HIROSAKI UNIVERSITY

Radiation Emergency Medicine 2012 Vol.1 No.1-2 62-69

REM
Radiation Emergency Medicine

Full Reconstitution of Hematopoietic System by Murine Allogeneic Umbilical Cord Blood Transplantation

Koichi Ito[1], Masashi Sato[2], Makie Chiba[2], Yusuke Okui[2], Akira Nakano[3], Ayumi Wakayama[4], Kyoko Ito[1], Fujimi Kudo[1], Manabu Nakano[1] and Hideaki Sato[5]

[1]Department of Biomedical Sciences, Hirosaki University Graduate School of Health Sciences, 66-1 Hon-cho, Hirosaki, Aomori 036-8564, Japan.
[2]Department of Medical Technology, Hirosaki University School of Health Sciences, 66-1 Hon-cho, Hirosaki, Aomori 036-8564, Japan.
[3]Department of Cellular Biochemistry and Molecular Biology, Hirosaki University Graduate School of Health Sciences, 66-1 Hon-cho, Hirosaki, Aomori 036-8564, Japan.
[4]Transfusion and Cell Therapy Center, Hakodate Municipal Hospital, 1-10-1 Minato-cho, Hakodate, Hokkaido 041-8680, Japan.
[5]Cell Technology Center, Stem Cell Institute Inc., 5-22-10 Shimbashi, Minato-ku, Tokyo 105-0004, Japan.

(Received 3 November 2011; revised 11 January 2012; accepted 13 January 2012)

Murine allogeneic umbilical cord blood cells (UCBCs) were studied for their ability to reconstitute the hematopoietic system. UCBCs obtained from fetuses of C57BL/6 mice, which were transgenic for green fluorescent protein (GFP), were transplanted into RAG2(-/-)BALB/c mice. For comparison, bone marrow cell (BMC) transplantation was also performed. At 16 weeks after transplantation, phenotypically mature GFP-positive immune cells of donor origin, including T cells, B cells, monocytes, and granulocytes, were detected in the recipients' peripheral blood, even after major histocompatibility complex-mismatched UCBC transplantation. Functional analysis showed that mice with allogeneic UCBC transplants accepted skin grafts from both BALB/c and C57BL/6 mice. However, these chimeric mice completely rejected skin grafts from third-party C3H/HeJ mice, indicating that both $CD8^+$ killer and $CD4^+$ helper T cells were functionally mature in the recipients. In addition, 2,4,6-trinitrophenyl-keyhole limpet hemocyanin (TNP-KLH) immunized mice with UCBC transplant produced both TNP-specific IgM and IgG antibodies. These findings indicate the ability of recipient mice to develop antibody responses with Ig class switching to T-cell-dependent antigens, thereby confirming that both B cells and $CD4^+$ helper T cells derived from allogeneic UCBC were immunologically competent. Furthermore, we have demonstrated for the first time that B-1a cells, which produce natural IgM antibodies against pathogens such as *Streptococcus pneumoniae* and the influenza virus, can be generated from UCBCs but not from BMCs.

Key words: Cord blood, Stem cell transplantation, Major histocompatibility complex, Hematopoietic system, Immune function, Lymphocytes.

Koichi Ito: Hirosaki University Graduate School of Health Sciences
66-1 Hon-cho, Hirosaki, Aomori 036-8564, Japan.
E-mail: kohito@cc.hirosaki-u.ac.jp

Copyright © 2012 by Hirosaki University. All rights reserved.

1. Introduction

Umbilical cord blood cell (UCBC) transplantation has been applied as a strategy for the treatment of not only various hematological diseases[1-3] but also accidental total-body radiation exposure. UCBC transplantation has several advantages over bone marrow cell (BMC) transplantation, including the much larger size of the available donor pool, the rich proportion of hematopoietic progenitor cells[4], the low content of mature T cells that might cause a graft-versus-host reaction[5, 6], and the low risk of cytomegalovirus infection[7]. In clinical situations, a perfect major histocompatibility complex (MHC) matching cannot be expected in UCBC transplantation, which is based essentially on unrelated donor-recipient combinations. Although the low content of mature T cells allows the use of even MHC-mismatched UCBCs[6, 8], the extent to which lymphocytes derived from MHC-mismatched UCBC transplantation recover their immune function remains unclear because of the lack of convenient animal models. In human patients, analysis of the differentiation capacity of allogeneic UCBCs has been limited to clinical observation after transplantation[1-3].

To clarify this issue, Sato et al. previously evaluated the functional maturation of lymphocytes derived from UCBC-hematopoietic stem cells (HSCs) in a fully MHC-mismatched combination using a murine model of UCBC transplantation[9]. In the present study, the immunological competence of allogeneic UCBC-derived T and B lymphocytes was investigated further using additional samples, and reconstitution of B-1a cells[10], which produce natural IgM antibodies against various pathogens, was compared between UCBC and BMC transplantation. Clinical application of allogeneic UCBC transplantation would not only expand the number of potential donors but also facilitate faster emergency medicine without MHC-matching for patients who have suffered accidental radiation exposure.

2. Materials and methods

Mice

Female C57BL/6 (B6: H-2^b), BALB/c (H-2^d) and C3H/HeJ (C3H: H-2^k) mice were purchased from CLEA Japan, Inc. (Tokyo). B6-TgN (act-EGFP) OsbY01 (green fluorescent transgenic mice on B6 background[11], H-2^b: mentioned as GFP.B6 in this paper) and RAG2 knockout BALB/c (T cell and B cell deficient mice on BALB/c background, H-2^d: mentioned as RAG2(-/-)BALB/c in this paper) were kindly provided by Dr. Masaru Okabe (Osaka University) and Dr. Michio Shimamura (Mitsubishi Kagaku Institute of Life Sciences), respectively. Mice were maintained in a specific pathogen-free facility of Hirosaki University. The experiment was approved by the Animal Research Committee of Hirosaki University and performed in accordance with the Guidelines for Animal Experimentation, Hirosaki University.

Preparation of UCBCs and BMCs

UCBCs were collected from (GFP.B6×B6) F1 fetuses at 18.5 days of gestation, as described previously[12]. BMCs were collected from the femur and pelvic bones of (GFP.B6×B6) F1 adults. Both UCBCs and BMCs were obtained from (GFP.B6×B6) F1 fetuses and adults, because the strong fluorescence emitted by GFP-homozygous cells was not observed for GFP-negative cells in the same plotting field on flow cytometry. Thereafter, mature T cells present in the UCBC and BMC populations were depleted by induction of complement-dependent cytotoxicity with anti-CD4 (GK1.5) and anti-CD8 (83-12-5) monoclonal antibodies[13, 14].

Transplantation

To deplete natural killer (NK) cells, which interfere with the engraftment of HSCs[15], RAG2(-/-)BALB/c recipients were intraperitoneally administered 50 μl of rabbit anti-asialo GM1 antiserum prepared in our laboratory 1 day before transplantation. On the following day, the recipients were lethally irradiated with 8 Gy using an X-ray irradiator (MBR-1505R2; Hitachi Medico Co., Tokyo, Japan) with a filter (Cu: 0.5 mm, Al: 2 mm) while monitoring the cumulative radiation dose. The irradiated mice were then immediately given a transplant containing a high dose (1×10^6 cells), medium dose (0.5×10^6 cells) or low dose (0.1×10^6 cells) of UCBCs or BMCs.

Flow cytometric analysis

Eight-Gy-irradiated RAG2(-/-)BALB/c mice that had received 0.5×10^6 GFP-transgenic UCBCs or BMCs were analyzed 16 weeks after the cell transfer. Engrafted cells in the recipients' peripheral blood were detected by staining with a biotinylated antibody against a lineage marker such as T cell receptor (CD3e; T cells), B220 (CD45R; B cells), Mac-1 (CD11b; macrophages) or Gr-1 (Ly-6G; granulocytes), followed by phycoerythrin (PE)-labeled streptavidin. In addition, to examine reconstitution of B-1a cells, peritoneal cells (PCs) were harvested carefully from the peritoneal cavity of UCBC and BMC recipients by rinsing with 2-3 ml of sterile PBS, avoiding any contamination with peripheral blood, after sacrifice. Harvested PCs were then triple-stained with PE-labeled anti-CD45R/B220, biotin-labeled anti-CD5, followed by PE-TexasRed labeled-streptavidin and PerCP-Cy5.5-labeled anti-IgM monoclonal antibodies, and the cells were analyzed on flow cytometry. All the antibodies were purchased from BD Biosciences (CA, USA)

Immunization and enzyme-linked immunosorbent assay

More than 16 weeks after transplantation, the chimeric RAG2(-/-)BALB/c mice were immunized with 2 biweekly intraperitoneal injections of 100 mg 2,4,6-trinitrophenyl-keyhole limpet hemocyanin (TNP-KLH; Biosearch Technologies Inc., CA, USA), initially with complete Freund's adjuvant and the second time without the adjuvant. The immunized mice were bled from the tail vein 2 weeks after the second immunization. Anti-TNP antibody production was examined by performing an enzyme-linked

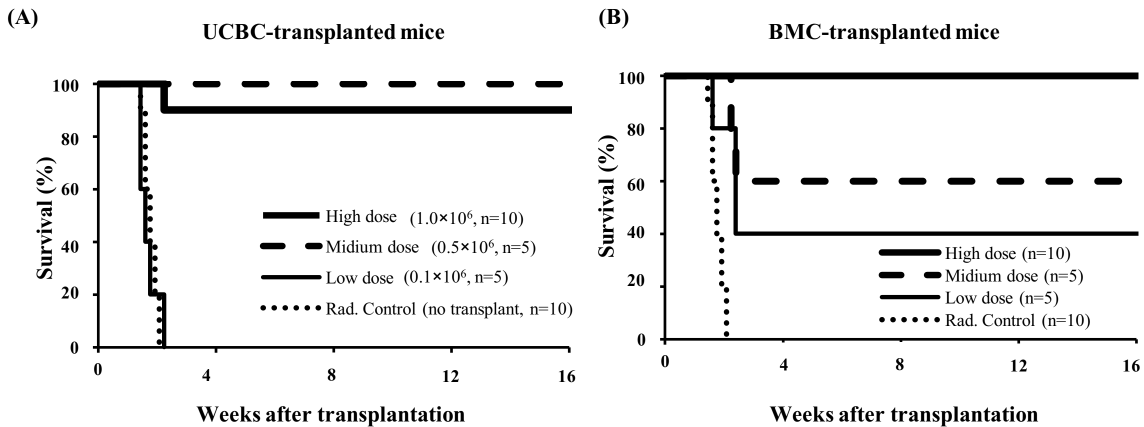

Fig. 1. Effect of cell dose on graft survival after transplantation.
Survival curves for mice receiving high (1.0×10^6 cells, bold line, n=10), medium (0.5×10^6 cells, bold dotted line, n=5), and low (0.1×10^6 cells, thin line, n=5) doses of UCBCs (A) or BMCs (B) for up to 16 weeks after transplantation. The negative control group indicated by a thin dotted line comprised 10 X-ray-irradiated RAG2(-/-)BALB/c mice that did not undergo any form of transplantation. Significance of differences was determined using the log-rank test. Survival rates of the mice in the low-dose UCBC group differed significantly from those of the mice in the low-dose BMC group ($P<0.05$).

immunosorbent assay (ELISA) with TNP-BSA-coated 96-well plates. TNP-BSA conjugates were also purchased from Biosearch Technologies Inc. Serum samples were diluted 1:1024 and added to the TNP-BSA-coated plates. Bound TNP-specific IgM and IgG were then detected using horseradish peroxidase (HRP)-conjugated rabbit anti-mouse IgM and anti-mouse IgG antibodies (Zymed, CA, USA), respectively, followed by addition of O-phenylenediamine dihydrochloride. Optical density (OD) was measured at 490 nm. In addition, total IgM and bacterial polysaccharide-reactive IgM in mice that had received UCBCs and BMCs were detected by ELISA. Pneumovax-NP (Banyu Pharm. Co.,Ltd), which contains polysaccharides from *Streptococcus pneumoniae* constituted by equal amounts of 23 capsular types, was used as an antigen for detection of anti-bacterial polysaccharide IgM production.

Skin grafting

For skin grafting, dermis was harvested from the tails of B6, BALB/c and C3H mice and placed on the shaved backs of the chimeric RAG2(-/-)BALB/c mice that had survived for more than 16 weeks after transplantation. The grafts were protected by immediately wrapping them with a bandage. After 7 days, the bandage was removed to allow observation. Rejection time was calculated as the number of days until the complete detachment of the grafted skin.

Statistical analysis

Data were analyzed statistically using the log-rank test, Tukey's test or Student's *t* test.

3. Results

Survival rate after transplantation

RAG2(-/-)BALB/c recipients were lethally irradiated at 8 Gy and then given a transplant containing a high dose (1×10^6 cells, n=10), medium dose (0.5×10^6 cells, n=5) or low dose (0.1×10^6 cells, n=5) of UCBCs or BM cells obtained from (B6.GFP×B6) F1 fetuses or (B6.GFP×B6) F1 mice, respectively. As a control, 10 mice that had been irradiated but not given a transplant were prepared. Low-dose UCBC transplantation is unable to rescue X-ray-irradiated recipients (Fig. 1A). In contrast, the mice transplanted with low doses of BMCs achieved a high survival rate of 40% with significant differences until 16 weeks after the transplantation (Fig. 1B). However, no significant difference was observed in the survival rate between the UCBC and BMC recipients in the medium-dose or high-dose group. Essentially, the survival rate was proportional to the number of transplanted cells in both chimeric recipients, indicating that the difference in the recovery of the hematopoietic system between UCBC- and BMC-transplanted recipients is attributable to the number of HSCs in the transplanted cell population[16] and not to the nature of HSCs. Collectively, these results suggest that UCBC-HSCs and BMC-HSCs are essentially similar with respect to their capacity for self-renewal and their ability to differentiate into cells of all hematopoietic lineages, even in an allogeneic environment.

Reconstitution of the hematopoietic system in peripheral blood

RAG2(-/-)BALB/c mice that had been irradiated with 8 Gy and received a medium dose (0.5×10^6) of UCBCs or BMCs derived from GFP-transgenic B6 mice were analyzed 16 weeks after transplantation (Fig. 2). Engrafted cells were detected in the peripheral blood of the recipient mice on flow cytometric analysis that was performed using biotin-labelled lineage-specific antibodies, followed by PE-labeled streptavidin. The analysis revealed that the transplanted cells had developed into T cells, B cells, Mac-1$^+$ cells, and granulocytes in the allogeneic UCBC- and BMC-transplanted recipients. Each cell lineage consisted of more than 95% GFP-positive cells, indicating that these phenotypically mature cells had developed from donor-derived HSCs. Although 4 cell populations in UCBC- and BMC-transplanted

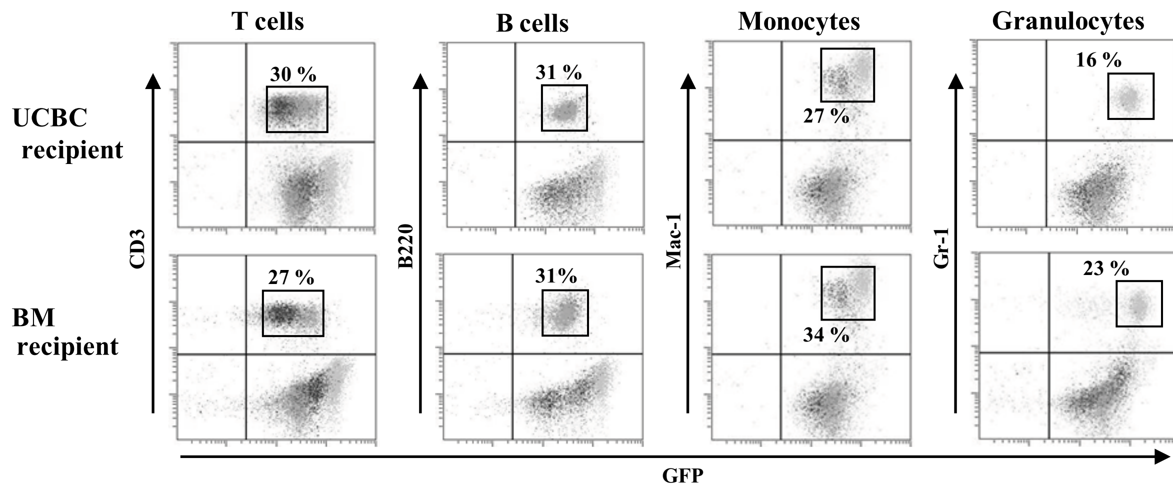

Fig. 2. Reconstitution of the hematopoietic system by donor cells in allogeneic recipients.
At 16 weeks after transplantation, peripheral blood cells of RAG2(-/-)BALB/c recipients were analyzed by flow cytometry utilizing biotin-labeled antibodies such as anti-CD3 (T cells), anti-B220 (B cells), anti-CD11b (macrophages) and anti-Gr-1 (granulocytes), followed by addition of streptavidin-PE. All GFP fluorescent lineage cells of donor origin including T cells, B cells, macrophages and granulocytes were observed in each representative recipient of transplanted UCBCs (upper four panels) or BMCs (lower four panels).

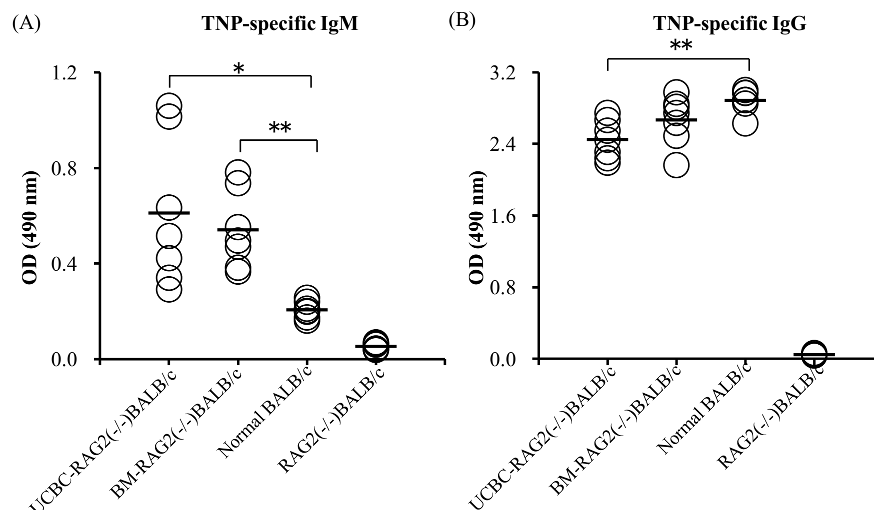

Fig. 3. B cell function in UCBC transplant recipients.
Over 16 weeks after allogeneic UCBC or BMC transplantation, the chimeric RAG2(-/-)BALB/c recipients were immunized with 2 biweekly intraperitoneal injections of 50 μg TNP-KLH, initially with complete Freund's adjuvant, and the second time without adjuvant. Two weeks after the last immunization, the TNP-specific IgM (A) and IgG (B) antibody titers in serum samples were determined by ELISA. Serum samples diluted 1:1024 were used in the assay. All OD values at 490 nm obtained for seven mice in each group were plotted (Tukey's test, *P<0.05, **P<0.01: comparison among all 4 groups).

mice appeared to comprise >100% GFP-positive cells, this may have been due to overlap of a monocyte population with a granulocyte population during flow cytometry. In addition, no non-GFP-positive Mac-1^+ and Gr-1^+ cells were observed in the peripheral blood of both RAG2(-/-)BALB/c recipients, indicating the replacement of recipient-derived by donor-derived HSCs in bone marrow after UCBC and BMC transplantation. It is likely that a small amount of recipient-derived non-GFP-positive Mac-1^+ and Gr-1^+ cells would also have been detectable if a large number of peripheral blood cells had been analyzed by flow cytometry. Similar results were obtained for recipients that had received high and low doses of UCBCs or BMCs (data not shown).

Functional analysis of UCBC-derived B cells

The most important aspect of this study was to determine whether allogeneic UCBC-derived lymphocytes attain functional maturity. First, the functional maturity of allogeneic UCBC- or BMC-derived B cells in RAG2(-/-)BALB/c mice was examined by intraperitoneal injection of TNP-KLH, a T-cell-dependent antigen. Recipient mice that had received a high dose (1×10^6) of UCBCs or BMCs derived from GFP-transgenic B6 mice were used for this purpose. In the absence of contaminating host-derived B cells, both IgM and IgG antibody responses to TNP were successfully induced in both the UCBC- and BMC-transplanted recipients (Fig. 3). Needless to say, production

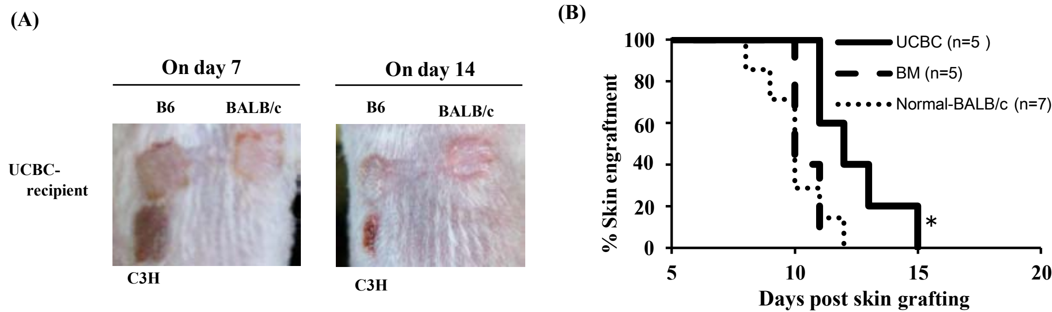

Fig. 4. T cell function in UCBC transplant recipients.
(A) Over 16 weeks after allogeneic UCBC or BMC transplantation, a piece of skin harvested from the tail of the BALB/c, B6 and C3H mice was simultaneously grafted onto the shaved backs of chimeric RAG2(-/-)BALB/c mice. The photographs show that on day 14 after skin grafting, UCBC-reconstituted chimeric mice rejected the third-party skin grafts from C3H mice, whereas the skin grafts from BALB/c and B6 mice were still maintained in these recipient mice at the same time point. (B) The time taken for rejection of the C3H skin was compared among five UCBC-transplanted and BMC-transplanted, and seven normal BALB/c mice. Although the UCBC chimeric mice completely rejected the skin grafts from the C3H mice, the time taken for graft rejection was significantly longer than that for the BMC chimeric and normal BALB/c mice, as determined by log-rank test (*$P<0.05$, UCBC-transplanted mice versus BMC-transplanted or normal BALB/c mice).

of TNP-specific antibody was not induced in the non-chimeric RAG2(-/-)BALB/c mice. These results indicate that in recipient mice, the reconstituted B cells derived from donor HSCs are capable of inducing antibody responses to T-cell-dependent antigens such as TNP-KLH. Further, Ig class switching from IgM to IgG confirmed that both helper T cells and B cells derived from the allogeneic UCBCs were immunologically competent. However, we found that TNP-specific IgM titers were higher (Fig. 3A) and TNP-specific IgG titers were lower (Fig. 3B) in the UCBC and BMC chimeric mice than in normal BALB/c mice, indicating that Ig class switching from IgM to IgG in response to TNP was delayed in these chimeric mice relative to that in normal BALB/c mice.

Functional analysis of UCBC-derived T cells

To examine the functional competence of allogeneic UCBC- and BMC-derived T cells, the chimeric RAG2(-/-) BALB/c mice received skin grafts from BALB/c, B6, and C3H mice. Rejection time was determined as the day when the grafted skin became completely detached. Recipient mice that had received a high dose (1×10^6) of UCBCs or BMCs derived from GFP-transgenic B6 mice were used. Representative photographs showed that on day 14 after skin grafting, UCBC-reconstituted chimeric mice rejected the third-party skin grafts from C3H mice, whereas the skin grafts from BALB/c and B6 mice were still maintained in these recipient mice at the same time point in the absence of contaminating host-derived T cells (Fig. 4A). In addition, the rejection time for skin grafts from C3H mice was compared among five UCBC and BMC chimeric mice and seven normal BALB/c mice (Fig. 4B). Although the graft rejection time for UCBC chimeric mice was significantly longer than that for BMC chimeric and normal BALB/c mice, the UCBC recipients completely rejected the allogeneic C3H skin. No significant differences in C3H skin graft rejection time were evident between the BMC chimeric and normal BALB/c mice. These findings indicated the presence of donor-derived functional $CD8^+$ killer T cells and $CD4^+$ helper T cells that activated killer T cells via interleukin-2 secretion and the ability of both types of T cells to discriminate between self and non-self antigens.

Reconstitution of peritoneal B-1a cells in recipients

Peritoneal cells were harvested from RAG2(-/-)BALB/c mice that had received a high dose of allogeneic UCBCs or BMCs, and triple-stained with anti-B220, anti-IgM and anti-CD5 antibodies. Flow cytometeric analysis showed that the number of GFP-positive B-1a cells ($CD5^+B220^{lo}IgM^{hi}$) was high in normal BALB/c mice (45.3%) and UCBC-transplanted mice (20.9%) but low in BMC-transplanted mice (4.6%) (Fig. 5A). Total IgM and bacterial polysaccharide-reactive IgM in UCBC- and BMC-recipient mice were measured by ELISA (Fig. 5B). The total IgM levels were similar among the three groups, although bacterial polysaccharide-reactive IgM antibodies were strongly produced in normal BALB/c mice, moderately produced in UCBC-transplanted mice, and not produced in BMC-transplanted mice, indicating that IgM production was correlated with the presence of B-1a cells. These results indicate that in hematopoietic stem cell transplantation, UCBCs have promising potential for reconstitution of natural IgM-producing B-1a cells involved in early innate defense.

4. Discussion

In our present study, cell lineages derived from HSCs were clearly identified as GFP-positive cells in the recipients. In addition, RAG2(-/-)BALB/c mice were used as allogeneic recipients because examination of the whole-body immune response of UCBC-derived T and B cells was not informative in the presence of BALB/c (host type) bone marrow-derived T and B cells. HSCs among allogeneic UCBCs differentiated into phenotypically mature GFP-positive T cells, B cells, $Mac\text{-}1^+$ cells, and $Gr\text{-}1^+$ cells in RAG2(-/-)

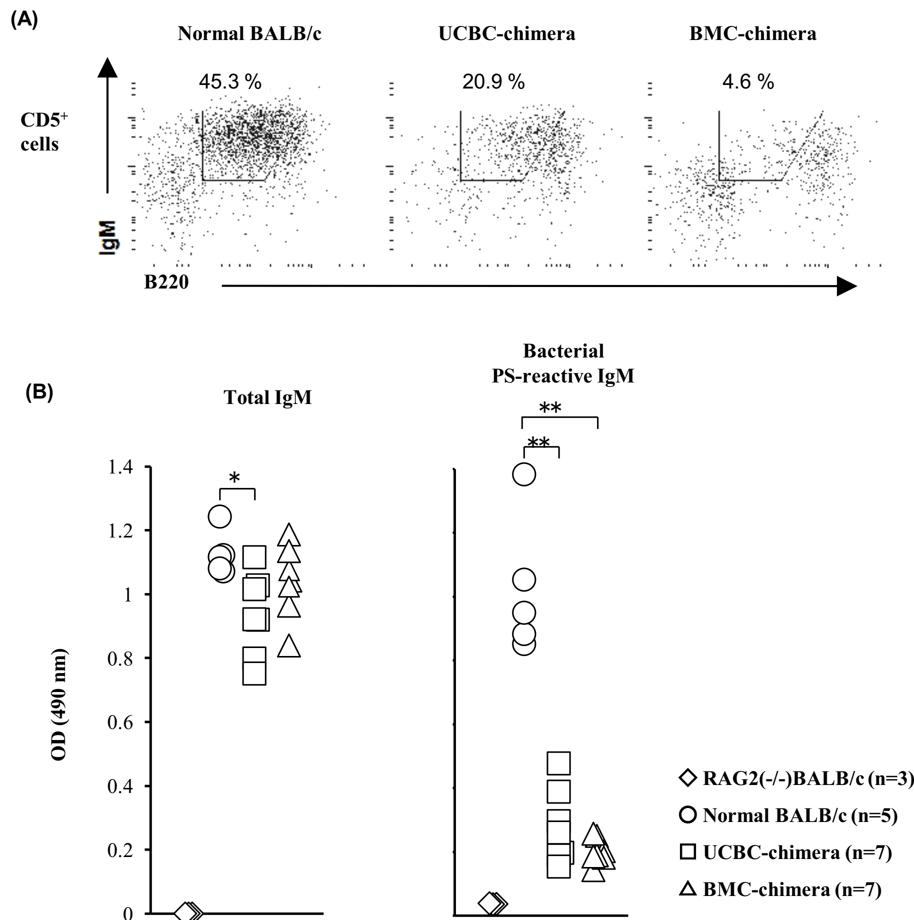

Fig. 5. Reconstitution of B-1a cells by UCBC transplantation.
The extent of B-1a cell reconstitution was compared between UCBC and BMC transplantation. (A) PCs were harvested from the peritoneal cavity of UCBC and BMC recipients. Harvested PCs were then stained with specific antibodies against CD45R/B220, CD5 and IgM, and the cells were analyzed by flow cytometry. (B) Total IgM and bacterial polysaccharide (PS)-reactive IgM antibodies in four groups (RAG2(-/-) BALB/c, n=3; normal BALB/c, n=5; UCBC chimera, n=7; BMC chimera, n=7) were measured by ELISA (Student's *t* test, *P<0.05, **P<0.01; comparison among all 4 groups).

BALB/c recipient mice. In addition, in the recipient mice, the reconstituted B cells derived from the UCBCs of the B6 mice mounted specific antibody responses to T-cell-dependent antigen and exhibited Ig class switching in the absence of host-derived T and B cells. Further, rejection of the allogeneic skin graft by both types of chimeric mice indicates the functional maturity of both $CD8^+$ killer and $CD4^+$ helper T cells derived from UCBC-HSCs and BMC-HSCs.

In an allogeneic environment, low-dose UCBC transplantation could not the rescue X-ray-irradiated recipients. However, transplantation with medium and high doses of UCBCs resulted in a high survival rate comparable to that with BMCs. This observation possibly corresponds to that of previous studies suggesting the different numbers of potent HSCs in UCBCs (1 cell per 4×10^4 UCBCs)[17] and in BMCs (1 cell per 2.5×10^4 BMCs)[12] that contribute to long-term engraftment. In addition, allogeneic UCBC-transplanted mice showed survival rates comparable to those obtained after syngeneic UCBC transplantation (GFP.B6 > RAG2 (-/-) B6) at 3 different doses (data not shown). Collectively, our results indicate that UCBC-HSCs and BMC-HSCs have almost equal potential to reconstitute the hematopoietic system.

Although our results indicate that T and B cells derived from allogeneic UCBCs are immunologically competent, the functional ability of the reconstituted lymphocytes in both of the chimeric mice was lower than that of lymphocytes in normal mice, as indicated by a delayed Ig class switching in B cells (Fig. 3A and B) and T cell-induced skin graft rejection (Fig. 4B). Chen et al.[18] have reported delayed hematopoietic engraftment and impaired immune reconstitution in wild-type BALB/c mice that received allogeneic UCBCs harvested from B6 fetuses. This finding is possibly attributable to decreased numbers of reconstituted peripheral T and B cells. However, we did not detect any decrease in the number of reconstituted immune cells in UCBC-transplanted recipients, which indicates the presence of other defects in the immune system (data not shown). Talvensaari et al.[19] suggested that UCBC

transplantation leads to a board T-cell repertoire diversity and a favorable long term immune reconstitution in patients. At present, we are examining the detailed functions of reconstituted T and B cells in UCBC- and BMC-transplanted recipients by using an *in vitro* assay.

Because the quantity of individual cord blood samples is limited, the use of pooled cord blood might be required in clinical practice. In mixed UCBC transplantation[20, 21], MHC restriction can be more complex. As shown in Figure 4A, chimeric RAG2(-/-)BALB/c mice accepted skin grafts from both B6 and BALB/c mice. In these recipients, the repertoire of T and B cell receptors were composed by antigen-presenting cells from both BALB/c and B6 mice. Thus, in such cases, a clear understanding of the mechanisms underlying MHC restriction is necessary.

It remains unclear whether UCBC and BMC transplantation reconstitute all immune cells. B cell population is composed mainly of 3 different subsets: B-1a, B-1b, and B-2 cells. Among these, B-1a cells spontaneously secrete natural IgM antibodies against bacteria and viruses localized primarily in the peritoneal and pleural cavities[10]. In this study, we compared the extent of B-1a cell reconstitution between UCBC and BMC transplantation. Flow cytometric analysis revealed that the number of GFP-positive peritoneal B-1a cells was relatively high in UCBC recipients and low in BMC recipients. Bacterial polysaccharide-reactive IgM antibodies were produced predominantly in UCBC recipients, indicating that IgM production is correlated with the presence of B-1a cells. This finding is clinically valuable for hematopoietic stem cell transplantation.

In conclusion, in RAG2(-/-)BALB/c mice, hematopoietic stem cells present among allogeneic UCBCs and BMCs differentiated into phenotypically mature T cells, B cells, Mac-1$^+$ cells, and granulocytes. In addition, UCBCs and BMCs had essentially similar abilities for reconstitution of functional T and B lymphocytes even in an allogeneic environment, because both types of chimeric mice generated a specific antibody response to TNP-KLH as a T-cell-dependent antigen and also rejected the third-party skin grafts. Furthermore, this study demonstrated that UCBCs have promising potential for reconstitution of B-1a cells, thereby replenishing polysaccharide-reactive IgM as a front-line defensive factor against invading pathogens. On the basis of our finding, allogeneic UCBC transplantation can allow recovery of the normal hematopoietic system in patients who have been accidentally exposed to radiation.

Acknowledgement

We wish thank all members of the Experimental Animal Laboratory, Hirosaki University, for their skilled technical assistance. This work was supported by a Grant for Co-medical Education Program in Radiation Emergency Medicine by the Ministry of Education, Culture, Sport, Science and Technology, Japan, and by a Grant for Hirosaki University Institutional Research.

References

1. Gluckman E, et al (1989) Hematopoietic reconstitution in a patient with Fanconi's anemia by means of umbilical-cord blood from an HLA-identical sibling. N Engl J Med 321: 1174-1178.
2. Gluckman E, et al (1997) Outcome of cord-blood transplantation from related and unrelated donors. N Engl J Med 337:373-381.
3. Broxmeyer HE, et al (1989) Human umbilical cord blood as a potential source of transplantable hematopoietic stem/progenitor cells. Proc Natl Acad Sci USA 86:3828-3832.
4. Wang JCY, Doedens M and Dick JE (1997) Primitive human hematopoietic cells are enriched in cord blood compared with adult bone marrow or mobilized peripheral blood as measured by the quantitative in vivo SCID-repopulating cell assay. Blood 89: 3919-3924.
5. Bofill M, et al (1994) Immature CD45RA^{low}RO^{low} T cells in the human cord blood. J Immunol 152:5613-5623.
6. Madrigal JA, et al (1997) Does cord blood transplantation result in lower graft-versus host disease? Hum Immunol 56:1-5.
7. Rubinstein P, et al (1993) Stored placental blood for unrelated bone marrow reconstitution. Blood 81: 1679-1690.
8. Wagner JE. et al (1996) Successful transplantation of HLA-matched and HLA-mismatched umbilical cord blood from unrelated donors: analysis of engraftment and acute graft-versus-host disease. Blood 88:795-802.
9. Sato H, et al (2010) Reconstitution of the immune system after murine allogeneic umbilical cord blood transplantation. Hirosaki Med J 61:35-45.
10. Choi Y-S and Baumgarth N (2008) Dual role for B-1a cells in immunity to influenza virus infection. J Exp Med 205: 3053-3064.
11. Okabe M, et al (1997) 'Green Mice' as a source of ubiquitous green cells. FEBS Lett 407:313-319.
12. Migishima F, et al (2003) Full reconstitution of hematopoietic system by murine cord blood. Transplantation 75:1820-1826.
13. Park SY, et al (1992) Effective blocking of natural cytotoxicity of young rabbit serum on murine thymocytes by high concentration of glucose in complement-dependent cytotoxicity method. J Immunol Method 154:109-119.
14. Ho VT and Soiffer RJ (2001) The history and future of T-cell depletion as graft-versus-host disease prophylaxis for allogeneic hematopoietic stem cell transplantation. Blood 98: 3192-3204.
15. Tiberghien P, et al (1990) Anti-asialo GM1 antiserum treatment of lethally irradiated recipients before bone marrow transplantation: evidence that recipient natural killer depletion enhance survival, engraftment, and hematopoietic recovery. Blood 76: 1419-1430.
16. Uchida N, et al (1998) High doses of purified stem cells cause early hematopoietic recovery in syngeneic and allogeneic hosts. J Clin Invest 101: 961-966.
17. Oikawa A, et al (1997) Development of immunocompetent lymphocytes in vivo from murine umbilical cord blood cells. Transplantation 84: 23-30.
18. Chan B-J, et al (2002) A comparison of murine T-cell-depleted adult bone marrow and full-term fetal blood cells in hematopoietic engraftment and immune reconstitution. Blood 99: 364-371.
19. Talvensaari et al (2002) A broad T-cell repertoire and an efficient thymic function indicate a favorable long-term immune reconstitution after cord blood stem cell transplantation. Blood 99: 1458-1464.

20. Barker JN, et al (2005) Transplantation of 2 partially HLA-matched umbilical cord blood units to enhance engraftment in adults with hematologic malignancy. Blood 105:1343-1347.

21. Nauta AJ, et al (2005) Enhanced engraftment of umbilical cord blood-derived stem cells in NOD/SCID mice by cotransplantation of a second unrelated cord blood unit. Exp Hematol 33:1249-1256