower levels (1.9%) in the adult BM cells. In addition, the ratio of CD117$^+$ cells is very low (0.5%) in the CB and no CD117$^+$ cells were detected in the spleen, lymph nodes and peripheral blood monocytes. These results suggest that CD117$^+$ cells and hematopoietic stem/progenitor cells exist in the swine bone marrow and other lymphoid tissues at a ratio that is comparable with human and mouse.

3.3. *Colony assay for the multipotency of CD117$^+$ cells*

To analyze the multipotency of CD117$^+$ BM cells *in vitro*, the swine BM MNCs were collected and stained

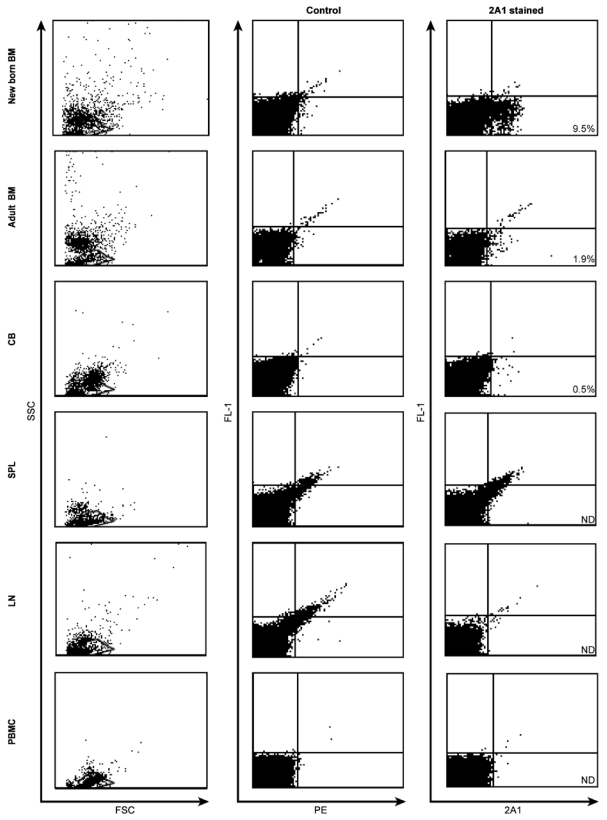

Figure 2. Abundance of swine CD117$^+$ cells in newborn BM relative to five other lymphoid tissue cells (adult BM, CB, SPL, LN and PBMC). Lymphoid tissue cells were stained with 2A1 Mabs. Control means the secondary antibody-stained cells. The percentage of 2A1 stained CD117$^+$ cells within the lymphoid-gate are shown in each panel. ND; not detected. Representative data of five independent assays are shown.

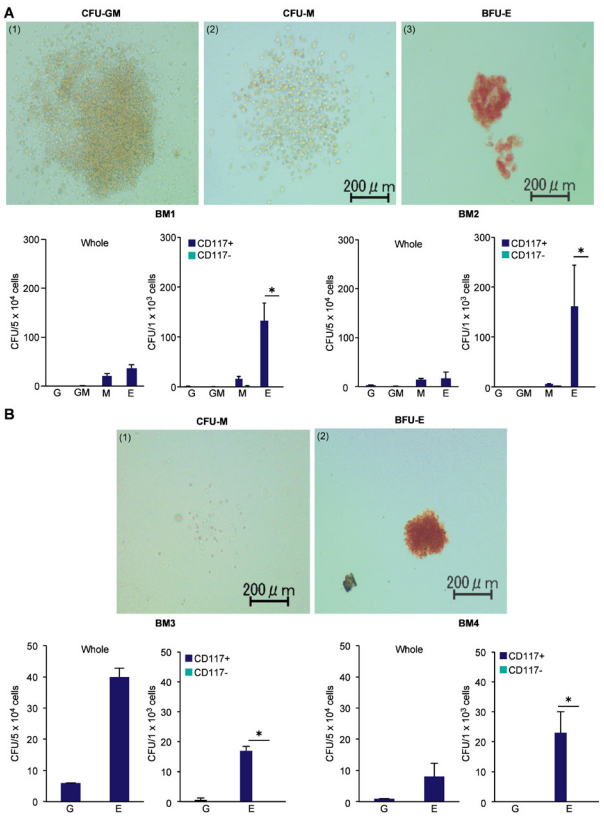

Figure 3. Swine CD117+ cells extensively develop into erythroid-lineage cells *in vitro*. BM cells were sorted as shown in Supplementary Figure 3 and the sorted cells were used in the colony assay. **(A)** Colony assay using the human cytokines SCF, IL-3, erythropoietin and G-CSF. **(1)** CFU-GM, **(2)** CFU-M, **(3)** BFU-E. (G; granulocyte, GM; granulocyte and monocyte, M; monocyte, E; erythrocyte). The data were obtained from two separate swine BM experiments, BM1 and BM2, for the human cytokine environment. Three independent cultures were performed for both of the two BMs and the colonies were counted and compared. The mark * represents the significant difference by *t*-test ($p < 0.05$). Two independent assays and representative photographs are shown here. **(B)** Colony assay using the mouse cytokines SCF, IL-3, erythropoietin and G-CSF. **(1)** CFU-G, **(2)** BFU-E. All pictures were taken by KEYENCE BIOREVO BZ-90000, Object lens: ×10 Plan Fluor NA 0.30 Ph1. The data were obtained from two separate swine BM experiments, BM3 and BM4, for the human cytokine environment. Three independent cultures were performed for both of the two BMs and the colonies were counted and compared. The mark * represents the significant difference by *t*-test ($p < 0.05$). Two independent assays and representative photographs are shown here.

with 2A1. The CD117$^+$ cells were purified as shown in Suppl. Figure 3 (*http://www.biosciencetrends.com/docindex.php?year=2014&kanno=6*). CD117$^+$ cells and CD117- cell fractions were partially purified by magnetic beads sorting at first and then to over 98% purity levels by additional sorting using a FACSaria (Suppl. Figure 3A). Both of the CD117 positive and negative cell fractions were then used in a colony assay by culturing the cells in the presence of the human cytokines, SCF, IL-3, erythropoietin and G-CSF. As a result of the cellular purifications and colony assays four types of cellular clones were identified in the BM cell cultures. These were granulocytes (G), granulocytes/monocytes (GM), monocytes (M) and erythrocytes (E). Notably, CD117$^+$ cells in the colony assay generated erythroid burst-forming units (BFU-E) with significantly higher efficiency than either the BM cells or CD117$^-$ cells. The generation of colony forming units (CFU) were also observed for G, M and GM, but at significantly lower levels for the CD117$^+$ cell fraction (Figure 3A). Similar results were obtained when the cells were cultured in the colony assay using the mouse instead of the human cytokines (Suppl. Figure 3B). In two of these experiments, the frequency of BFU-E in the CD117$^+$ cell fraction was significantly higher than in the CFU-M assay or in the CD117$^-$ cell fraction. The CFU of the other cell types, G and GM, were low or undetectable (Figure 3B and Suppl. Figure 3B). Compared to human cytokine conditions, the colony forming ability of BM cells was less effective with the mouse cytokines.

Overall, these results suggest that the swine CD117$^+$ BM cells have the potential to develop into erythroid-monocyte lineage *in vitro*.

3.3. *Development of swine hematopoietic cells in immunodeficient NOD/SCID/IL-2gc-null (NOG) mouse*

While mouse cytokines can support the development of swine erythroid cells and monocytes, other cell lineages

could not be detected by the *in vitro* colony assay. Therefore, we transplanted the swine hematopoietic cells into severely immunodeficient NOG mice to examine if lymphoid lineage cells could be developed from the CD117$^+$ cells *in vivo*. Swine CD117$^+$ cells were purified and transplanted into irradiated NOG mice and analysed by cytometry using different gate settings to detect erythroid cells, lymphoid cells and monocytes (Figure 4A). After 2 and 12 weeks post transplantation, lymphoid organs were collected and the presence of swine blood cells were examined by flow cytometry using the lymphoid gate setting. As a result,

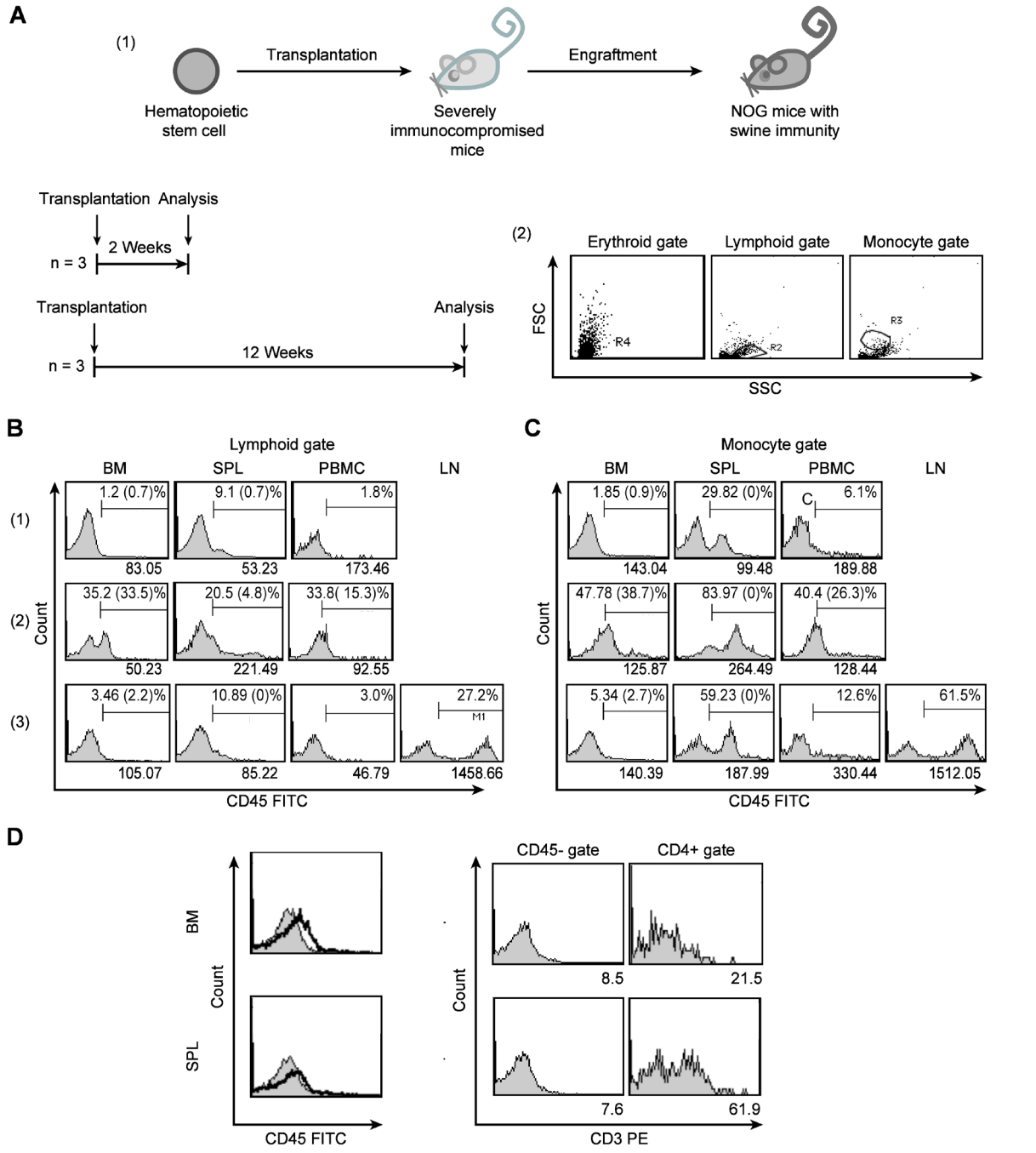

Figure 4. Repopulation of CD117$^+$ swine BM cells in immunodeficient NOG mice. (A) (1) Protocol for CD117$^+$ cell transplantation into NOG mice. In short term (2 w) and long term (12 w) analysis after the transplantation, lymphoid cells were analyzed for the swine cell engraftment either two weeks or twelve weeks after transplantation. **(2)** Flow cytometrical gates used for the analyses of erythroid (R4), lymphoid (R2) and monocyte (R3) cells were set by FSC and SSC. **B** and **C**. Flow cytometrical analysis of engrafted swine cells. Swine leukocytes were detected by swine CD45 expression using an anti-swine CD45-FITC antibody. Lymphoid-gated cells are shown in **B** and monocyte-gated cells in **C**. **(B) (1)** Non-transplanted control mice. **(2)** Two weeks after the transplantation. **(3)** Twelve weeks after the transplantation. Some of the SPL cells are stained with CD45-FITC. These are some non-specific binding of the antibodies to the cells as we analyzed by 2D-flow cytometry analysis (data not shown). The numbers show the shifted cell percentages compared to control mice without transplantation. In the brackets, shifted percentages of no-stain controls are shown. **(D)** Lymphoid-gated cells were analyzed for the expression of CD3. Swine CD45-FITC and CD3-PE double staining of the BM and SPL cells are shown in the panels. The mean fluorescent intensity (MFI) is shown below the panels. Representative data of three independent assays are shown.

swine CD45$^+$ cells were observed in BM, spleen (SPL) and the peripheral blood of NOG mice transplanted with CD117$^+$ cells 2 weeks after the transplantation (Figure 4B). Higher amounts of the CD45$^+$ cells were observed through the monocyte gate of the SPL cells 2 weeks after transplantation (Figure 4C). CD45$^+$ cells were also observed in the lymph node 12 weeks after transplantation. These results indicate that the engrafted swine white blood cells had developed successfully in the NOG mice. We used a TER119 antibody, which recognizes mouse erythrocyte to distinguish between the mouse and swine erythrocytes, and found that the NOG mice included TER119$^-$ cells in the erythrocyte gate, and that the swine CD117$^+$ cells might be differentiated into erythrocytes, which were TER119 (mouse erythroid marker) negative, *in vivo* (Suppl. Figure 4, *http://www.biosciencetrends.com/docindex.php?year=2014&kanno=6*).

The CD45$^+$ cells of the mice SPL were examined for the CD3 expression in lymphoid gate 12 weeks after the transplantation. As shown in Figure 4D, significant amount of CD3$^+$ cells were observed in the SPL.

These results show that the transplanted swine CD117$^+$ cells had developed into erythroid, myeloid and lymphoid cells in NOG mice and suggest that the swine CD117$^+$ cells are hematopoietic stem/progenitor cells that can be transplanted successfully to a different species under the right conditions of engraftment.

4. Discussion

CD117 is a cytokine receptor tyrosine kinase type III expressed on the surface of hematopoietic stem cells and when activated by the cytokine SCF can regulate the maintenance, proliferation, migration and differentiation of HSCs in the BM. In the swine, the stem cell activity of CD117$^+$ cells have been previously evaluated by using SCF to activate their stem cell potential to differentiate into various cellular lineages (*20,21*). Although a swine CD117 Mab has been developed and characterized (*24*), the species specificity of the Mab and xeno-transplantation of CD117$^+$ cells have not been reported. They checked *in vitro* development of erythroid and myeloid cells from Mab-enriched cells, but they could not detect lymphoid cell development. Swine SCF-reacting cells previously reported could not develop lymphoid cells, either (*25*). Because the species specificity of both tools was unclear, xeno-transplantation experiments using a species specific CD117 Mab might not be undertaken until we prepared our own highly species-specific anti-CD117 Mab clone. This Mab clone, 2A1, enabled us to evaluate the multipotency of swine CD117$^+$ cells *in vitro* and *in vivo*. The Mab reacted against the swine CD117 receptor expressed by a recombinant DNA vector N576 in HEK293 or A2 cells, was specific for the detection of CD117 in swine and did not cross react with the CD117 cells of mice or humans. This specificity allowed us to develop the *in vivo* xeno-transplantation system using NOG mice because the porcine specific CD117 antibody did not cross-react with other cells examined. In this *in vivo* system, we demonstrated that CD117$^+$ cells could develop into T cells, which indicate that the CD117$^+$ cells involve hematopoietic stem cells.

Consequently, on the basis of the specificity of Mab 2A1, we found that CD117$^+$ cells were abundant in the newborn swine BM, but not in CB or adult BM. Moreover, the CD117$^+$ cells from the newborn BM were found to develop into erythroid cells or monocytes using the *in vitro* colony assay and into lymphoid cells by the xeno-transplantation *in vivo* system. Thus, both the *in vitro* and the *in vivo* experiments confirmed that the CD117$^+$ cells from the newborn swine BM are hematopoietic stem/progenitor cells.

The similarity between our *in vitro* data for CD117$^+$ cells from the newborn BM and the data reported by Dor *et al*. (*25*) in the spleen or BM of young pigs less than 1 year old suggests a high efficiency of CD117$^+$ cell purification with SCF-binding affinity in their system. In our *in vitro* study, we also confirmed that the CD117$^+$ cells differentiated into the erythrocytes in the presence of mouse or human cytokines SCF, IL-3, erythropoietin and G-CSF. While the swine CD117$^+$ cells developed into erythroid colonies in our *in vitro* studies and those of others (24), we could not definitely conclude the potential of the CD117$^+$ cells to develop into erythroid cells in the mouse transplantation system because of the absence of a good erythrocyte monitoring system. We could only suggest that the TER119-negative erythroid gated cells were observed early after the CD117$^+$ cell transplantation. On the other hand, we found white blood cells including CD3$^+$ cells in the peripheral lymph organs of the transplanted NOG mice, suggesting that the CD117$^+$ cells had differentiated into the monocyte and lymphoid lineages. Human HSC that were transplanted into NOG mice developed into myeloid and lymphoid lineages, but not into erythroid lineage cells *in vivo* (*13,26*). The cytokine, erythropoietin, is a known requirement for the differentiation of CD117 stem cells into erythroid precursor cells and erythrocytes (*27*). The contrary results obtained in the *in vitro* and the *in vivo* systems respectively may be due to the presence of sufficient amount of erythropoietin in the *in vitro* system and its reduced amount in the *in vivo* system. Alternatively, other synergistic factors are missing or inhibitory factors are in play preventing the CD117 stem cells from developing into erythrocytes in the xeno-transplantation environment.

In conclusion, we produced a highly specific anti-swine CD117 Mab and used it to confirm that the swine CD117$^+$ stem cells have a multipotency that can differentiate into erythroid cells, monocytes and lymphoid cells depending on the *in vitro* or *in vivo* system chosen for analysis. The swine-NOG mouse transplantation system in conjunction with

specific detection reagents like the Mabs 2A1 shows an important experimental potential for the study of hematopoietic cells *in vivo*, especially for understanding the factors required in erythroid cell development from hematopoietic stem/progenitor cells.

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