Long-term survival and differentiation of human thymocytes in human thymus-grafted immunodeficient mice

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Aim: Thymus transplants have produced encouraging clinical outcomes in achieving thymopoiesis and T-cell development. This study was aimed to investigate whether human thymus contains self-renewing lymphoid progenitors capable of maintaining long-term T-cell development. **Materials & methods:** Immunodeficient mice were transplanted with human thymic tissue along with autologous GFP-expressing or allogeneic CD34⁺ cells and followed for human thymopoiesis and T-cell development from the thymic progenitors versus CD34⁺ cells, which can be distinguished by GFP or HLA expression. **Results:** In both models, long-term thymopoiesis and T-cell development from the thymic grafts were detected. In these mice, human thymic progenitor-derived T cells including CD45RA⁺CD31⁺CD4⁺ new thymic emigrants were persistently present in the periphery throughout the observation period (32 weeks). **Conclusion:** The results indicate that human thymus contains long-lived lymphoid progenitors that can maintain durable thymopoiesis and T-cell development.

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Thymus transplants have produced encouraging results in reconstituting thymopoiesis and T-cell development in patients with T-cell deficiencies, such as DiGeorge syndrome [1,2] and FOXP1 mutation [3]. Thymopoiesis, which is the differentiation of lymphoid progenitors into mature T cells in the thymus, requires an appropriate thymic microenvironment and seeding of the lymphoid progenitors into the thymus. In addition to thymus transplantation, thymopoiesis is also important for other types of immunotherapy, such as *in vivo* generation of cancer- or virus-reactive T cells [4,5]. How the lymphoid progenitor pool is maintained in the thymus has been disputed. An earlier study suggested that self-renewing lymphoid progenitors may reside in the adult thymus [6], but it has been generally believed that thymopoiesis relies on the continuing input of bone marrow-derived progenitor cells [7,8]. This dogma was challenged by recent studies showing that T-cell development continues following thymic transplantation into a host devoid of T-cell progenitors [9,10]. However, these previous studies were performed in mice, and it is unclear whether sustained thymopoiesis and T-cell development require continuing migration of lymphoid progenitors into the thymus in humans. In the present study, we addressed this question using immunodeficient mice transplanted with human fetal thymic tissue and CD34⁺ hematopoietic stem cells.

Materials & methods

Animals & human tissues & cells

NOD.Cg-Prkdc^{scid} Il2rg^{tm1Wjl}/SzJ (NSG) mice from the Jackson Laboratory (ME, USA) were used to prepare humanized mice. All mice were housed in micro-isolator cages in a specific pathogen-free animal facility. All animal studies were approved by the Institutional Animal Care and Use Committee (IACUC). Discarded human fetal tissues (thymus and liver; gestational age of 17–20 weeks) were obtained from Advanced Bioscience Resource (CA, USA) or the First Hospital of Jilin University. The thymic tissue was cut into small fragments (approximately 1 mm³ in size), which were used immediately or cryopreserved in liquid nitrogen for later use. Human CD34⁺ cells were purified from fetal liver cells (FLCs) by incubation with antihuman CD34 microbeads followed by positive selection using MACS (Miltenyi Biotech, CA, USA). The prepared human CD34⁺ FLCs were either used freshly or cryopreserved in liquid nitrogen for later use. Experiments involving the use of discarded human tissues were approved by the Institutional Review Board of Columbia University Medical Center or the First Hospital of Jilin University.

Preparation of GFP lentivirus & transduction of CD34⁺ cells

Recombinant lentiviruses were produced by transfection of 293FT cells with a three-plasmid system consisting of the transfer vector GFP (pLVTHM 10 µg) and two packaging plasmids (psPAX2 7.5 µg, pMD2G 2.5 µg). The transfected 293FT cells were cultured for 48 h, then the virus-containing culture supernatant was collected and ultracentrifuged at 50,000 *g* for 2 h, and the concentrated viral solution was stored at -80°C until use. Viral transduction of human CD34⁺ cells was performed as previously described [11,12]. Briefly, human CD34⁺ cells were prestimulated overnight in retroinfection-coated plates (Takara Bio, Inc., Shiga, Japan) in media containing 50 ng/ml of human SCF, 50 ng/ml of human Flt-3 ligand, 25 ng/ml of human TPO, and 10 ng/ml of human IL-3 (human cytokines were purchased from R&D, MN, USA or Ebioscience, CA, USA), then viral stock was added for another 12 h. The virally transduced CD34⁺ cells were washed and used immediately.

Humanized mouse preparation

NSG mice were conditioned with sublethal (2 Gy) total body irradiation, and received a fresh or cryopreserved human fetal thymic tissue fragment (measuring about 1 mm³, under the subcapsular space of a kidney) plus autologous or allogeneic (with respect to the thymic graft) CD34⁺ FLCs (1–5 × 10⁵/mouse, iv.), as previously described [13,14]. Blood and tissues were prepared from the humanized mice at the indicated times, and the levels of human hematopoietic cell chimerism were determined by flow cytometric (FCM) analysis using various combinations of fluorescence-conjugated mAbs against human cluster of differentiation (CD) antigens (CD45, CD3, CD4, CD8, CD19, CD45RA and CD31). Anti-HLA-A9 was used to distinguish between human thymus- and CD34⁺ FLC-derived cells in humanized mice made of allogeneic human tissues/cells. The cells were also stained with antimouse CD45 and Ter119, and appropriate isotype control mAbs for better identification of human cells. All antibodies used were purchased from BD Pharmingen (CA, USA). Peripheral blood mononuclear cells (PBMCs) were prepared by density gradient centrifugation using Histopaque 1077 (Sigma-Aldrich, MO, USA). The samples were acquired on a LSR II or FACSCanto (Becton Dickinson, CA, USA) with dead cells excluded by propidium iodide staining.

Statistical analysis

The level of significant differences in group means was determined by the student's *t*-test for parametric data sets using Prism 5 (GraphPad Software, CA, USA). A *p*-value of ≤0.05 was considered significant in all analyses.

Results & discussion

Implantation of fetal human thymus and liver tissues beneath the renal capsule of immunodeficient mice has been shown to achieve durable human thymopoiesis and T-cell development [15], which is further improved by injection iv. of human CD34⁺ hematopoietic stem/progenitor cells (HSPCs) [16,17]. Because significant human T-cell reconstitution was not observed in mice receiving human thymic tissue alone, human T cells in these humanized mice (hu-mice) were thought to develop from lymphoid progenitors derived from the fetal liver graft and iv. injected CD34⁺ HSPCs. Subsequently, we showed that robust human thymopoiesis and T-cell development can also be achieved in mice receiving implantation (under the renal capsule) of fetal human thymic tissue (without liver) and iv. injection of CD34⁺ HSPCs [18]. Here, we used this hu-mouse model to investigate whether human thymus

Figure 1. Pre-existing T-cell precursors in the thymic graft contribute to sustained thymopoiesis and T-cell development in hu-mice. (A) Schematic showing preparation of hu-mice. Human CD34⁺ FLC were transduced with GFP-lentiviruses and injected into sublethally irradiated NSG mice that were grafted with fresh (B–D) Or cryopreserved-thawed (E–G) Human thymic tissues (n = 5 per group). (B & E) Percentages of GFP⁺ cells in GFP-transduced CD34⁺ cells (i.e., GFP transduction efficiency). (C & F) Percentages of GFP⁺ human B and T cells in peripheral blood mononuclear cells at the indicated time points (mean ± standard error of the mean). (D & G) FACS profile showing the percentage of GFP⁺ cells in human CD45⁺ thymocytes from representative human thymic grafts at week 26 post-transplantation. FLC: Fetal liver cell; TBI: Traumatic brain injury.

contains lymphoid progenitors that can sustain durable thymopoiesis and T-cell development. To distinguish between thymic graft- and CD34⁺ HSPC-derived thymocytes and T cells, CD34⁺ HSPCs were transduced with *GFP* prior to injection in mice that also received implantation of thymic tissue from the same fetus (Figure 1A & B). All human B cells in the hu-mice should be derived from iv. injected CD34⁺ cells, and consistent with this, the proportion of GFP⁺ cells in the human B-cell population was stable throughout the experiment (Figure 1C) and comparable to the transduction efficiency in CD34⁺ cells (Figure 1B & C). However, the proportion of GFP⁺ cells in the human T-cell population was significantly lower than that in the human B-cell population (Figure 1C) and the thymic graft comprised primarily GFP⁻ thymocytes (Figure 1D), indicating that many of the human T cells in these hu-mice were derived from the GFP⁻ T-cell progenitors in the thymic graft. Although we do not have direct evidence to rule out the possibility of contamination of the fetal thymic tissue by HSCs, this is unlikely as the mice were grafted only with a very small piece of thymic tissue (approximately 1 mm³) that had been prewashed with medium. In support of this, previous studies found no evidence for thymic graft-derived HSCs in immunodeficient mice following transplantation of newborn thymus [9].

It has been shown that cryopreservation with thawing and pipetting can significantly eliminate pre-existing thymocytes within the thymus [12,19]; therefore, we next compared the proportion of GFP⁺ cells in the human B- and T-cell populations in hu-mice that were injected with GFP-transduced CD34⁺ cells and grafted with cryopreserved-thawed and thoroughly pipetted human thymic tissue fragments (Figure 1E–G). In these hu-mice, the proportion of GFP⁺ cells in the human T-cell population was lower than that in the human B-cell population at the early time, but became comparable by 12 weeks and both were similar to the proportion of GFP⁺ cells in the transduced CD34⁺ cells (Figure 1E & F). Furthermore, the proportion of GFP⁺ thymocytes in the human thymic grafts (Figure 1G) was also similar to that of GFP⁺ cells in the transduced CD34⁺ cells (Figure 1E). Although we have not directly compared the levels of human T and B cells between hu-mice grafted with fresh

versus cryopreserved human thymic tissues, the significantly lower percentage of GFP⁺ cells in human T cells relative to B cells in the former but not latter group of hu-mice indicates that both the thymic graft- and CD34⁺ HSPC-derived lymphoid progenitors can contribute to long-term human thymopoiesis and T-cell development in hu-mice.

We further measured long-term human chimerism in hu-mice transplanted with fetal thymic tissue and allogeneic CD34⁺ cells. Although both human T cells and non-T cells were detected initially, the non-T-cell population disappeared completely (i.e., all human CD45⁺ cells were CD3⁺ T cells) in PBMCs by 14 weeks after transplantation (Figure 2A & B). It has been shown that, in hu-mice receiving fetal human thymus and allogeneic CD34⁺ cells, thymic graft-derived T cells that matured prior to migration of iv. injected CD34⁺ cell-derived APCs into the thymic graft are capable of rejecting the allogeneic cells [12,19]. Thus, the disappearance of the non-T-cell population in these hu-mice is likely due to allorejection of iv. injected CD34⁺ cells and their derivatives by thymic graft-derived human T cells. Given that APCs are highly effective in inducing intrathymic deletion of alloreactive thymocytes, there were presumably no or extremely low numbers of allogeneic HSC-derived APCs in the thymic grafts in these hu-mice, in which CD34⁺ cell-derived cells were completely rejected (Figure 2). The data also implicate that thymic graft-derived human T cells were functional and capable of rejecting allogeneic cells. In support of this possibility, successful engraftment and multilineage chimerism were achieved in mice transplanted with the same cohort of CD34⁺ HSPCs plus autologous thymic tissues (i.e., CD34⁺ HSPCs and thymic tissue were from the same fetus; Figure 3). Previous studies have shown that T cell–MHC interaction is important for T-cell survival and homeostatic expansion in the periphery [20–22]. In line with this, the levels of human T-cell chimerism in mice receiving thymus and allogeneic HSPCs were considerably low after 14 weeks when human non-T-cell populations disappeared, despite the maintenance of a functional human thymic graft. Thus, it would be important to avoid using HLA-fully mismatched donors for clinical thymus transplantation.

The data detailed in Figure 2A and B suggest that the human T cells detected in the hu-mice grafted with human fetal thymic tissue and allogeneic CD34⁺ cells were all derived from the T-cell progenitors in the thymic grafts. To confirm this, we analyzed the origin of long-term surviving human T cells in hu-mice that received fetal thymic tissue (HLA-A9⁺) and allogeneic (HLA-A9⁻) CD34⁺ cells. FACS analysis at week 32 revealed that human CD45⁺ cells detected in all tissues analyzed (PBMCs, spleen, bone marrow, liver and lung) were CD3⁺ T cells, and were all derived from HLA-A9⁺ fetal thymic grafts, but not from allogeneic CD34⁺ cells (Figure 2C; Supplementary Figure 1). The human CD3⁺ T cells comprised both CD4⁻ and CD8⁻ T cells, and approximately 45–50% of CD4⁺ T cells expressed a CD45RA and CD31 (Figure 2C), a phenotype of new thymic emigrants [23,24]. Furthermore, the human thymic grafts in these mice appeared macroscopically healthy and maintained a cellularity of approximately 1×10^8 human CD45⁺ thymocytes (Figure 2D). Flow cytometry revealed that the human thymocytes had normal phenotypic profiles as indicated by staining with anti-CD4, CD8, CD3 and MHC class I mAbs (Figure 2E). These results indicate that thymocytes pre-existed in the human thymic grafts maintained durable (>30 weeks) thymopoiesis and T-cell development.

As shown in Figure 2, all human CD45⁺ cells were CD3⁺ T cells at the later times in hu-mice transplanted with human thymic tissue and allogeneic CD34⁺ cells and notably, the level of human CD3⁺ T cells in these hu-mice was much lower (Figure 2 & Supplementary Figure 2) than that in mice receiving human thymic tissue and autologous CD34⁺ cells (Figure 3) [14,25]. Because antigen presenting cells are required for T-cell survival and homeostatic expansion [20,26], we propose that the low level of human T-cell reconstitution in these hu-mice was caused primarily by the lack of non-T-cell lineage human cells, leading to poor survival and homeostatic expansion of human T cells in the periphery. Thus, although the thymic grafts are capable of sustaining human thymopoiesis and T-cell development, bone marrow-derived antigen presenting cells are required to maintain the peripheral T-cell pool.

Conclusion

This study provides direct evidence that, similar to the observations in mice, T-cell progenitors in the human thymus can survive long-term and maintain durable thymopoiesis and T-cell development.

Future perspective

The thymus functions as a site for T-cell generation or thymopoiesis. Thymus transplants have produced encouraging results in correction of immunodeficiency in patients with thymus degeneration caused by complete DiGeorge syndrome or *FOXP1* mutation. The current study confirms that human thymus harbors T-cell progenitors that can

Figure 2. Pre-existing T-cell precursors in the thymic grafts sustain long-term thymopoiesis and functional T-cell development. (A & B) Hu-mice were made by transplantation of human fetal thymic tissues together with allogeneic CD34⁺ cells (n = 3). (A) Percentages of human CD45⁺ cells in PBMCs (left) and of human CD3⁺ T cells in gated human CD45⁺ PBMCs (right). (B) Representative FACS profiles showing presence of both human CD3⁺ T cells and CD19⁺ B cells at the early time (6 weeks; left) and only CD3⁺ T cells at the later time (18 weeks; right) after transplantation. (C–E) FACS analysis of PBMCs, spleen cells and human thymic graft cells from a representative hu-mouse sacrificed 32 weeks after transplantation of HLA-A9⁺ fetal human thymic tissue and allogeneic (HLA-A9⁻) CD34⁺ cells. (C) Staining profiles of PBMCs and spleen cells: 1st row: human CD45⁺ cell chimerism; 2nd row: expression of human CD3 and HLA-A9 on gated human CD45⁺ cells; 3rd row: CD4 and CD8 expression on gated human CD3⁺ cells; and 4th row: human CD45RA and CD31 expression on gated human CD4⁺ cells. (D) Macroscopic image of a thymic graft (on the top of a kidney). (E) Human thymic graft cells stained with antimouse CD45 versus antihuman CD45 (left panel), and the expression of human CD4 versus CD8 and CD3 versus HLA-A9 in gated human CD45⁺ thymocytes (right panel). PBMC: Peripheral blood mononuclear cell.

Figure 3. Human hematopoietic reconstitution in hu-mice made by transplantation of human fetal thymic tissue and CD34⁺ fetal liver cells from the same fetus. (A) Percentages of human CD45⁺ cells in PBMCs at the indicated time points (n = 4; each symbol represents an individual animal). (B) Representative FACS profiles showing human CD45⁺, CD3⁺ and CD19⁺ cell chimerism in PBMCs 18 weeks after human fetal thymus/CD34⁺ fetal liver cell (FLC) transplantation. Human CD34⁺ FLCs from the same fetus were used in the experiment presented in Figure 2. PBMC: Peripheral blood mononuclear cell.

sustain thymopoiesis and T-cell development in immunodeficient mice, suggesting that thymus transplantation may produce T cells without the supply of T-cell progenitors from the recipient bone marrow. Thus, thymus transplants could also be a therapeutic option for patients with T-cell intrinsic deficiencies. With the rapid development of novel gene editing techniques, thymus transplant is expected to have even broader clinical applications. For example, transplantation of human thymic tissues that have been virally transduced with antigen-specific T-cell receptor or chimeric antigen receptor genes could potentially lead to *in vivo* generation of antigen-specific effector T cells, offering an effective means of immunotherapy for viral infections and cancers.

Summary points

- Thymopoiesis requires an appropriate thymic microenvironment and seeding of the lymphoid progenitors into the thymus.
- In human thymus, how the lymphoid progenitor pool is maintained remains elusive.
- Human thymus contains T-cell progenitors that can survive long-term following transplantation in immunodeficient mice.
- Human thymic tissue transplantation leads to durable thymopoiesis and T-cell development in immunodeficient mice.
- Thymus transplantation may potentially reconstitute donor-derived thymopoiesis and T-cell development in patients with T-cell intrinsic deficiency.

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Authors' contributions

Y Tang, J Xia and Z Hu performed experiments and analyzed data; YG Yang analyzed data and wrote the paper; O Bai, Z Hu and J Xia designed studies, analyzed data and wrote paper.

Financial & competing interests disclosure

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Ethical conduct of research

All protocols involving the use of human tissues and animals were approved by the Institutional Review Board and Institutional Animal Care and Use Committee, respectively. The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

References

1. Markert ML, Alexieff MJ, Li J *et al*. Postnatal thymus transplantation with immunosuppression as treatment for DiGeorge syndrome. *Blood* 104(8), 2574–2581 (2004).
2. Markert ML, Devlin BH, Alexieff MJ *et al*. Review of 54 patients with complete DiGeorge anomaly enrolled in protocols for thymus transplantation: outcome of 44 consecutive transplants. *Blood* 109(10), 4539–4547 (2007).
3. Markert ML, Marques JG, Neven B *et al*. First use of thymus transplantation therapy for FOXP1 deficiency (nude/SCID): a report of 2 cases. *Blood* 117(2), 688–696 (2011).
4. Gschwend E, De Oliveira S, Kohn DB. Hematopoietic stem cells for cancer immunotherapy. *Immunol. Rev.* 257(1), 237–249 (2014).
5. Zhen A, Carrillo MA, Kitchen SG. Chimeric antigen receptor engineered stem cells: a novel HIV therapy. *Immunotherapy* 9(5), 401–410 (2017).
6. Ezine S, Papiernik M, Lepault F. Persistence of stem cell activity within the murine thymus after transfer of a bone marrow fraction enriched in CFU-S. *Int. Immunol.* 3(3), 237–243 (1991).
7. Shortman K, Wu L. Early T lymphocyte progenitors. *Ann. Rev. Immunol.* 14(1), 29–47 (1996).
8. Schwarz BA, Bhandoola A. Trafficking from the bone marrow to the thymus: a prerequisite for thymopoiesis. *Immunol. Rev.* 209(1), 47–57 (2006).
9. Martins VC, Ruggiero E, Schlenner SM *et al*. Thymus-autonomous T cell development in the absence of progenitor import. *J. Exp. Med.* 209(8), 1409–1417 (2012).
10. Peadeceerf L, Lemos S, Galgano A *et al*. Thymocytes may persist and differentiate without any input from bone marrow progenitors. *J. Exp. Med.* 209(8), 1401–1408 (2012).
11. Hu Z, Xia J, Fan W, Wargo J, Yang Y-G. Human melanoma immunotherapy using tumor antigen-specific T cells generated in humanized mice. *Oncotarget* 7(6), 6448–6459 (2016).
12. Xia J, Hu Z, Yoshihara S *et al*. Modeling human leukemia immunotherapy in humanized mice. *EBioMedicine* 10, 101–108 (2016).
13. Lan P, Wang L, Diouf B *et al*. Induction of human T-cell tolerance to porcine xenoantigens through mixed hematopoietic chimerism. *Blood* 103(10), 3964–3969 (2004).
14. Lan P, Tonomura N, Shimizu A, Wang S, Yang YG. Reconstitution of a functional human immune system in immunodeficient mice through combined human fetal thymus/liver and CD34⁺ cell transplantation. *Blood* 108(2), 487–492 (2006).
15. McCune J, Namikawa R, Kaneshima H, Shultz L, Lieberman M, Weissman I. The SCID-hu mouse: murine model for the analysis of human hematolymphoid differentiation and function. *Science* 241(4873), 1632–1639 (1988).
16. Lan P, Tonomura N, Shimizu A, Wang S, Yang YG. Reconstitution of a functional human immune system in immunodeficient mice through combined human fetal thymus/liver and CD34⁺ cell transplantation. *Blood* 108, 487–492 (2006).
17. Melkus MW, Estes JD, Padgett-Thomas A *et al*. Humanized mice mount specific adaptive and innate immune responses to EBV and TSST-1. *Nat. Med.* 12(11), 1316–1322 (2006).
18. Hu Z, Yang YG. Human lymphohematopoietic reconstitution and immune function in immunodeficient mice receiving cotransplantation of human thymic tissue and CD34(+) cells. *Cell. Mol. Immunol.* 9(3), 232–236 (2012).
19. Kalscheuer H, Danzl N, Onoe T *et al*. A model for personalized in vivo analysis of human immune responsiveness. *Sci. Transl. Med.* 4(125), 125ra130 (2012).
20. Onoe T, Kalscheuer H, Chittenden M, Zhao G, Yang Y-G, Sykes M. Homeostatic expansion and phenotypic conversion of human T cells depend on peripheral interactions with APCs. *J. Immunol.* 184(12), 6756–6765 (2010).
21. Ge Q, Rao VP, Cho BK, Eisen HN, Chen J. Dependence of lymphopenia-induced T cell proliferation on the abundance of peptide/MHC epitopes and strength of their interaction with T cell receptors. *Proc. Natl Acad. Sci. USA* 98(4), 1728–1733 (2001).
22. Min B, McHugh R, Sempowski GD, MacKall C, Foucras G, Paul WE. Neonates support lymphopenia-induced proliferation. *Immunity* 18(1), 131–140 (2003).
23. Fink PJ. The biology of recent thymic emigrants. *Annu. Rev. Immunol.* 31, 31–50 (2013).

24. Kimmig S, Przybylski GK, Schmidt CA *et al*. Two subsets of naive T helper cells with distinct T cell receptor excision circle content in human adult peripheral blood. *J. Exp. Med.* 195(6), 789–794 (2002).
25. Hu Z, Van Rooijen N, Yang YG. Macrophages prevent human red blood cell reconstitution in immunodeficient mice. *Blood* 118(22), 5938–5946 (2011).
26. Martin B, Bourgeois C, Dautigny N, Lucas B. On the role of MHC class II molecules in the survival and lymphopenia-induced proliferation of peripheral CD4⁺ T cells. *Proc. Natl Acad. Sci. USA* 100(10), 6021–6026 (2003).