Early development of human hematopoietic and acquired immune systems in new born NOD/Scid/Jak3null mice intrahepatic engrafted with cord blood-derived CD34+ cells

doi: 10.1007/s12185-008-0215-z

Early development of human hematopoietic and acquired immune systems in new born NOD/Scid/Jak3^{null} mice intrahepatic engrafted with cord blood-derived CD34^{+} cells.

Seiji Okada, Hideki Harada, Takaaki Ito, Takashi Saito, and Shinya Suzu,*

Running title: Generation of the human acquired immune system in mice

Main category: A. Erythrocytes, Leukocytes & Hematopoiesis

Subcategory: A. 13) Hematopoiesis-others

Type of manuscript: Rapid communications

S. Okada, H. Harada, S. Suzu

Division of Hematopoiesis, Center for AIDS Research, Kumamoto University

2-2-1, Honjo, Kumamoto, 860-0811, Japan

T. Ito

Department of Pathology and Experimental Medicine, Graduate School of Medical Sciences, Kumamoto University

T. Saito

Laboratory for Cell Signaling, RIKEN Research Center for Allergy and Immunology, Yokohama 230-0045, Japan,

***Address Correspondence to**: Seiji Okada, M.D., Division of Hematopoiesis, Center for AIDS Research, Kumamoto University, 2-2-1 Honjo, Kumamoto 860-0811, Japan.

TEL: +81-96-373-6522 Fax: +81-96-373-6523

e-mail: okadas@kumamoto-u.ac.jp

Abstract

An animal model in which the human immune system can be reconstituted is necessary to study acquired immunity *in vivo*. We report here a novel model, the NOD/SCID/JAK3^{null} mouse, for the human immune system's development. Newborn mice transplanted with human cord blood CD34^{+} cells intrahepatically, developed human T and B cells, and myeloid and plasmacytoid dendritic cells. The T and B cells had a naïve to memory phenotype, and included plasma cells. The human acquired immune system can be reconstituted from CD34^{+} cells in NOD/SCID/JAK3^{null} mice. This model is a powerful tool for the study of human immunity.

Key Words: hematopoietic stem cell, immunodeficient mice, CD34, transplantation

1 Introduction

Xenotransplantation of human cells into immunocompromised mice has become an invaluable tool for studying the function of the human hematopoietic and immune systems [1, 2]. NOD/SCID mice have been widely used for the evaluation of human hematopoietic stem cell activity, because they exhibit deficiencies in NK cell activity, macrophage and DC function, and complement activation as well as T and B cell deficiencies. Recent advances have revealed that the complete abolishment of NK cell activity by anti-NK antibody treatment or intercrossing with NK defective genetically modified mice results in a high degree of engraftment with human hematopoietic cells and T cell development. Notably, recently established NOD/SCID/common γ chain knockout ($\gamma c^{-/-}$) and Balb/C-Rag-$2^{-/-}\gamma c^{-/-}$ mice offered superior engraftment capacity [3-5].

JAK3, a tyrosine kinase crucial for mediating signaling from the common γ-chain of cytokine receptors, is limited to cells that actively participate in the immune response to allografts [6]. Recent tests in stringent preclinical studies suggest that JAK3 inhibitiors are effective in preventing of allograft rejection with a narrow side-effect profile [7]. On the basis of these findings, NOD/SCID were crossed with $JAK3^{null}$ mice [8] to produce a novel strain, NOD/SCID/$JAK3^{null}$, resulting in a complete lack of T, B, NK, and NKT cell function. We successfully reproduced myelo-lymphoid maturation by injecting human cord blood-derived $CD34^{+}$ cells into the liver of newborn NOD/SCID/$JAK3^{null}$ mice. Our data show that the NOD/SCID/$JAK3^{null}$ newborn system provides a valuable tool with which to reproduce human hemato-lymphoid development.

2 Materials and methods

2.1 Mice

The NOD/SCID/JAK3^{null} strain was established by backcrossing JAK3 ^{null} mice [8] with the NOD.Cg-Prkdc^{scid} strain for 10 generations. All experiments were performed according to the guidelines of the Institutional Animal Committee of Kumamoto University.

2.2 Cell preparation and transplantation

Umbilical cord blood cells were collected during normal full-term deliveries after obtaining informed consent, according to institutional guidelines approved by The Faculty of Medical and Pharmaceutical Sciences, Kumamoto University. CD34^{+} cells were isolated using the CD34 Progenitor Cell Isolation Kit (Miltenyi Biotec, Sunnyvale, CA). The purity of the CD34^{+} cells after isolation was >90 %, and contamination of CD3^{+} cells was less than 5%. The CD34^{+} cells ($5x10^{4}$) were transplanted into the liver of irradiated (1.2 Gy) newborn mice.

2.3 Flow cytometry

Mouse spleen cells were stained with DX5-FITC (pan NK marker), mCD122 (IL-2Rβ)-PE, mCD19-APC and mCD3-PE/Cy7 (eBiosciences, San Diego, CA) to detect the murine lymphocytes. Eight to sixteen wks after transplantation, peripheral blood samples were obtained from the retroorbital sinus. At the time of sacrifice, the thymus, the spleen and BM were collected, stained with mAbs for differentiation antigens, and analyzed using LSR II (BD Biosciences, San Jose, CA). The mAbs used were human (h) CD3-APC, hCD4-FITC, hCD11c-APC, hCD33-FITC, hCD34-PE, hCD45-FITC, and mCD45.1-PE (BD Biosciences, San Diego, CA), hCD3-PE/Cy7, hCD20-PE, hCD38-PE, hCD56-PE, hCD123-PE, and hCD138-FITC (eBiosciences),

hCD8-PB, hCCR7-PE, hCD19-PB, hCD27-APC, and hCD45-PB (Dako Cytomation, Glostrup, Denmark), BDCA-1(CD1c)-PE, BDCA-2(CD303)-FITC, BDCA-3(CD141)-FITC (Miltenyi Biotech), IgD-FITC (Sigma, St. Louis, MO), and hCD45RA-ECD (Beckman Coulter, Fullerton, CA). Data were analyzed with FlowJo (Tree Star, San Carlos, CA).

2.4 Cytotoxicity assay

The cytotoxic activity of the murine NK cells was measured by flowcytometry-based cytotoxicity assay with CFSE-labeled Yac-1 cells and propidium iodide (PI) as described by Marcusson-Stahl et al. [9,10]. Briefly, target Yac-1 cells were stained with 2 μM of fluorescence labeling reagent, 5-(6)-carboxy-fluorescein succinimidyl ester (CFSE, Molecular Probes, OR) as described in manufacturer's instruction. Fifty μl aliquots of CFSE-labeled Yac-1 cells ($1x10^5$cell/ml) were placed in 5-ml round-bottomed tubes, and 400 μl of two-fold serial diluted mononuclear cells from spleen was added into the tubes as effector cells. Effector or target cells alone were used as negative controls. After 4 h incubation at 37°C, 50 μl of 20 μg/ ml PI was added and cells were incubated for an additional 15 min. Target cells in FSC (forward scatter) vs. SSC (side scatter) dot plots were gated, and CFSE and PI were measured by LSR II flowcytometer. Cytotoxic activity was calculated as follows: A/(A+B)x100-C (%), where A is the percentage of PI^+EGFP^+ cells; B is the percentage of PI^-GFP^+ cells at each E/T ratio; C is the percentage of spontaneous PI^+ cells without effector cells (A/(A+B)x100 (%) at E/T ratio =0).

2.5 Histologic analysis

Tissue samples were fixed with 10% neutral-buffered formalin embedded in paraffin, cut into 4-μm sections, and immunostained with mouse anti-CD19, anti-CD3, and

antiCD4 antibodies (Dako Cytomation, Carpinteria, CA). After rinsing, the sections were incubated with goat anti-mouse-Ig horseradish peroxidase complex (Nichirei, Tokyo, Japan) for 30 min and visualized with the use of 3,3'-diaminobenzidine (DAKO, Glostrup, Denmark) in 0.05M acetate buffer containing 0.015% H_2O_2.

2.6 Proliferative responses to PHA and human IL-2.

Spleen cells of englafted mice were aseptically prepared as single-cell suspension. The cells were cultured in RPMI-1690 containing 20% human AB plasma with PHA (1 μg/ml) or human IL-2 (200U/ml. Peprotech,Rocky Hill, NJ) at $3x10^5$ cells/100μl in microtiter plate. After incubation for 4 days, Tetrazolium dye methylthiotetrazole (MTT,0.5 mg/ml final conc.) (Sigma) was added to each well. After 3 hrs of additional incubation, 100μl of a solution containing 10% SDS plus 0.01N HCl was added to dissolve the crystal. The absorption values at 570 nm were determined with an automatic ELISA plate reader (Multiskan, Thermo ElectronVantaa, Finland).

3 Results and Discussion

3.1 Development of severe immunodeficient NOD/SCID/JAK3^{null} mice

The NOD/SCID/JAK3^{null} mice were established by backcrossing C57BL/6- JAK3^{null} mice [8] with NOD/SCID mice for 10 generations. Flow cytometric analysis a complete absence of $CD3^+$ T cells and NKT cells, $CD19^+$ B cells, and $DX5^+CD122(IL\text{-}2R\beta)^+$ NK cells in the spleen (Figure 1A). NK cell activity was also hardly detectable in NOD/SCID/JAK3^{null} mice in functional examinations (Figure 1B). NOD strain is known to have functional defects for macrophages and dendritic cells, and compliment deficiency [1, 2]. Thus, NOD/SCID/JAK3^{null} mice have severely impaired immunologic function.

3.2 Early T cell reconstitution in NOD/SCID/JAK3^{null} mice

To establish the human hemolymphoid system, newborn NOD/SCID/JAK3^{null} mice were transplanted intrahepatically with 5x10^4 CD34$^+$ CB cells. Peripheral blood was collected at various intervals for up to 16 wks, and analyzed by flow cytometry for the presence of human cells expressing the common human leukocyte antigen CD45 and the T lineage marker CD3. Seven wks after transplantation, human CD45$^+$ cells were already detected (53.6 ±16.2 %) (Figure 2A, B), though most of them were CD19$^+$ B lymphocytes (data not shown), and only a few CD45$^+$CD3$^+$ T lymphocytes (1.2 ± 0.4 % of hCD45$^+$ cells) were observed. However, human T lymphocytes began to emerge and proliferate after 12 wks and made up to 62.8 ±19.5 % of the CD45$^+$ cells at 16 wks. Representative profiles of CD3$^+$ cell kinetics from the same individual NOD/SCID/JAK3^{null} mice are shown in Fig 2B. The reconstitution of T cells occurred earlier in the in NOD/SCID/JAK3^{null} newborn system than in the adult recipient system, where it usually takes more than 13 wks [3].

3.3 Human acquired immune system reconstitution from CD34$^+$ cells in NOD/SCID/JAK3^{null} mice

At 16 wks after transplantation, the thymus, the spleen and bone marrow were harvested and analyzed by flow cytometry. Myeloid differentiation from cord blood-derived hematopoietic stem cells was observed in the bone marrow of the transplanted NOD/SCID/JAK3^{null} mice, including CD34$^-$CD33$^+$ myeloid cells (Figure 3A), CD14$^+$ monocytes (data not shown), and CD34$^+$ hematopoietic stem cells. Human CD45$^+$ cells comprised 64.2 ± 11.5 % of the spleen cells of engrafted NOD/SCID/JAK3^{null} mice.

The human cells were mainly $CD19^+$ B cells and $CD3^+$ T cells with a few $CD56^+CD3^-$ NK cells and $CD56^+CD3^+$ NKT cells. To further characterize the T lymphocytes emerging in the NOD/SCID/$JAK3^{null}$ mice, cells harvested from thymus and spleen were analyzed for T cell markers. All thymi contained double-positive, as well as CD4 and CD8 single positive, T cells (Fig. 3C). Based on the expression of CD45RA and CCR7, both human $CD4^+$ and $CD8^+$ T cells can be classified as naïve ($CD45RA^+CCR7^+$), central memory ($CD45RA^-CCR7^+$), effector memory ($CD45RA^-CCR7^-$), and terminally differentiated ($CD45RA^+CCR7^-$) cells with different homing and effector capacities [11, 12]. All these fractions were observed in the spleen of transplanted mice. These results demonstrate that human T cell progenitors derived from $CD34^+$ cells can proliferate and differentiate into mature T cells in the NOD/SCID/$JAK3^{null}$ thymic and splenic microenvironment.

As for the development of B cells, 77.8 ± 10.2 % of human $CD45^+$ cells in the bone marrow were $CD19^+$ B cells. Both immature CD20-negative and mature CD20-positive $CD19^+$ B cells were detected in bone marrow of engrafted mice (Figure 3D). In the secondary lymphoid tissue, CD38 and Ig-D have been useful in classifying important developmental stages in the pathway from naïve to memory B cells (Bm1-Bm5) [13]. All of these stages of B cells were detected in spleen of reconstituted mice. Moreover, $CD138^+CD38^+CD27^+$ plasma cells were detected in bone marrow and spleen, indicating that full B cell maturation occurred in reconstituted NOD/SCID/$JAK3^{null}$ mice. Human DCs can be divided into $CD11c^{high}$ myeloid DCs (MDC) and $CD123^+CD303^+$ plasmacytoid DCs [14]. $CD11c^{high}$ myeloid DCs are subdivided into MDC1 and MDC2 by the expression of CD1c and CD141. All three subsets were present in BM and spleen of reconstituted mice. Immunohistochemical staining for the expression of CD20, CD3,

and CD4 showed that human lymphocytes in the spleen of reconstituted mice possessed follicular structures (Figure 3F).

Next, we cultured the unseparated splenocytes with PHA or human IL-2. As shown in Figure 4, splenocytes from these engrafted mice showed a vigous proliferative response to these T cell mitogens. Although we observed human T cell proliferation with mitogen or human IL-2, it is still unknown whether generated T cells have fully functional or not. Indeed, low or absent immune response were observed in HIV-1-exposed immunodeficeint mice transplanted with human CD34+ cells [15-17], which is explained by insufficient MHC selection of human T cells in the mouse thymus and possible lack of some cross reactive cytokines and chemokines in the xenogenic environment [18]. This speculation is supported by the fact that co-transplantation of human fetal/liver tissues and human $CD34^{+}$ cells enabled the mice to induce the antigen-specific T-cell response and T cell dependent antibody production [19]. Detailed analysis and additional ingenuity is needed to induce the functional human immune response.

The Rag-$2^{-/-}\gamma c^{-/-}$ newborn, and NOD/SCID/$\gamma c^{-/-}$ adult and newborn models have already provided definitive evidence that functional T cells, B cells, and DCs can develop from hematopoietic stem cells in immunodeficient mice [3-5], which is recently called “humanized mice” [1,20]. Consistent with these previous models, the NOD/SCID/$JAK3^{null}$ newborn model provided full T and B cell maturation as well as DC development. The engrafted mice looked healthy and there were no signs and symptoms of apparent xenogenic graft versus host disease (GVHD) until 6 months after birth, which is also consistent with previous models [3, 21]. Then the mice were gradually going to die after 6 months with unknown reason. Further histological

examination is needed to clarify the cause of death including the late occurring GVHD or the influence of irradiation at the time of birth. There are two advantages to our NOD/SCID/JAK3^{null} newborn model. First, T cell ontogeny occurs earlier than in adult models. The full T cell reconstitution takes 12 wks after birth in our model (Figure 2), whereas adult models take 23 wks (Transplantation into 7-wk-old adult mice and reconstitution takes 16 wks) [3]. This is extremely important for enabling long term experimentation because the life span of the NOD/SCID strain and irradiated mice is relatively short [22,23]. Second, it is relatively easy to transplant the hematopoietic stem cells into newborn liver via the cutaneous rather than intravenous route [3, 5]. In addition, since relatively small number of CD34^{+} cells ($5x10^4$/mouse) can reconstitute the human hematopoietic and immune system in the NOD/SCID/JAK3^{null} mice reproducibly, a single human stem cell donor can seed sufficient number of mice for testing novel therapeutic strategies such as proof-of-concept mechanistic models and models of human infectious disease.

In summary, we showed that the NOD/SCID/JAK3^{null} newborn system efficiently supports the development of human B and T lymphocytes from cord blood-derived CD34^{+} cells, passing through physiological developmental intermediates, and the development of human dendritic cells. NOD/SCID/JAK3^{null} mice are a powerful and versatile tool that can be used to analyze the human hematopoietic and immune systems in response to infection, cancer, and drug regimens without putting patients at risk.

Acknowledgment We are grateful to Dr. K. Matsui and his coworkers (Fukuda Hospital, Kumamoto, Japan) for providing cord blood. We thank I. Suzu for technical assistance and Y. Endo for secretarial assistance. This work was supported in part by

Health and Labour Sciences Research Grants from the Ministry of Health, Labour and Welfare of Japan (H-16-AIDS-003 and H19-AIDS-003), by grants from the Ministry of Education, Science, Sports, and Culture of Japan, and by a Grant from the Uehara Memorial Foundation.

References

1. Shultz DL, Ishikawa F, Greiner DL. Humanized mice in translational biomedical research. Nature Rev Immunol. 2007;7:118-130.
2. Legrand N, Weijer K, Spits H. Experimental models to study development and function of the human immune system in vivo. J Immunol. 2006;176:2053-2058.
3. Yahata T, Ando K, Nakamura, Y, Ueyama Y, Shimamura K, Tamaoki N et al. Functional human T lymphocyte development from cord blood CD34+ cells in Nonobese Diabetic/Shi-Scid, IL-2 receptor γ null mice. J Immunol. 2002;169:204-209.
4. Traggiai E, Chicha L, Mazzucchelli L, Bronz L, Piffaretti JC, Lanzavecchia A et al. Development of a human adaptive immune system in cord blood cell-transplanted mice. Science. 2004;304:104-107.
5. Ishikawa F, Yasukawa M, Lyons B, Yoshida S, Miyamato T, Yoshimoto G, et al. Development of functional human blood and immune systems in NOD/SCID/IL2 receptor γ chain^{null} mice, Blood.2005;106:1565-1573.
6. Suzuki K, Nakajima H, Saito Y, Saito T, Leonard WJ, Iwamoto I. Janus kinase 3 (Jak3) is essential for common cytokine receptor gamma chain (gamma(c))-dependent signaling: comparative analysis of gamma(c), Jak3, and gamma(c) and Jak3 double-deficient mice, Int Immunol. 2000;12: 123-132.
7. Borie DC, O'Shea JJ, Changelian PS. JAK3 inhibition, a viable new modality of immunosuppression for solid organ transplants. Trends Mol Med. 2004;10:532-541.
8. Park SY, Saijo K, Takahashi T, et al. Developmental defects of lymphoid cells in Jak3 kinase-deficient mice, Immunity. 1995;3:771-782.

9. Marcusson-Stahl M, Cederbrant K. A flow-cytometric NK-cytotoxicity assay adapted for use in rat repeated dose toxicity studies, Toxicology. 2003;193 :269-279.

10. Koga T, Harada H, TS Shi TS, Okada S, Suico MA, Shuto T, et al. Hyperthermia suppresses the cytotoxicity of NK cells via down-regulation of perforin/granzyme B expression. Biochem Biophysys Res Commun. 2005;337:1319-1323.

11. De Rosa SC, Herzenberg LA, Herzenberg LA, Roederer M. 11-color, 13-parameter flow cytometry: identification of human naive T cells by phenotype, function, and T-cell receptor diversity. Nature Med. 2001;7:245-248.

12. Schaerli P, Moser B. Chemokines: control of primary and memory T-cell traffic. Immunol Res. 2005;31:57-74.

13. Bohnhorst JO, Bjorgan MB, Thoen JE, Navtig JB, Thompson KM. Bm1-Bm5 classification of peripheral blood B cells reveals circulating germinal center founder cells in healthy individuals and disturbance in the B cell subpopulations in patients with primary Sjogren's syndrome. J Immunol. 2001;167:3610-3618.

14. Dzionek A, Fuchs A, Schmidt P, Cremer S, Zysk M, Miltenyi S et al. BDCA-2, BDCA-3, and BDCA-4: three markers for distinct subsets of dendritic cells in human peripheral blood. J Immunol. 2000;165:6037-6046.

15. Zhang L, Kovalev GI, Su L. HIV-1 infection and pathogenesis in a novel humanized mouse model. Blood 2007;109:2978-2981.

16. An DS, Poon B, Ho Tsong Fang R, Weijer K, Blom B, Spits H, Chen IS, Uittenbogaart CH. Use of a novel chimeric mouse model with a functionally active human immune system to study human immunodeficiency virus type 1 infection. Clin. Vaccine Immunol. 2007;14:391-396.

17. Watanabe S, Terashima K, Ohta S, Horibata S, Yajima M, Shiozawa Y, Dewan MZ,

Yu Z, Ito M, Morio T, Shimizu N, Honda M, Yamamoto N. Hematopoietic stem cell-engrafted NOD/SCID/IL2Rγ^{null} mice develop human lymphoid systems and induce long-lasting HIV-1 infection with specific humoral immune responses. Blood 2007;109:212-218.

18. Baenziger S, Tussiwand R, Schlaepfer E, Mazzucchelli L, Heikenwalder M, Kurrer MO, Behnke S, Frey J, Oxenius A, Joller H, Aguzzi A, Manz MG, Speck RF. Disseminated and sustained HIV infection in CD34^{+} cord blood cell-transplanted Rag2$^{-/-}$γc$^{-/-}$ mice. Proc Natl Acad Sci USA. 2006;103:15951-15956.
19. Tonomura N, Habiro K, Shimizu A, Sykes M, Yang YG. Antigen-specific human T-cell responses and T cell-dependent production of human antibodies in a humanized mouse model. Blood. 2008;111:4293-4296.
20. Macchiarini F, Manz MG, Palucka K, Shultz LD. Humanized mice: are we there yet ? J Exp Med. 2005;202:1307-1311.
21 Shultz LD, Lyons BL, Burzenski LM, Gott B, Chen X, Chaleff S, Kotb M, Gillies SD, King M, Maggada J, Greiner DL, Hndgretinger R. Human lymphoid and myeloid cell development in NOD/LtSz-scid IL2Rγ^{null} mice engrafted with mobilized human hemopoietic stem cells. J Immunol. 2005:174:6477-6489.
22. Goldman JP, Blundell MP, Lopes L, Kinnon C, Di Santo JP, Thrasher AJ. Enhanced human cell engraftment in mice deficient in RAG2 and common cytokine receptor γ chain. Brit J Haematol. 1998;103:335-342.
23. Watanabe S, Ohta S, Yajima M, Terashima K, Ito M, Mugishima H, Fujiwara S, Shimizu K, Honda M, Shimizu N, Yamamoto N. Humanized NOD/SCID/IL2Rγ^{null} mice transplanted with hematopoietic stem cells under nonmyeloablative conditions show prolonged life spans and allow detailed analysis of human immunodeficiency

virus type 1 pathogenesis. J. Virol. 2007;81:13259-13264.

Figure Legends

Figure 1. Lymphocytes and NK cell activity in NOD/SCID/JAK3^{null} mice.

(A) Spleen cells from mice were stained with DX5-FITC (pan NK marker), CD122(IL-2RB)-PE, CD19-APC and CD3-PE/Cy7. No NK T, and B cells were observed in the spleens of NOD/SCID/JAK3^{null} mice. (B) Absence of NK activity in NOD/SCID?JAK3^{null} mice. Spleen cells from Balb/c, NOD/SCID, and NOD/SCID/JAK3^{null} mice were used. The formula for percentage cytotoxicity is described in "Materials and methods". Each value represents the mean of data from four mice.

Figure 2. Reconstitution of human T lymphocytes in CD34^{+} cell-engrafted NOD/SCID/JAK3^{null} recipients.

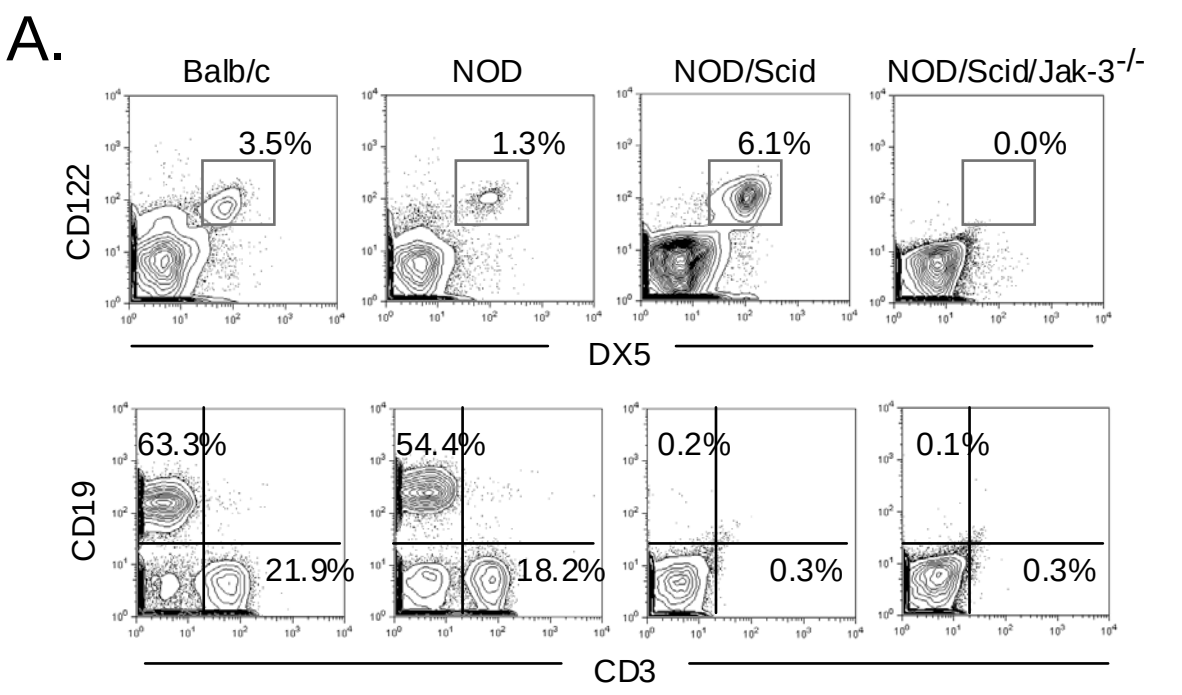
(A) Kinetics of human cells in NOD/SCID/JAK3^{null} newborn mice (a litter, n=8) transplanted with human cord blood CD34^{+} cells. Peripheral blood cells were collected at various intervals from CD34^{+} cell-transplanted mice and stained with anti-mouse CD45.1, and anti-human CD45, CD3, and CD19.. The cells were then analyzed by flow cytometry. Human hematopoietic cells were distinguished from mouse cells by the expression of human CD45. CD3^{+} cells among the human CD45^{+} cells were considered as human T lymphocytes. (B) Representative profiles of CD45^{+}CD3^{+} peripheral blood cells in an individual NOD/Scid/Jak3^{null} newborn mouse transplanted with human cord blood CD34^{+} cells. A representative results from 5 independent experiments.

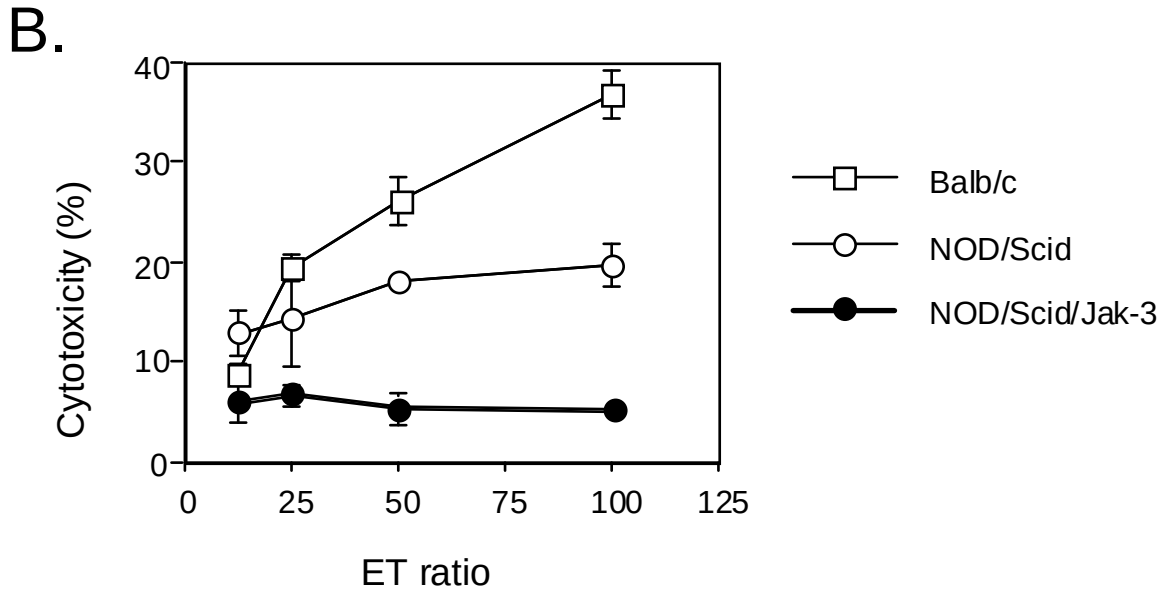
Figure 3. Analysis of human hematopoietic cells in NOD/SCID/JAK3^{null} recipients.

(A) Bone marrow contained $CD33^{-}CD34^{+}$ immature hematopoeitic stem cells and $CD33^{+}$ myeloid progenitors. (B) Spleen consisted of both human ($hCD45^{+}$) and murine ($mCD45.1^{+}$) white blood cells. Human white blood cells consisted of B cells($CD19^{+}$), T cells ($CD3^{+}$), NK cells ($CD3^{-}CD56^{+}$) and NKT cells ($CD3^{+}CD56^{-}$). (C) The majority of cells in the thymus were CD4/CD8 double positive thymocytes. The CD3+ spleen cells included CD4+ or CD8+ single positive mature T cells. Both CD4+ and CD8+ T cells included naïve ($CD45RA^{+}CCR7^{+}$), central memory ($CD45RA^{-}CCR7^{+}$), effector memory ($CD45RA^{-}CCR7^{-}$), and terminally differentiated ($CD45RA^{+}CCR7^{-}$) cells. (D) Bone marrow contained $CD19^{+}CD20^{-}$ immature and $CD19^{+}CD20^{+}$ mature B cells. Naïve ($CD19^{+}Ig\text{-}D^{+}CD38^{+}$) to memory ($CD19^{+}Ig\text{-}D^{-}CD38^{-}$) B cell differentiation was observed in the spleen. $CD138^{+}CD38^{+}CD27^{+}$ plasma cells were detected in bone marrow. (E) BM contained $hCD45^{+}CD11c^{+}$ human DCs. CD11c+ DCs included $CD1c^{+}$ MDC1 and $CD141^{+}$ MDC2. BM also contained CD123/CD303 double positive human plasmacytoid DCs. (F) Immunohistochemistry of engrafted NOD/Scid/$Jak3^{null}$ mouse spleen showed follicular structures in $CD20^{+}$ and $CD3^{+}$ human lymphocytes.

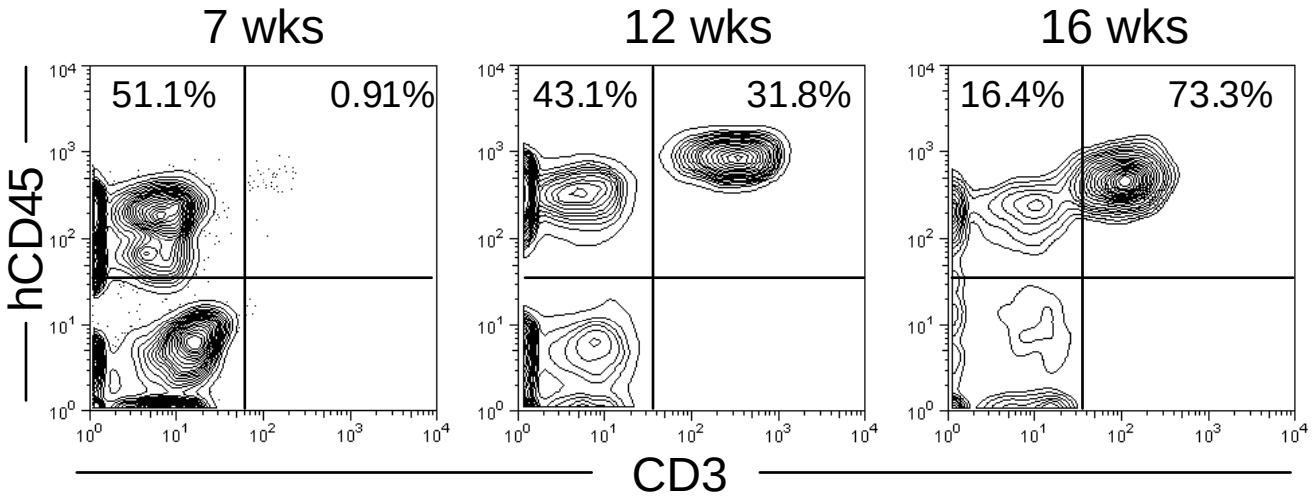
Figure 4. Proliferative response of spleen cells from engrafted NOD/SCID/$JAK3^{null}$ mice.

Proliferative response of unfractionated splenocytes obtained from 16 weeks old NOD/SCID/$JAK3^{null}$ mice. Data shown are mean ± SD of three mice.

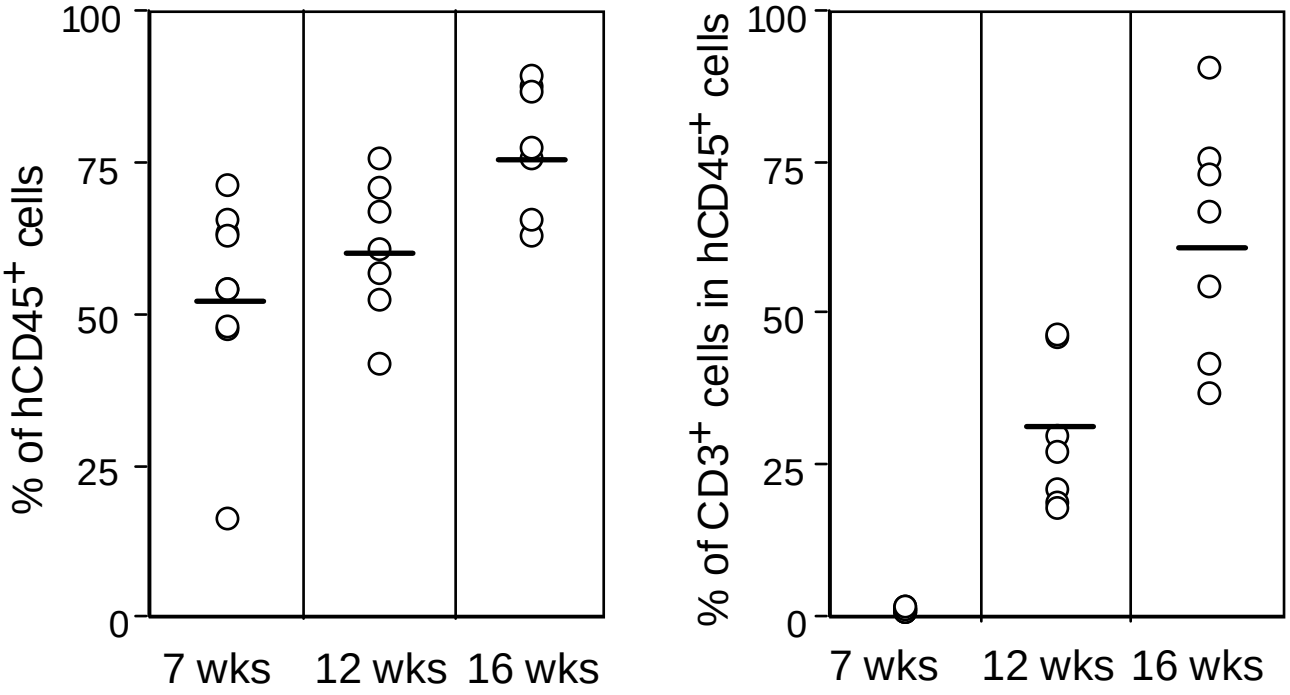

A.
Balb/c
NOD
NOD/Scid
NOD/Scid/Jak-3-/-
CD122
3.5%
1.3%
6.1%
0.0%
DX5
CD19
63.3%
21.9%
54.4%
18.2%
0.2%
0.3%
0.1%
0.3%
CD3


B.
Cytotoxicity (%)
0
10
20
30
40
0
25
50
75
100
125
ET ratio
Balb/c
NOD/Scid
NOD/Scid/Jak-3

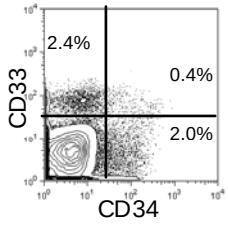
A.


7 wks
12 wks
16 wks
51.1%
0.91%
43.1%
31.8%
16.4%
73.3%
hCD45
CD3

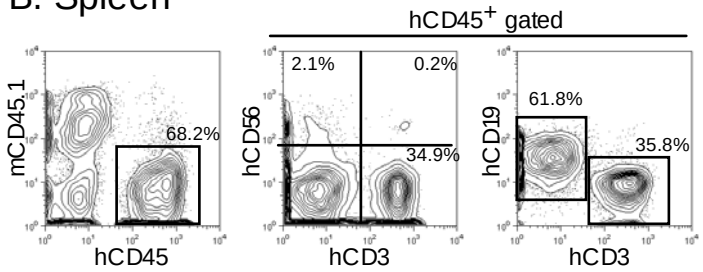
B.


% of hCD45+ cells
% of CD3+ cells in hCD45+ cells
100
75
50
25
0
7 wks
12 wks
16 wks

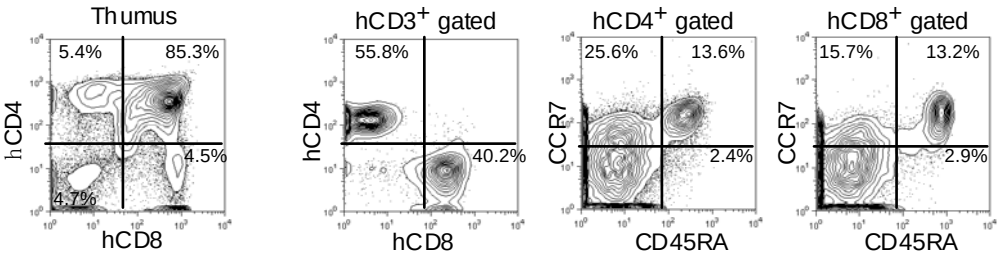
A.Bone marrow



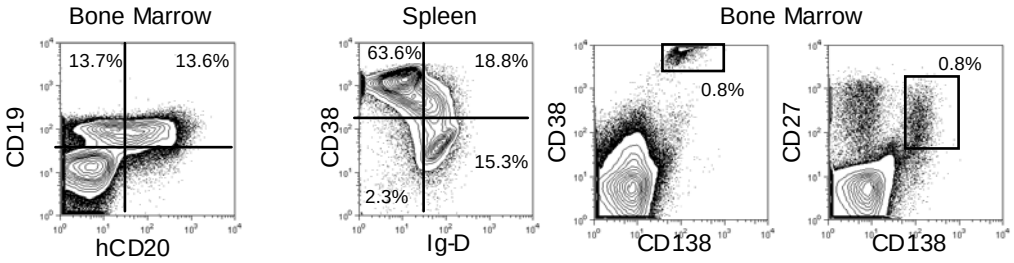
B. Spleen



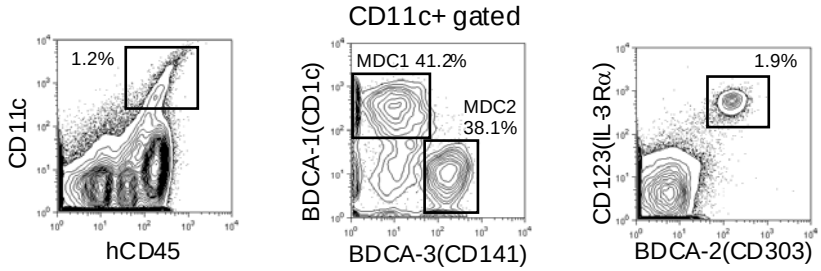
C. T lymphocytes



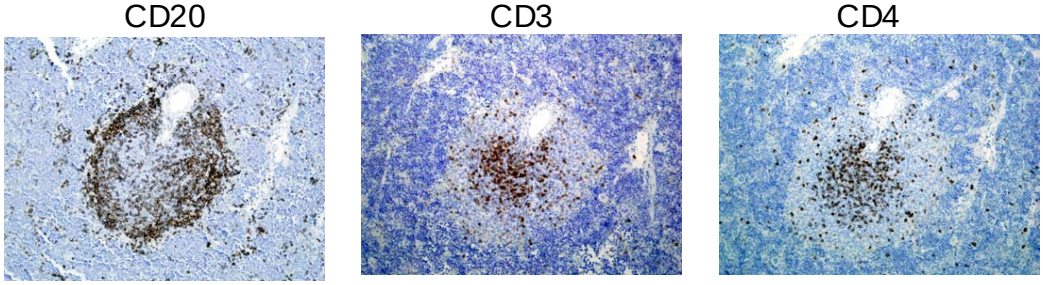
D. B lymphocytes

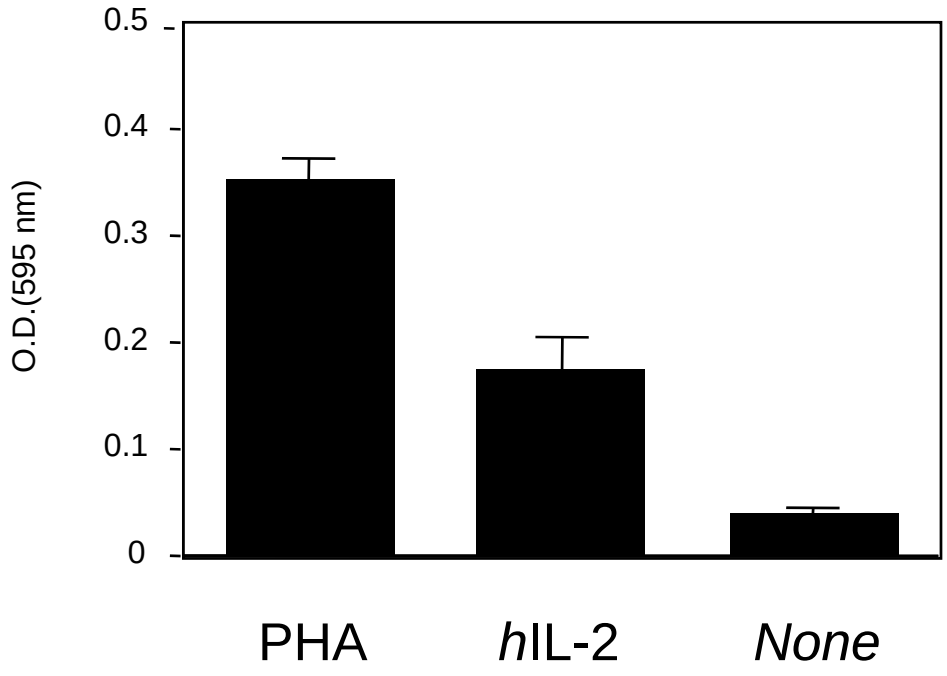


E. Dendritic cells



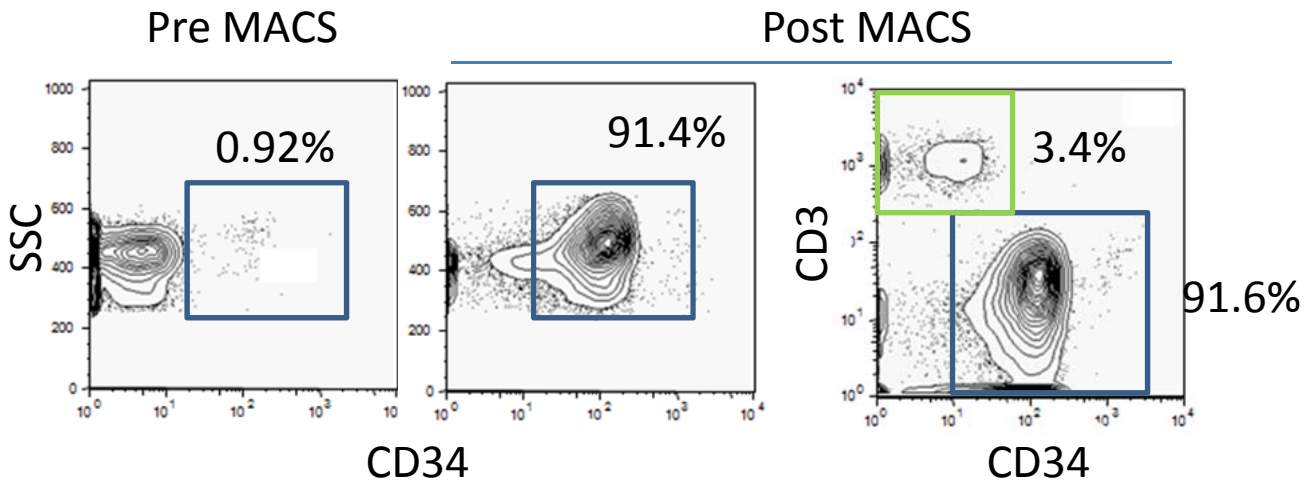
F. Spleen




0.5
0.4
0.3
0.2
0.1
0
O.D.(595 nm)
PHA
hIL-2
None

Supplement 1.

Enrichment of CD34+ cells by MACS



Percent of CD34+ cells were 0.3-1.5% / cord blood derived mononuclear cells.

Cord blood derived mononuclear cells include 0.3-1.5 % of CD34+ cells. We used the CD34+ cells when the purity of CD34+ cells are >90% and contaminated CD3+ cells are <5%.