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Propagating Humanized BLT Mice for the Study of Human Immunology and Immunotherapy

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The humanized bone marrow-liver-thymus (BLT) mouse model harbors a nearly complete human immune system, therefore providing a powerful tool to study human immunology and immunotherapy. However, its application is greatly limited by the restricted supply of human $CD34^+$ hematopoietic stem cells and fetal thymus tissues that are needed to generate these mice. The restriction is especially significant for the study of human immune systems with special genetic traits, such as certain human leukocyte antigen (HLA) haplotypes or monogene deficiencies. To circumvent this critical limitation, we have developed a method to quickly propagate established BLT mice. Through secondary transfer of bone marrow cells and human thymus implants from BLT mice into NSG (NOD/SCID/IL-2R$\gamma^{-/-}$) recipient mice, we were able to expand one primary BLT mouse into a colony of 4–5 proBLT (propagated BLT) mice in 6–8 weeks. These proBLT mice reconstituted human immune cells, including T cells, at levels comparable to those of their primary BLT donor mouse. They also faithfully inherited the human immune cell genetic traits from their donor BLT mouse, such as the HLA-A2 haplotype that is of special interest for studying HLA-A2-restricted human T cell immunotherapies. Moreover, an EGFP reporter gene engineered into the human immune system was stably passed from BLT to proBLT mice, making proBLT mice suitable for studying human immune cell gene therapy. This method provides an opportunity to overcome a critical hurdle to utilizing the BLT humanized mouse model and enables its more widespread use as a valuable preclinical research tool.

Keywords: humanized BLT mice, human immunology, human immunotherapy, propagating, CD34, HSC

Introduction

Small animal models that harbor a human immune system and support human immune cell development are valuable tools for the study of human immunology and the preclinical development of human immunotherapy. In the past decades, various humanized mouse models were developed toward enabling the long-term engraftment of a complete human immune system [1–3]. As the first step, immunodeficient mouse strains were identified or developed that accept xenogeneic transplanted human immune cells. These mouse strains carry spontaneous or targeted mutations of immune regulatory genes such as the *Prkdc* gene (spontaneous mutation in SCID or NOD/SCID mice) or *RAG1*/*RAG2* genes that are involved in the formation of antigen receptors on B and T cells, or the IL-2 receptor gamma-chain (*IL-2Rγ*) gene that is the common signaling subunit of the receptors for multiple cytokines (IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21) and is, thus, involved in the development of lymphoid and natural killer (NK) cells [1–3]. At present, NOD/SCID/IL-2R$\gamma^{-/-}$ (NSG) or BALB/c/RAG2$^{-/-}$/IL-2R$\gamma^{-/-}$ (BRG) mice are the standard recipients in generation of humanized mice, because they are deficient of murine T cells, B cells, and NK cells. NSG mice have shown stronger engraftment, presumably due to their expression of a signal regulatory protein alpha (*SIRP-α*) gene that is more homologous to the human *SIRP-α* gene and that delivers a more effective ''do-not-eat-me'' signal resulting in decreased phagocytosis of engrafted human cells [4,5]. As the second step, various human immune cell xenograft protocols were developed. The initial protocol involves injecting human peripheral blood mononuclear leukocytes (PBL) into SCID

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mice. The resulting Hu-PBL-SCID mice contain limited lineages of human immune cells and do not support de novo human immune cell development [6]. Transfer of human $CD34^+$ hematopoietic stem and progenitor cells (HSPCs) into newborn NSG or BRG mice results in long-term engraftment of $CD34^+$ cells and reconstitution of multilineage human immune cells [7,8]. However, a key limitation of such HIS (human immune system) mice is the aberrant development of human T cells in the mouse thymus that affects the MHC selection and functional maturation of human T cells [7,8]. Human T cells generated in these HIS mice are selected on mouse MHC molecules, making them incapable of recognizing human antigen-presenting cells (APCs) and mounting human HLA-restricted T cell responses [7,8]. The issue of T cell education was circumvented by another protocol wherein transfer of human fetal liver and thymus tissue under the kidney capsule of recipient SCID mice gave rise to a human organoid that was capable of supporting proper human T cell development [9]. Although human T cells are abundant in these SCID-hu mice, they are mainly confined to the organoid and, thus, the reconstitution of the other organs is poor [9]. The most comprehensive transfer protocol combined the strengths of these early protocols and involves the transfer of human $CD34^+$ cells into the most supportive NSG recipient mice, as well as implanting human fetal thymus and liver tissue under the kidney capsule of these mice [10,11]. The resulting humanized mice, named bone marrow-liver-thymus (BLT) mice, support the long-term engraftment and systemic reconstitution of a nearly complete human immune system, including multilineage human adaptive and innate immune cells consisting of T cells, B cells, NK cells, dendritic cells, and macrophages [10,11]. Importantly, human immune cells developed in BLT mice, especially T cells, are functional, and they have shown productive responses to skin xenografts and various viral/bacterial infections [10–13].

Because it supports the development and maintenance of a nearly complete and functional human immune system, the humanized BLT mouse model is a promising tool to study human hematopoiesis and immune cell activities under healthy and disease conditions [1,2]. It is particularly useful for studies of human immunodeficiency virus (HIV) infection because of the high frequencies of human T cells in the lymphoid and mucosal tissues of BLT mice, as well as because of the proper maturation status and lineage differentiation of these human T cells [14–16]. To date, studies using BLT mice have generated valuable knowledge in many aspects of HIV infection, including prevention, mucosal transmission, HIV-specific innate and adaptive immunity, viral latency, and novel anti-retroviral and immune-based therapies for suppression and reservoir eradication [14–17]. The humanized BLT mouse model is also ideal for the study of hematopoietic stem cell (HSC)- and T cell-based immunotherapies, because of the long-term engraftment of human HSCs and T cells in BLT mice [10,11]. We and others have utilized BLT mice for the preclinical development of gene-modified HSC-based immunotherapies for treating cancer and HIV [18,19]. Despite its potential as a valuable research tool, the application of BLT mice is greatly limited by the restricted supply of human $CD34^+$ cells and human fetal liver and thymus tissues that are required to generate these mice. The restriction is especially problematic for the study of human immune systems with special genetic traits, such as certain human leukocyte antigen (HLA) haplotypes or immune monogene deficiencies [19–22].

To circumvent this critical limitation, we have developed a method to quickly propagate established BLT mice without the need of additional human tissues. We hypothesized that human $CD34^+$ cells engrafted into the bone marrow of a primary BLT mouse retained their HSC potential and could repopulate a human immune system in multiple naïve NSG mice through secondary bone marrow transfer; meanwhile, the human thymus organoid established in a primary BLT mouse maintained a human thymus structure and could be split and transplanted into the secondary recipient NSG mice to provide a human thymus microenvironment supporting proper human T cell development. In the present article, we demonstrated the feasibility of this new method to expand a single primary BLT mouse into a colony of 4–5 proBLT (propagated BLT) mice in 6–8 weeks, and we provided evidence to support the potential research value of these proBLT mice.

Materials and Methods

Mice and materials

NOD.Cg-Prkdc^{SCID}Il2rg^{tm1Wjl}/SzJ (NOD/SCID/IL-2R$\gamma^{-/-}$, NSG) mice were purchased from the Jackson Laboratory and maintained in the animal facilities at the University of California, Los Angeles (UCLA). Six- to 10-week-old females were used for all experiments, unless otherwise indicated. All animal experiments were approved by the Institutional Animal Care and Use Committee of UCLA.

X-VIVO-15 cell culture medium was purchased from Lonza. Recombinant human Flt3 ligand, stem cell factor (SCF), thrombopoietin (TPO), IL-3, and Fixable Viability Dye eFluor506 were purchased from affymetrix eBioscience. Retronectin® was purchased from Clontech.

Antibodies and flow cytometry

Fluorochrome-conjugated antibodies that were specific for human CD45, TCR$\alpha\beta$, CD11b, CD11c, CD14, CD19, CD56, and HLA-A2 were purchased from BioLegend; those specific for human CD34 were purchased from BD Biosciences. Human Fc Receptor Blocking Solution (TruStain FcX™) was purchased from BioLegend, whereas mouse Fc Block (anti-mouse CD16/32) was purchased from BD Biosciences. Cells were stained as previously described and analyzed by using an MACSQuant Analyzer 10 flow cytometer (Miltenyi Biotec) [23]. FlowJo software was used to analyze the data.

Tissue processing for flow cytometry analysis

For flow cytometry analysis, all tissues were processed into mononuclear cells (MNCs) and lysed of red blood cells (RBCs). Blood and bone marrow samples were directly lysed with Tris-buffered ammonium chloride (TAC) buffer, following a standard protocol (Cold Spring Harbor Protocols). Spleens were smashed against a 70 μm cell strainer (Corning) to prepare single cells and then lysed with TAC. Livers were cut into small pieces by using a pair of scissors, smashed against a 70 μm cell strainer to prepare single cells, and passed through a 33% Percoll gradient isolation (Sigma) to remove hepatocytes, followed by TAC lysis.

Human CD34$^+$ cells and thymus tissues

Human fetal liver CD34$^+$ HSPCs, as well as fetal thymus tissues, were obtained from the CFAR Gene and Cellular Therapy Core Laboratory at UCLA, without identification information under federal and state regulations. CD34$^+$ cells were sorted from fetal liver cells through magnetic-activated cell sorting by using a Direct CD34 Progenitor Cell Isolation Kit (Miltenyi Biotec) following the manufacturer's instructions [18]. The purity of CD34$^+$ cells was more than 97%, as evaluated by flow cytometry.

pMND-EGFP lentiviruses

pMND-EGFP lentiviral vector was constructed by inserting an EGFP reporter gene into the lentivector that contains the MND retroviral LTR U2 region as an internal promoter [24]. VsVg-pseudotyped pMND-EGFP lentiviruses were produced by using HEK293.T cells, following a standard calcium precipitation protocol and an ultracentrifugation concentration protocol as previously described [25].

Generation of humanized BLT Mice

Humanized BLT mice were generated as previously described, with certain modifications [10,11]. In brief, human CD34$^+$ cells were cultured for no more than 48 h in X-VIVO-15 cell culture medium containing recombinant human Flt3 ligand (50 ng/mL), SCF (50 ng/mL), TPO (50 ng/mL), and IL-3 (20 ng/mL) in nontissue culture-treated plates coated with Retronectin. Viral transduction, when applicable, was performed at 24 h by adding concentrated pMND-EGFP lentiviruses directly to the culture medium. At around 48 h, CD34$^+$ cells were collected and *i.v.* injected into NSG recipient mice (~ 0.5–1×10^6 CD34$^+$ cells per recipient) that had received 270 rads of total body irradiation. Then, 1–2 fragments of human fetal thymus ($\sim 1\,mm^3$), as well as donor-matched fetal liver CD34$^-$ cells, when available ($\sim 4.5 \times 10^6$), were implanted under the kidney capsule of each recipient NSG mouse. The mice were maintained on trimethoprim/sulfmethoxazole (TMS) chow in a sterile environment for 8–12 weeks until analysis or use for further experiments.

Generation of humanized proBLT Mice

Humanized proBLT (propagated BLT) mice were generated through adoptive transfer of bone marrow cells and human thymus organoid implant fragments from BLT mice into secondary recipient NSG mice that had received 270 rads of total body irradiation. On the day of transfer, one primary BLT mouse was dissected. Total bone marrow cells harvested from the femur and tibia of the BLT mouse were split equally and *i.v.* injected into 4–5 recipient NSG mice. On average, about 40–50×10^6 total bone marrow cells were harvested from each primary BLT donor mouse, whereas about 10×10^6 total BLT bone marrow cells were given to each NSG recipient mouse. Meanwhile, human thymus organoid was dissected out from the kidney capsule of a BLT mouse, cut into pieces of $\sim 1\,mm^3$, and surgically implanted under the kidney capsule of the secondary recipient NSG mice (1–2 human thymus organoid pieces per recipient). The mice were maintained on TMS chow in a sterile environment for 8–12 weeks until analysis or use for further experiments.

Immunohistology

Primary human fetal thymus tissue or human thymus organoid implants dissected out from the experimental BLT or proBLT mice were fixed in 10% neutral-buffered formalin and embedded in paraffin for sectioning (4 μm thickness), followed by hematoxylin and eosin (H/E) staining or antibody staining (for human CD45 or CD3) by using standard procedures (UCLA Translational Pathology Core Laboratory). The sections were imaged by using an Olympus BX51 upright microscope equipped with an Optronics Macrofire CCD camera (AU Optronics) at 40×, 100×, and 400× magnifications. The images were analyzed by using Optronics PictureFrame software (AU Optronics).

Statistical analysis

Student's two-tailed t test was used for paired comparisons. Data are presented as mean ± SEM, unless otherwise indicated. $P < 0.05$ was considered significant.

Results

Generation of BLT mice

We generated humanized BLT mice by following previously established procedures, with certain modifications (Materials and Methods section; Fig. 1A). In brief, cryopreserved human fetal liver CD34$^+$ cells were thawed and cultured for no more than 48 h in X-VIVO-15 medium containing recombinant human Flt3 ligand, SCF, TPO, and IL-3. Viral transduction, when applicable, was performed at 24 h by adding concentrated lentiviral vectors directly to the culture medium. At around 48 h, CD34$^+$ cells were collected and *i.v.* injected into NOD/SCID/IL-2Rγc$^{-/-}$ (NSG) recipient mice (~ 0.5–1×10^6 CD34$^+$ cells per recipient) that had received 270 rads of total body irradiation. On the same day, 1–2 fragments of human fetal thymus ($\sim 1\,mm^3$), as well as donor-matched fetal liver CD34$^-$ cells, when available ($\sim 4.5 \times 10^6$), were implanted under the kidney capsule of each recipient NSG mouse. The resulting BLT mice were allowed to reconstitute a human immune system, whereas periodic bleedings were performed to monitor the presence of human immune cells (gated as hCD45$^+$, Fig. 1B, D). We started to detect human immune cells in BLT mice at 4 weeks post human tissue transplantation. The levels of human immune cells gradually increased over time and then peaked and stabilized at around week 12 (Fig. 1B). In our experiments, we routinely obtained ∼30–80% human immune cell reconstitution in the blood of BLT mice. Data from a representative experiment showing ∼40% human immune cell reconstitution are presented (Fig. 1B, D). In our studies, we found that transfer of fetal liver (either tissue fragments or CD34$^-$ cells) was optional for making BLT mice. We also found that human CD34$^+$ cells and human fetal thymus used for making BLT mice did not need to be donor matched, making it flexible to use CD34$^+$ cells isolated from fetal liver, cord blood, adult bone marrow, or G-CSF-mobilized adult peripheral blood. The reconstitution efficiency was ranked as the following: fetal liver CD34$^+$ cells > cord blood CD34$^+$ > adult CD34$^+$ cells (data not shown). In this article, data from BLT mice made with fetal liver CD34$^+$ cells (either donor matched or -unmatched with

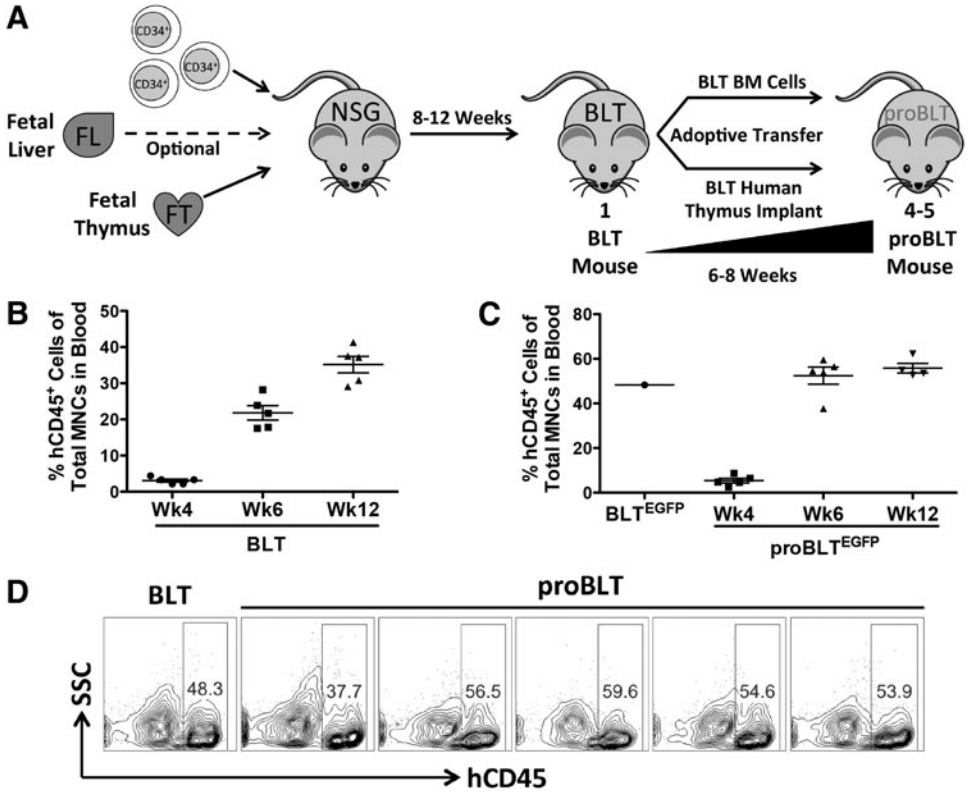

FIG. 1. Generation of proBLT mice through secondary transfer of bone marrow cells and human thymus implants from primary BLT mice to naïve NSG mice. The experiments were repeated over six times. Representative results are presented. **(A)** Schematic representation of the experimental design to generate humanized BLT and proBLT mice. **(B)** Scatter plot showing the time-course detection of human $CD45^+$ cells (gated as $hCD45^+$ of total MNCs) in the blood of BLT mice post human tissue transplantation ($n=5$). Data are presented as individual mouse measurements and mean ± SEM of all experimental mice at the indicated time points. **(C)** Scatter plot showing the time course detection of human $CD45^+$ cells (gated as $hCD45^+$ of total MNCs) in the blood of proBLT mice post secondary transfer of bone marrow cells and human thymus implants from a single primary BLT donor mouse ($n=4$–5). Data are presented as individual mouse measurements and mean ± SEM of all experimental mice at the indicated time points. **(D)** Representative FACS plots showing the presence of human $CD45^+$ cells in the blood of proBLT mice at 6 weeks post secondary transfer ($n=5$). NSG, NOD/SCID/IL-2R$\gamma^{-/-}$ mice; BLT, human bone marrow-liver-thymus implanted NSG mice; proBLT, propagated BLT mice; BM, bone marrow; MNCs, mononuclear cells.

fetal thymus tissue) are presented. Notably, when nondonor matched $CD34^+$ cells and fetal thymus are used to produce BLT mice, such BLT mice can still be valuable tools to study human immune cell development, but certain precautions need to be taken when using these mice to study human T cell immunity. In these BLT mice, T cell function may be affected by the HLA mismatch between human T cells developed in these mice (selected on fetal thymus HLAs) and human APCs generated in these mice (derived from $CD34^+$ cells and, therefore, carrying their HLAs). For certain research, a partial HLA match (e.g., HLA-$A2^+$) may still allow for the study of defined types of T cell responses (e.g., HLA-A2-restricted T cell response).

Generation of propagated BLT mice

Humanized propagated BLT (proBLT) mice were generated through secondary transfer of bone marrow cells and human thymus organoid fragments harvested from BLT mice into naïve recipient NSG mice that had received 270 rads of total body irradiation (Fig. 1A). Primary BLT mice at 12 weeks post primary human tissue transplant were used as donor mice. Total bone marrow cells harvested from one BLT mouse (~ 40–50×10^6 cells) were split equally and *i.v.* injected into 4–5 recipient NSG mice ($\sim 10\times10^6$ cells per recipient). Meanwhile, human thymus organoid dissected out from the BLT mouse was cut into pieces of $\sim 1\,mm^3$, and it was then surgically implanted under the kidney capsule of the recipient NSG mice (1–2 human thymus organoid pieces per recipient). The resulting proBLT mice were allowed to reconstitute a human immune system, whereas periodic bleedings were performed to monitor the generation of human immune cells (gated as $hCD45^+$; Fig. 1C, D). Interestingly, human immune cells were reconstituted more quickly in proBLT mice compared with those in BLT mice, peaking and stabilizing at around week 6 (Fig. 1C). The faster human immune cell reconstitution in proBLT mice was likely due to their inheritance of both early and intermediate human hematopoietic progenitor cells, as well as mature human immune cells, from the bone marrow of donor BLT mice. Notably, because the bone marrow cells and human thymus organoid implant harvested from a single BLT mouse could be used to generate 4–5

proBLT mice, these proBLT mice contain a human immune system including a human T cell educational microenvironment genetically identical to that of their donor BLT mouse and, thus, can be considered ''clonal'' (Fig. 1A). Post stabilization, the human immune cell reconstitution levels are consistent among individual ''clonal'' proBLT mice and similar to those of the primary BLT donor mouse (Fig. 1C, D). Therefore, the proBLT approach allows the expansion of established BLT mice to a large homogeneous colony in a short period of time, while also maintaining the ''clonal'' nature of the engrafted human immune system. These features are especially attractive for studies that are large scale and require specific genetic traits of human immune cells such as MHC haplotypes or immune monogene deficiencies [19–22].

Reconstitution of multilineage human immune cells in proBLT mice

To study the human immune cell reconstitution in proBLT mice, we performed a systemic analysis of these mice in comparison with their ''parental'' BLT mouse. Data from a representative analysis are presented in Fig. 2. High percentages of human immune cells were detected in all immune-homing tissues of proBLT mice, including peripheral blood (~80%), central lymphoid organs such as bone marrow (~80%) and spleen (~80%), and immune regulatory organs such as liver (>90%), at levels similar to those of their primary donor BLT mouse (Fig. 2A). Lineage analysis revealed the reconstitution of a nearly complete human immune system in these proBLT mice, including adaptive immune cells such as TCR$\alpha\beta^+$ T cells and $CD19^+$ B cells, as well as innate immune cells such as $CD56^+$ natural killer (NK) cells, $CD11b^+$ myeloid cells, $CD11c^+$ dendritic cells, and $CD14^+$ monocytes/macrophages, with a composition similar to that of the primary BLT mouse (Fig. 2B–D). Notably, in the primary BLT mice, we detected high numbers of human $CD34^+$ HSPCs (gated as $hCD45^+$ Lin^-hCD34^+) that were enriched in the bone marrow (comprising ~10% of total $hCD45^+$ cells) but not in other tissues such as liver and spleen (comprising <0.4% of total $hCD45^+$ cells) (Fig. 2B). This observation suggests that the initial transplants of human fetal liver $CD34^+$ HSPCs were able to home to the proper HSC niche in the recipient NSG mice and expand, while still maintaining their characteristic longevity and multi-potential to repopulate a nearly complete human immune system. Encouragingly, a similarly high percentage of human $CD34^+$ HSPCs (~10%) were detected in the bone marrow of proBLT mice, indicating the ability of these human $CD34^+$ HSPCs to survive the secondary bone marrow transfer and to repopulate the secondary NSG recipient mice, which was key to the success of the proBLT method (Fig. 2C).

Reconstitution of human thymus and human T cells in proBLT mice

The most attractive feature of the BLT model is its capacity to support long-term systemic reconstitution of properly matured human T cells, which benefits from the presence of an authentic human thymus component [10,11]. Post insertion under the kidney capsule of NSG recipient mice, the implanted human fetal thymus fragments (~1 mm^3 in size; 1–2 pieces per implantation site) grow into human thymus organoids that support human T cell development [10,11]. In our experiments, these human thymus organoids could grow to a size of ~20–200 mm^3. The image of a representative organoid is presented in Fig. 3A. When generating proBLT mice, such an organoid was cut into ~1 mm^3 fragments and 1–2 pieces of these fragments were implanted under the kidney capsule of each secondary NSG recipient mouse (Materials and Methods section). In proBLT mice, secondary human thymus organoids were observed and grown to a size similar to that of the primary BLT mice. An immunohistology study revealed that both the BLT and proBLT human thymus organoids displayed a typical human thymus structure similar to that of the primary human fetal thymus, comprising a cortex region that could support the positive selection of human thymocytes for HLA recognition, and a medulla region that could support the negative selection of thymocytes for autoreactive T cell depletion (Fig. 3B, C). These human thymus organoids were populated with developing human thymocytes that had undergone T cell receptor (TCR) selection, evidenced by the positive immunochemical staining for human CD45 and CD3 markers (Fig. 3B). Interestingly, in the medulla of these human thymus organoids, we also observed abundant numbers of Hassall's corpuscles (HCs), structures that are unique to human thymus and have been implicated in regulating the development of human $FoxP3^+$ regulatory T cells (Fig. 3C) [26]. These results indicate the reconstitution of a proper human thymus microenvironment in both BLT and proBLT mice.

Next, we analyzed the development and reconstitution of human T cells in proBLT mice. Similar to that in the human thymus organoid of BLT mice, human thymocytes (gated as $hCD45^+$) in the human thymus organoid of proBLT mice expressed rearranged human $\alpha\beta$ TCR receptors (stained as $hTCR\alpha\beta^+$), and they seemed to follow a classical human T cell developmental path from DP (gated as $CD4^+CD8^+$) to CD4 or CD8 SP (gated as $CD4^+CD8^-$ or $CD4^-CD8^+$, respectively) stages (Fig. 3D) [27]. Large numbers of mature human T cells (gated as $hCD45^+hTCR\alpha\beta^+$) were detected in various peripheral tissues of proBLT mice, including blood, spleen, bone marrow, and liver (Figs. 2 and 3). These T cells comprised both $CD4^+$ helper and $CD8^+$ cytotoxic T cell subsets, at a ratio similar to that observed in the primary BLT mice (Fig. 3E, G, H). Therefore, proBLT mice are able to support the proper development and systemic reconstitution of human T cells, at levels comparable to those of the primary BLT mice.

Inheritance of human immune cell genetic traits from BLT to proBLT mice

Certain genetic traits, such as HLA haplotypes that play important roles in regulating the development and functionality of human T cells, are critical for the study of human immunity [28]. For example, our knowledge of antigen-specific human T cell responses to viral infections and cancers are largely based on the studies of HLA-A2-restricted T cell reactions [29]. Humanized BLT mice that harbor a human immune system of HLA-A2 haplotype (denoted as BLT^{A2+}) are valuable tools for studying HLA-A2-restricted T cell responses and developing T cell-based immunotherapies. However, the supply of human $CD34^+$ cells and fetal thymus tissues of HLA-A2 haplotype needed

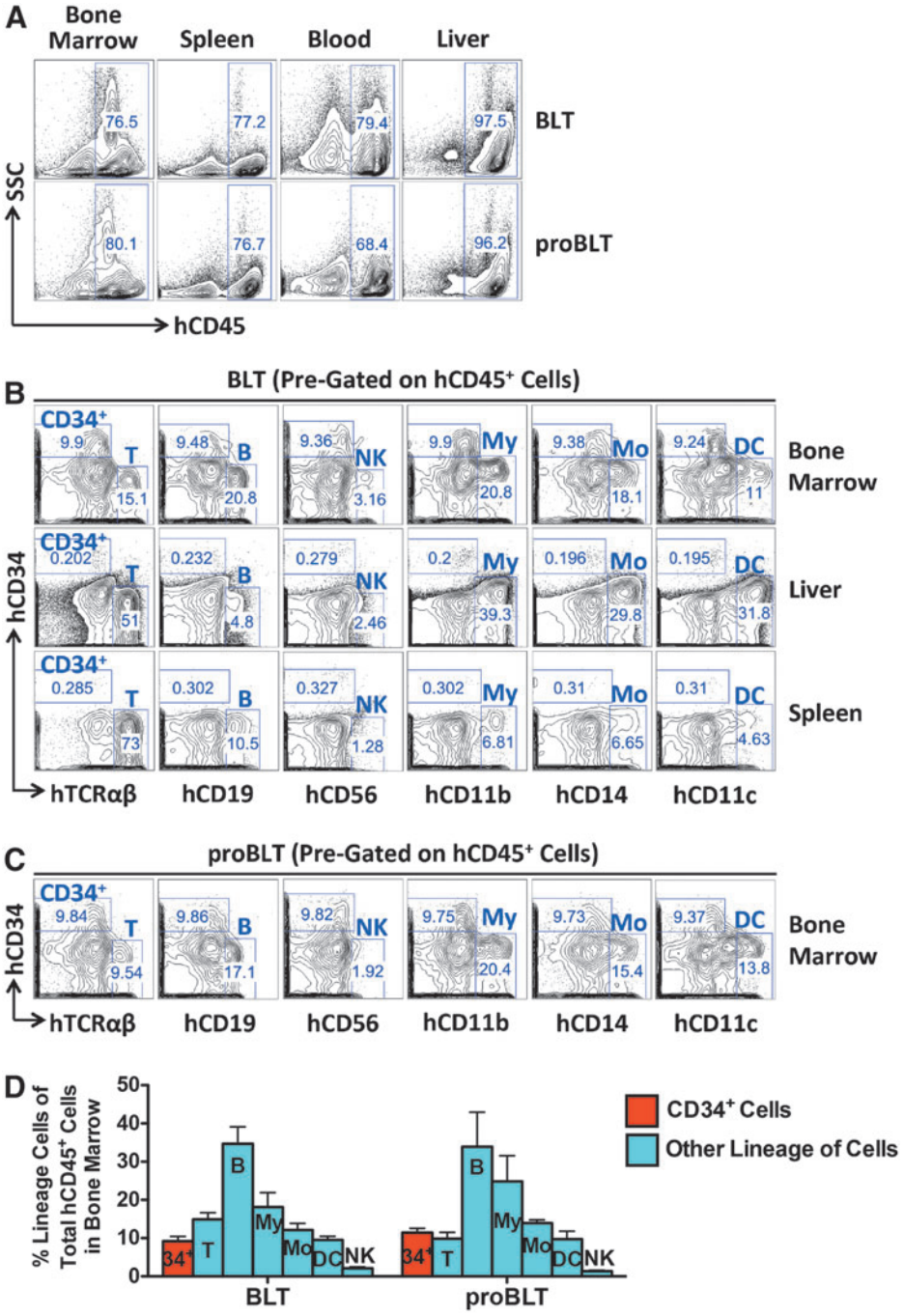

FIG. 2. Reconstitution of multilineage human immune cells in proBLT mice. The experiments were repeated at least three times. Representative results are presented from BLT and proBLT mice at 8–12 weeks post the primary human tissue or secondary BLT tissue transfer ($n = 4$–5 per experimental group). All tissues were processed into mononuclear cells and lysed of red blood cells for flow cytometry analysis (Materials and Methods section). **(A)** FACS plots showing the detection of human $CD45^+$ cells (gated as $hCD45^+$ cells of total mononuclear cells) in various lymphoid and immune-regulatory tissues of BLT and proBLT mice. **(B)** FACS plots showing the detection of multilineage human immune cells (pregated on human $CD45^+$ cells) in various lymphoid and immune-regulatory tissues of BLT mice. Note the homing of human $CD34^+$ cells to the bone marrow of BLT mice. **(C)** FACS plots showing the detection of multilineage human immune cells (pregated on human $CD45^+$ cells) in the bone marrow of proBLT mice. Note the persistence of human $CD34^+$ cells in proBLT mice post secondary transfer of BLT mice bone marrow cells. **(D)** Bar graphs showing the comparison of human immune cell composition in the bone marrow of BLT and proBLT mice. Data are presented as mean ± SEM. $CD34^+(34^+)$: $CD34^+$ hematopoietic stem and progenitor cells (gated as $hCD45^+Lin^-hCD34^+$); T, T cells (gated as $hCD45^+hTCR\alpha\beta^+$); B, B cells (gated as $hCD45^+hCD19^+$); NK, natural killer cells (gated as $hCD45^+hCD56^+$); My, myeloid cells (gated as $hCD45^+hCD11b^+$); Mo, monocytes/macrophages (gated as $hCD45^+hCD14^+$); DC, dendritic cells (gated as $hCD45^+hCD11c^+$).

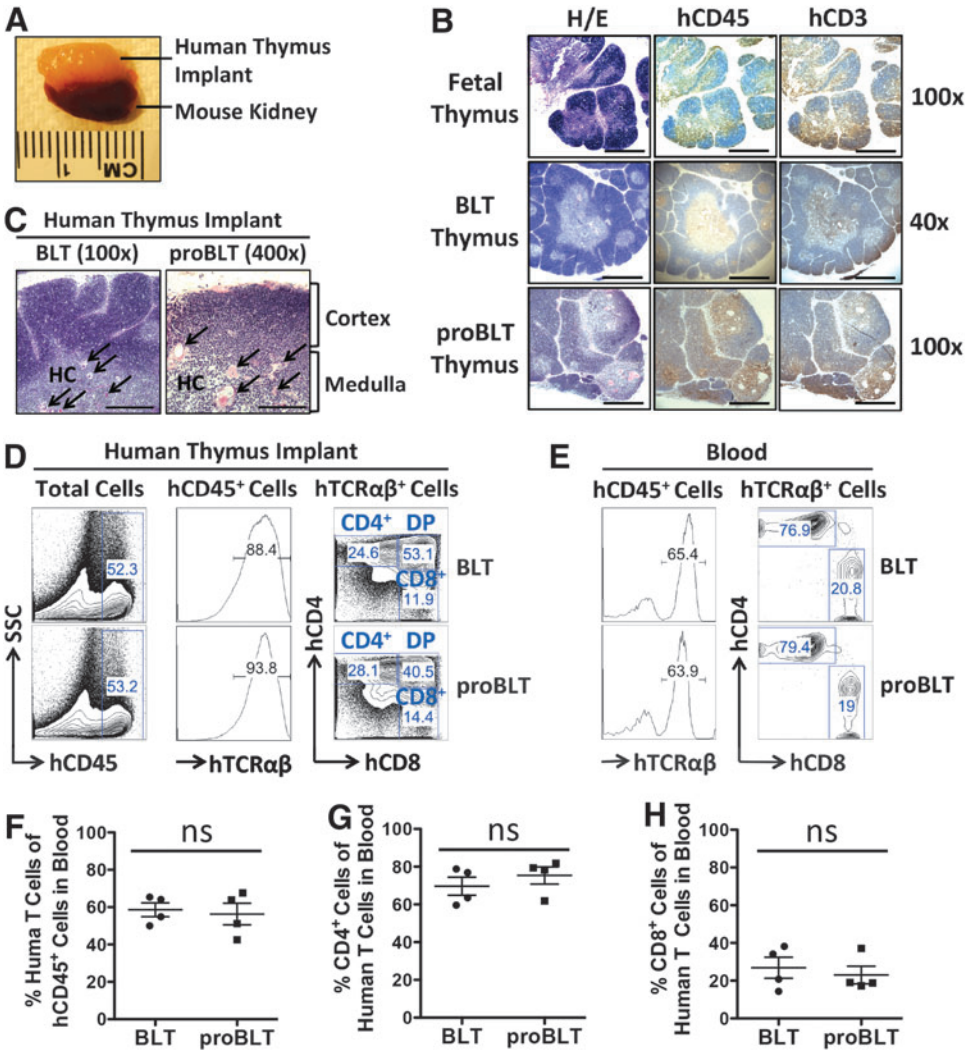

FIG. 3. Reconstitution of human thymus and human T cells in proBLT mice. The experiments were repeated at least three times. Representative results are presented from BLT and proBLT mice at 8–12 weeks post the primary human tissue or secondary BLT tissue transfer ($n = 4$–5 per experimental group). **(A)** Representative image of a human thymus implant growing on a mouse kidney. Data from a representative BLT mouse is shown. **(B)** Immunohistology analysis showing the H/E staining and antibody staining of sections of primary human fetal thymus or human thymus organoids from BLT or proBLT mice. Bars: 1,000 μm (40× magnification); 500 μm (100× magnification). **(C)** Histological analysis showing the H/E staining of representative human thymus organoid sections. Data from representative BLT and proBLT mice are shown. Note the presence of cortex and medulla regions that are typical of human thymus, as well as Hassall's corpuscles (HCs) that are unique to human thymus. Bars: 500 μm (100× magnification); 5,000 μm (400× magnification). **(D)** FACS plots showing the detection of human $CD45^+$ cells in human thymus organoid implants from BLT and proBLT mice. Analysis of human TCRαβ expression (pregated on human $CD45^+$ cells) and CD4/CD8 co-receptor expression (pregated on $hCD45^+hTCR\alpha\beta^+$ cells) are presented. DP, $CD4^+CD8^+$ double-positive thymocytes; $CD4^+$, CD4 single-positive thymocytes; $CD8^+$, CD8 single-positive thymocytes. **(E)** FACS plots showing the detection of human T cells (gated as $hCD45^+hTCR\alpha\beta^+$) and their CD4/CD8 subsets (further gated as $CD4^+$ or $CD8^+$) in the blood of BLT and proBLT mice. **(F–H)** Scatter plots showing the presence of human T cells (gated as $hCD45^+hTCR\alpha\beta^+$; **F**) and their CD4 (further gated as $CD4^+$; **G**) and CD8 (further gated as $CD8^+$; **H**) subsets in the blood of BLT and proBLT mice. Data are presented as individual mouse measurements and mean ± SEM of all mice in an experimental group. H/E, hematoxylin and eosin; ns, not significant.

to produce such BLT^{A2+} mice are especially limiting. We proposed to overcome this hurdle by maximizing the utilization of the limited supply of $HLA\text{-}A2^+$ human tissues, through expanding a small number of established BLT^{A2+} mice into a large colony of $proBLT^{A2+}$ mice without the need for additional primary human tissues. As shown in Fig. 4, the resulting $proBLT^{A2+}$ mice faithfully inherited the HLA-A2 haplotype genetic trait and reconstituted a human immune system, including the human T cell compartment that expressed HLA-A2.

Persistence of human immune cell gene modifications from BLT to proBLT mice

Humanized BLT mice are potent tools that are used to study gene-modified human immune cell therapies, especially gene-

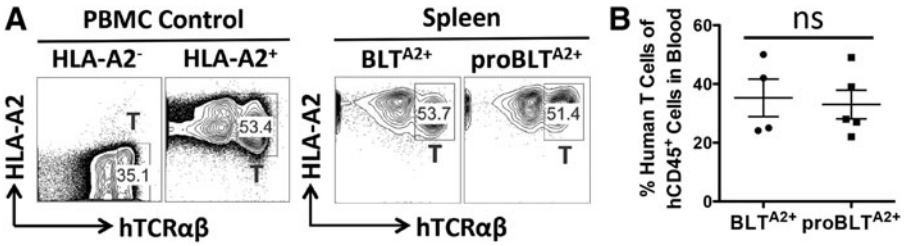

FIG. 4. Inheritance of human immune cell genetic traits from BLT to proBLT mice. HLA-A2$^+$ human fetal liver CD34$^+$ cells and matching fetal thymus were used to generate the primary BLT mice (denoted as BLT^{A2+}), which were then utilized to generate the secondary proBLT mice (denoted as proBLT^{A2+}). The experiments were repeated at least three times. Representative results are presented from BLT and proBLT mice at 8–12 weeks post the primary human tissue or secondary BLT tissue transfer ($n=4$–5 per experimental group). **(A)** FACS plots showing the inheritance of HLA-A2 haplotype on reconstituted human immune cells (pregated as hCD45$^+$) from the spleen of BLT^{A2+} and proBLT^{A2+} mice. HLA-A2$^-$ and HLA-A2$^+$ PBMCs were included as controls. PBMC, peripheral blood mononuclear cells from healthy human donors. T, human T cells (gated as hCD45$^+$hTCRαβ$^+$). **(B)** Scatter plot showing the detection of human T cells (gated as hCD45$^+$hTCRαβ$^+$) in the blood of BLT^{A2+} and proBLT^{A2+} mice. Data are presented as individual mouse measurements and mean ± SEM of all mice in an experimental group. ns, not significant.

modified HSC therapies for treating diseases such as cancer, HIV, or primary immune deficiencies [30–34]. To evaluate whether the proBLT approach may be useful for such applications, we transduced human CD34$^+$ cells with a lentivector pMND-EGFP encoding an enhanced green fluorescence (EGFP) reporter gene, and we then used these genetically modified CD34$^+$ cells to generate BLT mice (Fig. 5A). In the resulting BLT mice (denoted as BLT^{EGFP}), we observed high expression of the EGFP transgene in a high portion of human immune cells (gated as hCD45$^+$EGFP$^+$ comprising ~60% of total hCD45$^+$ cells; Fig. 5B). proBLT mice generated from these BLT^{EGFP} mice, denoted as proBLT^{EGFP}, repopulated a human immune system that persistently expressed high levels of EGFP transgene with the appearance of hCD45$^+$ cells starting from 4 weeks post secondary bone marrow transfer (Fig. 5B, C). Transgene expression was detected in multilineages of human immune cells, including CD4$^+$ helper and CD8$^+$ cytotoxic human T cells (gated as hCD45$^+$hTCRαβ$^+$CD4$^+$ or hCD45$^+$hTCRαβ$^+$CD8$^+$, respectively; Fig. 5D). Moreover, the levels of human immune cell gene modifications were very consistent among individual proBLT mice and were similar to those of the primary BLT^{EGFP} mice (~60% of total hCD45$^+$ cells; Fig. 5C). Therefore, proBLT mice are suitable to support large-scale studies of gene-modified human immune cell therapies.

Discussion

In this article, we describe a new method for propagating humanized BLT mice for the study of human immunology and immunotherapy. Through secondary transfer of bone marrow cells and human thymus implants from BLT mice into naïve NSG recipient mice, we were able to expand one primary BLT mouse into a colony of 4–5 proBLT mice in 6–8 weeks (Fig. 1). These proBLT mice reconstituted human immune cells, including T cells, at levels comparable to those of their primary BLT donor mice (Figs. 2 and 3). They also faithfully inherited the human immune genetic traits from their donor BLT mice, such as the HLA-A2 haplotype that is of special interest for studying antigen-specific T cell activities and T cell-based immunotherapies (Fig. 4). Moreover, an EGFP reporter gene engineered into the human immune system was stably passed from BLT to proBLT mice, making them suitable for studying gene-modified human immune cell gene therapies, especially gene-modified HSC therapies (Fig. 5). Therefore, these proBLT mice can be considered expanded ''clones'' of the established primary BLT mice. Through bypassing the need for additional human CD34$^+$ cells and fetal thymus tissues, the proBLT approach provides an opportunity to overcome a critical hurdle to utilizing the BLT humanized mouse model and enables its more widespread use as a valuable preclinical research tool.

There are two key factors that make the proBLT approach successful. One is the persistence of human CD34$^+$ HSPCs through secondary bone marrow transfer in NSG recipient mice; the other is the regeneration of a functional human thymus structure in the secondary NSG recipient mice through implanting the human thymus organoids harvested from the primary BLT mice. Despite their original sources (e.g., fetal liver, cord blood, adult bone marrow, or adult G-CSF-mobilized peripheral blood), human CD34$^+$ cells post adoptive transfer always preferentially homed to the bone marrow of NSG mice, likely because NSG mouse bone marrow provides a nurturing environment that supports the engraftment and long-term maintenance of these cells (Fig. 2B) [4]. The ability of BLT bone marrow-experienced human CD34$^+$ HSPCs to survive the secondary bone marrow transfer, while maintaining their longevity and multipotential, validates the supporting function of the NSG mouse bone marrow niche. Meanwhile, the ability of the human thymus organoids generated in BLT mice to develop into a functional human thymus structure in the proBLT mice is intriguing (Fig. 3). A human thymus graft is critical for the proper development, functional maturation, and systemic reconstitution of human T cells in humanized mice [9]. In addition to thymocytes that are derived from HSPCs, a functional human thymus comprises thymic epithelial/dendritic cells that mediate the positive and negative selections of human T cells, as well as the programming of special human T cell sublineages such as CD4$^+$CD25$^+$FoxP3$^+$ regulatory T cells (Tregs) [27]. In our experiments, we observed a typical cortex/medulla structure in human thymus organoids harvested from both BLT and proBLT mice, suggesting that human thymocytes and thymic epithelial/dendritic

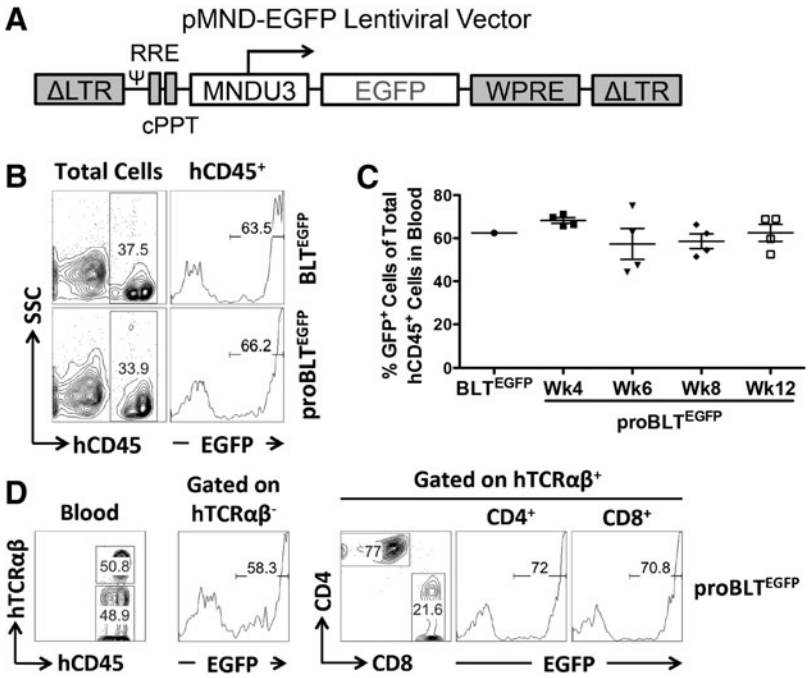

FIG. 5. Persistence of human immune cell gene modifications from BLT to proBLT mice. Human $CD34^+$ cells transduced with pMND-EGFP lentiviruses were used to generate the primary BLT mice modified with the EGFP reporter gene (denoted as BLT^{EGFP}), which were then utilized to generate the secondary proBLT mice (denoted as $proBLT^{EGFP}$). The experiments were repeated at least three times. Representative results are presented ($n = 4$–5 per experimental group). **(A)** Schematic of the pMND-EGFP lentiviral vector. ΔLTR, self-inactivating long-term repeats; MNDU3, internal promoter derived from the MND retroviral LTR U3 region; Ψ, packaging signal with the splicing donor and splicing acceptor sites; RRE, rev-responsive element; cPPT, central polypurine tract; WPRE, woodchuck responsive element; EGFP, enhanced green fluorescence protein reporter gene. **(B)** Representative FACS plots showing the detection of EGFP reporter gene expression in human immune cells (gated as $hCD45^+$) from the blood of BLT^{EGFP} and $proBLT^{EGFP}$ mice at 8 weeks post the primary human tissue or secondary BLT tissue transfer. **(C)** Scatter plot showing the time course detection of EGFP reporter gene expression in human immune cells (gated as $hCD45^+$) from the blood of $proBLT^{EGFP}$ mice post secondary BLT tissue transfer ($n = 4$). Data are presented as individual mouse measurements and mean ± SEM of all experimental mice at the indicated time points. Note the stable and persistent expression of EGFP transgene from the primary BLT^{EGFP} to the secondary $proBLT^{EGFP}$ mice. **(D)** Representative FACS plots of blood from $proBLT^{EGFP}$ mice at 8 weeks post secondary BLT tissue transfer, showing the detection of high EGFP transgene expression in various reconstituted human immune cells (gated as $hCD45^+hTCR\alpha\beta^{+/-}$), in particular in human T cells (gated as $hCD45^+hTCR\alpha\beta^+$) and their CD4/CD8 subsets (further gated as $CD4^+$ or $CD8^+$).

cells were present and organized properly in these organoids (Fig. 3B). In particular, we observed abundant numbers of HCs in the medulla of these human thymus organoids (Fig. 3C). HCs are structures unique to human thymus, formed from eosinophilic type VI epithelial reticular cells arranged concentrically, and have been implicated in the development of human Tregs [26]. Thymic stromal lymphopoietin expressed by thymic epithelial cells within the HCs has been indicated to activate thymic $CD11^+$ dendritic cells that then mediate the secondary positive selection of human Tregs [26]. Taken together, these observations verified the presence of an authentic human thymus environment in proBLT mice that is responsible for the functional reconstitution of human T cells in these mice.

Based on the success of generating proBLT mice, it is intriguing to propose that the tertiary transfer of bone marrow cells and human thymus organoid harvested from the proBLT mice into NSG mice may further expand the BLT colony size by another four- to five-fold, allowing the propagation of a single primary BLT mouse to ~5 pro-BLT mice in 6–8 weeks and then to ~25 pro-proBLT mice in 3–4 months. Unfortunately, our initial attempts to generate pro-proBLT mice did not yield satisfactory results. Reconstitution of human immune cells in the pro-proBLT mice was low and variable. It seemed that the human $CD34^+$ cells had exhausted their long-term potential post the tertiary bone marrow transfer, a phenomenon similar to but more severe than that has been observed for mouse HSCs post series bone marrow transfers in the mouse model. It has been well recognized in the mouse model that series of bone marrow transfers induce stress and impair the longevity of HSCs. Furthermore, in the BLT humanized mouse model, the mouse bone marrow environment does not provide the optimal support for the long-term maintenance of human HSCs. Supplementing the primary BLT mice and proBLT mice with human cytokines that are important for human HSC maintenance, such as Flt3 ligand, SCF, TPO, and IL-3, may improve the longevity of engrafted human $CD34^+$ cells and allow for the further propagation of proBLT mice. If possible, such a preclinical animal model will allow the continuous ''passage'' of human immune cells in living animals for an extended period of time, therefore maximizing the research value of a limited supply of primary human immune cells and tissues.

proBLT mice are particularly valuable for the study of human immune systems with special genetic traits. These genetic traits often pose particular restrictions on the supply of human tissues that can be used. One example is the HLA haplotype. For instance, the study of HLA-A2-restricted T cell responses is of special interest for cancer immunotherapy research, whereas the study of HLA-B57-restricted T cell responses is of special interest for HIV latency research [35,36]. Generation of BLT mice for such studies requires HLA-$A2^+$ or HLA-$B57^+$ human $CD34^+$ HSPCs as well as HLA haplotype-matched fetal thymus tissues. Another example is monogene deficiencies of the human hematopoietic system. For instance, adenosine deaminase (ADA) deficiency is of interest for studying human ADA-deficient severe combined immunodeficiency (SCID), whereas β-globin deficiency is of interest for studying human sickle cell disease [20–22]. Over the past decades, gene-corrected autologous HSC transfer has become a promising therapy for these monogene deficiency-induced diseases and inspires further investigations [32]. Humanized animal models are valuable tools for the preclinical development of these therapies, but their application is greatly limited by the small number of $CD34^+$ cells that can be collected from patients, which are often too few to engraft enough animals for meaningful studies [20–22]. By expanding established BLT mice and reducing the need for additional primary human tissues, the proBLT approach provides an attractive solution to overcome this critical hurdle and makes the humanized BLT mouse model suitable for the preclinical study of such hematopietic stem cell-based gene therapies.

Despite its valuable research potential, the BLT model still has its own limitations and can be improved further to make it more representative of the human immune system in terms of the composition of various lineages of immune cells and their functions. These next-generation humanized mouse models utilize recipient mice such as NSG or BRG that are further genetically modified to allow for enhanced human immune cell reconstitution. Such modifications include the deficiency of murine *c-Kit* gene that supports improved human $CD34^+$ HSPC engraftment [37,38], the addition of human transgenes such as *SIRPα* to improve overall human hematopoietic cell engraftment [39], or knock-in of human immune regulatory genes such as TPO, IL-3, GM-CSF (granulocyte-macrophage colony-stimulating factor), and M-CSF (macrophage colony-stimulating factor) that promote the development and function of human monocytes, macrophages, and NK cells [40]. The proBLT approach should also be applicable to expand BLT mice that are produced with these advanced recipient mice and support the studies of a human immune system that more closely resembles the human situation.

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Author Disclosure Statement

No competing financial interests exist.

References

1. Shultz LD, MA Brehm, JV Garcia-Martinez and DL Greiner. (2012). Humanized mice for immune system investigation: progress, promise and challenges. Nat Rev Immunol 12:786–798.
2. Rongvaux A, H Takizawa, T Strowig, T Willinger, EE Eynon, RA Flavell and MG Manz. (2013). Human hemato-lymphoid system mice: current use and future potential for medicine. Annu Rev Immunol 31:635–674.
3. Theocharides AP, A Rongvaux, K Fritsch, RA Flavell and MG Manz. (2016). Humanized hemato-lymphoid system mice. Haematologica 101:5–19.
4. Brehm MA, A Cuthbert, C Yang, DM Miller, P DiIorio, J Laning, L Burzenski, B Gott, O Foreman, et al. (2010). Parameters for establishing humanized mouse models to study human immunity: analysis of human hematopoietic stem cell engraftment in three immunodeficient strains of mice bearing the IL2rgamma(null) mutation. Clin Immunol 135:84–98.
5. Takenaka K, TK Prasolava, JC Wang, SM Mortin-Toth, S Khalouei, OI Gan, JE Dick and JS Danska. (2007). Polymorphism in Sirpa modulates engraftment of human hematopoietic stem cells. Nat Immunol 8:1313–1323.
6. Mosier DE, RJ Gulizia, SM Baird and DB Wilson. (1988). Transfer of a functional human immune system to mice with severe combined immunodeficiency. Nature 335:256–259.
7. Traggiai E, L Chicha, L Mazzucchelli, L Bronz, JC Piffaretti, A Lanzavecchia and MG Manz. (2004). Development of a human adaptive immune system in cord blood cell-transplanted mice. Science 304:104–107.
8. Chicha L, R Tussiwand, E Traggiai, L Mazzucchelli, L Bronz, JC Piffaretti, A Lanzavecchia and MG Manz. (2005). Human adaptive immune system Rag2-/-gamma(c)-/- mice. Ann N Y Acad Sci 1044:236–243.
9. McCune JM, R Namikawa, H Kaneshima, LD Shultz, M Lieberman and IL Weissman. (1988). The SCID-hu mouse: murine model for the analysis of human hematolymphoid differentiation and function. Science 241:1632–1639.
10. Melkus MW, JD Estes, A Padgett-Thomas, J Gatlin, PW Denton, FA Othieno, AK Wege, AT Haase and JV Garcia. (2006). Humanized mice mount specific adaptive and innate immune responses to EBV and TSST-1. Nat Med 12:1316–1322.
11. Lan P, N Tonomura, A Shimizu, S Wang and YG Yang. (2006). Reconstitution of a functional human immune system in immunodeficient mice through combined human fetal thymus/liver and CD34+ cell transplantation. Blood 108:487–492.

12. Wege AK, MW Melkus, PW Denton, JD Estes and JV Garcia. (2008). Functional and phenotypic characterization of the humanized BLT mouse model. Curr Top Microbiol Immunol 324:149–165.
13. Brainard DM, E Seung, N Frahm, A Cariappa, CC Bailey, WK Hart, HS Shin, SF Brooks, HL Knight, et al. (2009). Induction of robust cellular and humoral virus-specific adaptive immune responses in human immunodeficiency virus-infected humanized BLT mice. J Virol 83:7305–7321.
14. Marsden MD and JA Zack. (2015). Studies of retroviral infection in humanized mice. Virology 479–480:297–309.
15. Karpel ME, CL Boutwell and TM Allen. (2015). BLT humanized mice as a small animal model of HIV infection. Curr Opin Virol 13:75–80.
16. Garcia JV. (2016). In vivo platforms for analysis of HIV persistence and eradication. J Clin Invest 126:424–431.
17. Tager AM, M Pensiero and TM Allen. (2013). Recent advances in humanized mice: accelerating the development of an HIV vaccine. J Infect Dis 208 Suppl 2:S121–S124.
18. Shimizu S, P Hong, B Arumugam, L Pokomo, J Boyer, N Koizumi, P Kittipongdaja, A Chen, G Bristol, et al. (2010). A highly efficient short hairpin RNA potently down-regulates CCR5 expression in systemic lymphoid organs in the hu-BLT mouse model. Blood 115:1534–1544.
19. Vatakis DN, RC Koya, CC Nixon, L Wei, SG Kim, P Avancena, G Bristol, D Baltimore, DB Kohn, et al. (2011). Antitumor activity from antigen-specific CD8 T cells generated in vivo from genetically engineered human hematopoietic stem cells. Proc Natl Acad Sci U S A 108:E1408–E1416.
20. Carbonaro DA, L Zhang, X Jin, C Montiel-Equihua, S Geiger, M Carmo, A Cooper, L Fairbanks, ML Kaufman, et al. (2014). Preclinical demonstration of lentiviral vector-mediated correction of immunological and metabolic abnormalities in models of adenosine deaminase deficiency. Mol Ther 22:607–622.
21. Hoban MD, GJ Cost, MC Mendel, Z Romero, ML Kaufman, AV Joglekar, M Ho, D Lumaquin, D Gray, et al. (2015). Correction of the sickle cell disease mutation in human hematopoietic stem/progenitor cells. Blood 125:2597–2604.
22. Romero Z, F Urbinati, S Geiger, AR Cooper, J Wherley, ML Kaufman, RP Hollis, RR de Assin, S Senadheera, et al. (2013). Beta-globin gene transfer to human bone marrow for sickle cell disease. J Clin Invest 123:3317–3330.
23. Smith DJ, S Liu, S Ji, B Li, J McLaughlin, D Cheng, ON Witte and L Yang. (2015). Genetic engineering of hematopoietic stem cells to generate invariant natural killer T cells. Proc Natl Acad Sci U S A 112:1523–1528.
24. Giannoni F, CL Hardee, J Wherley, E Gschweng, S Senadheera, ML Kaufman, R Chan, I Bahner, V Gersuk, et al. (2013). Allelic exclusion and peripheral reconstitution by TCR transgenic T cells arising from transduced human hematopoietic stem/progenitor cells. Mol Ther 21:1044–1054.
25. Yang L, H Yang, K Rideout, T Cho, KI Joo, L Ziegler, A Elliot, A Walls, D Yu, D Baltimore and P Wang. (2008). Engineered lentivector targeting of dendritic cells for in vivo immunization. Nat Biotechnol 26:326–334.
26. Watanabe N, YH Wang, HK Lee, T Ito, YH Wang, W Cao and YJ Liu. (2005). Hassall's corpuscles instruct dendritic cells to induce CD4+CD25+ regulatory T cells in human thymus. Nature 436:1181–1185.
27. Spits H. (2002). Development of alphabeta T cells in the human thymus. Nat Rev Immunol 2:760–772.
28. Choo SY. (2007). The HLA system: genetics, immunology, clinical testing, and clinical implications. Yonsei Med J 48:11–23.
29. Tscharke DC, NP Croft, PC Doherty and NL La Gruta. (2015). Sizing up the key determinants of the CD8(+) T cell response. Nat Rev Immunol 15:705–716.
30. Rossi JJ, CH June and DB Kohn. (2007). Genetic therapies against HIV. Nat Biotechnol 25:1444–1454.
31. Gschweng E, S De Oliveira and DB Kohn. (2014). Hematopoietic stem cells for cancer immunotherapy. Immunol Rev 257:237–249.
32. Kuo CY and DB Kohn. (2016). Gene Therapy for the Treatment of Primary Immune Deficiencies. Curr Allergy Asthma Rep 16:39.
33. Wang CX and PM Cannon. (2016). The clinical applications of genome editing in HIV. Blood 127:2546–2552.
34. Petz LD, JC Burnett, H Li, S Li, R Tonai, M Bakalinskaya, EJ Shpall, S Armitage, J Kurtzberg, et al. (2015). Progress toward curing HIV infection with hematopoietic cell transplantation. Stem Cells Cloning 8:109–116.
35. Kershaw MH, JA Westwood and PK Darcy. (2013). Gene-engineered T cells for cancer therapy. Nat Rev Cancer 13: 525–541.
36. Zaunders J and D van Bockel. (2013). Innate and Adaptive Immunity in Long-Term Non-Progression in HIV Disease. Front Immunol 4:95.
37. Cosgun KN, S Rahmig, N Mende, S Reinke, I Hauber, C Schafer, A Petzold, H Weisbach, G Heidkamp, et al. (2014). Kit regulates HSC engraftment across the human-mouse species barrier. Cell Stem Cell 15:227–238.
38. McIntosh BE, ME Brown, BM Duffin, JP Maufort, DT Vereide, Slukvin, II and JA Thomson. (2015). Non-irradiated NOD,B6.SCID Il2rgamma-/- Kit(W41/W41) (NBSGW) mice support multilineage engraftment of human hematopoietic cells. Stem Cell Rep 4:171–180.
39. Strowig T, A Rongvaux, C Rathinam, H Takizawa, C Borsotti, W Philbrick, EE Eynon, MG Manz and RA Flavell. (2011). Transgenic expression of human signal regulatory protein alpha in Rag2-/-gamma(c)-/- mice improves engraftment of human hematopoietic cells in humanized mice. Proc Natl Acad Sci U S A 108:13218–13223.
40. Rongvaux A, T Willinger, J Martinek, T Strowig, SV Gearty, LL Teichmann, Y Saito, F Marches, S Halene, et al. (2014). Development and function of human innate immune cells in a humanized mouse model. Nat Biotechnol 32:364–372.

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