Transplant Immunology 25 (2011) 173–179

Contents lists available at SciVerse ScienceDirect

Transplant Immunology

journal homepage: www.elsevier.com/locate/trim

Efficient peripheral construction of functional human regulatory $CD4^+CD25^{high}Foxp3^+$ T cells in NOD/SCID mice grafted with fetal human thymus/liver tissues and $CD34^+$ cells

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ARTICLE INFO

Article history:
Received 24 May 2011
Received in revised form 27 July 2011
Accepted 4 August 2011

Keywords:
Humanized mice
Transplant tolerance
Regulatory T cells
Foxp3

ABSTRACT

Regulatory T cells, especially $CD4^+CD25^+$ regulatory T cells are critical regulators of immune tolerance in humans and mice. Mice with humanized immunity have been developed by various transplantation strategies of human tissues or cells related to immunity, which are being extensively applied in biomedical research. However, it is unclear whether human $CD4^+CD25^+$ regulatory T cells can normally develop in human thymic grafts and efficiently populate in the periphery in NOD/SCID mouse recipients. In human thymic grafts, high percentage of mature human $CD4^+CD25^{high}$ regulatory T cells was detected. Human $CD4^+CD25^+$ regulatory T cells maturing in fetal human thymus grafts could subsequently output to the periphery of NOD/SCID mouse recipients. Importantly, these cells exhibited $Foxp3^+CD45RO^+CTLA4^+CD127^-$ phenotype, similarly to those in healthy individuals. In addition, human $CD4^+CD25^+$ regulatory T cells maturing in human thymic grafts suppressed proliferative response of $CD4^+CD25^-$ T cells to allogeneic antigens, though the peripheral $CD4^+CD25^+$ regulatory T cells in fetal human thymus-grafted NOD/SCID mice showed somewhat decreased immunosuppressive ability compared with normal $CD4^+CD25^+$ regulatory T cells. Thus, this humanized animal model is suitable for examining development and function of human $CD4^+CD25^+$ regulatory T cells in vivo.

1. Introduction

The regulation of T cell responses is an important aspect in the maintenance of self tolerance[1]. $CD4^+CD25^+$ regulatory T cells play a critical role in regulating the function of other immune cells and preventing potentially harmful autoimmune responses in humans and mice[2,3]. It is reported that suppressive $CD4^+CD25^+$ T cells constitute 5–15% of peripheral $CD4^+$ T cell population in humans and mice[3–6]. Like mice, they are mainly generated in thymus by expressing a high-affinity TCR for self peptides, undergo a selection process different from negative or positive selection[7] and populate in the periphery after encountering antigens[8,9]. In vitro, these T cells suppress both proliferation and cytokine production by $CD4^+CD25^-$ T cells in response to a number of different polyclonal or specific antigens in a contact- and dose-dependent manner, but not mediated by IL-10, TGF-β or IL-4[2,3]. The accumulating data show that human $CD4^+CD25^+$ regulatory T cells play a key regulatory role in autoimmune diseases[10–12], infection[13], graft versus host disease (GVHD)[14], transplant tolerance[15,16] and tumor [17,18].

Humanized nonobese diabetic/severe combined immunodeficient mice (NOD/SCID mice) have been successfully developed by transplanting human mature immune cells[19], hematopoietic stem cells (HSCs)[20–22], thymus/liver (Thy/Liv) tissues[23] or a combination of Thy/Liv tissues and $CD34^+$ cells[19], and the latter is better than others with regard to humanized level. These models have been applied to study the development of human immune cells[24,25], human autoimmune diseases[26], virus infections[27,28], transplantation biology[29] and tumor biology[30,31]. The co-transplantation strategy of fetal human Thy/Liv tissues and $CD34^+$ HSCs could sustain

Abbreviations: CPM, counts per minute; CTLA-4, cytotoxic T lymphocyte-associated antigen 4; DCs, dendritic cells; FCM, flow cytometry; FITC, fluorescein isothiocyanate; Foxp3, Forkhead box P3; GVHD, graft versus host disease; HSCs, hematopoietic stem cells; LNs, lymph nodes; mAbs, monoclonal antibodies; MLR, mix lymphocyte reaction; NOD/SCID, non-obese diabetic/severe combined immunodeficiency; PB, peripheral blood; PBMCs, peripheral blood mononuclear cells; PE, phycoerythrin; PI, propidium iodide; TCR, T cell receptor; WBI, whole body irradiation.

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doi:10.1016/j.trim.2011.08.003

development of multilineage of human immune cells, including T cells, B cells and dendritic cells (DCs)[19]. Especially, functional T cells could develop in thymic grafts and mature in periphery[24,32].

Interestingly, human thymic regulatory T cells might also be generated via thymic development evidenced by expression of CD25 and Foxp3 in humanized mice[33]. However, whether human $CD4^+CD25^+$ regulatory T cells could mature and populate in periphery, and further exert suppressive function on effecter T cells need to be clarified. To address them, humanized NOD/SCID mice were successfully constructed by transplantation of fetal human Thy/Liv tissues and $CD34^+$ cells. We demonstrated that human $CD4^+CD25^+$ T cells could develop in fetal human thymic grafts and output to periphery including peripheral blood, spleens and lymph nodes (LNs). Thymic and peripheral $CD4^+CD25^+$ T cells showed the phenotype of $CD45RO^+CTLA\text{-}4^+Foxp3^+CD127^-$ and displayed normal immunosuppressive function in this mouse model, similarly to the counterpart in healthy individuals. These results revealed that this humanized NOD/SCID mouse model may be used to investigate the development and function of human $CD4^+CD25^+$ regulatory T cells in vivo.

2. Materials and methods

2.1. Animals and fetal human tissues

NOD/SCID mice were kept under specific pathogen-free conditions and used at 6 to 8 weeks of age. Fetal human thymus and liver tissues of gestational age of 18 to 24 weeks were obtained from local hospitals in Beijing (China). Protocols were approved by Animal Research Committee of the Institute of Zoology and all of the experiments were performed in accordance with the protocols.

2.2. Monoclonal antibodies (mAbs) and reagents

The following mAbs were used for staining: phycoerythrin (PE)-PRA-T4 (anti-human CD4), PE-CY5-PRA-T4 (anti-human CD4), Fluorescein isothiocyanate (FITC)-M-A251 (anti-human CD25), PE-HIT8a (anti-human CD8), PE-UCHL1 (anti-human CD45RO), PE-BNI3 (anti-human CTLA-4), PE-PCH101 (anti-human Foxp3) and PE-hIL-7R-m21 (anti-human CD127). Isotype-specific negative control mAbs were FITC-MOPC-31 (mouse IgG1, k), PE-G155-178 (mouse IgG2a, k) and PE-R35-95 (rat IgG2a, k). Rat anti-mouse FcR mAb (2.4G2) was produced by 2.4G2 hybridoma (ATCC, Rockville, Maryland) in our laboratory.

Mitomycin C ($C_{15}H_{18}N_4O_5$) was obtained from Kyowa Hakko Co, Ltd. (Tokyo, Japan). [^{3}H] thymidine was purchased from China institute of atomic energy (Beijing, China). The culture medium used in this study was RPMI 1640 (Hyclone, Logan, UT) supplemented with 10% heat-inactivated FCS, 100U/ml penicillin, 100 μg/ml streptomycin, 2 mM L-glutamine, 10 mM HEPES and 50 μM 2-ME (Sigma, St. Louis, MO).

2.3. Purification of human $CD34^+$ cells

Fetal human liver cells were collected by Histopaque-1077 (Sigma Aldrich, St. Louis, MO, USA) density gradient centrifugation. $CD34^+$ cell fractions were isolated using AutoMACS (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany) according to the manufacturer's protocol. Purity was evaluated using flow cytometry and no less than 95% of the positively selected cells were $CD34^+$.

2.4. Transplantation procedures

NOD/SCID mice were conditioned with 2-Gy whole body irradiation. Fetal human Thy/Liv fragments (~1 mm^3) were implanted under the recipient double kidney capsules within 24 h after irradiation, then received human $CD34^+$ cells (1×10^5/mouse, intravenously) purified from fetal liver cells of the same donor on the day of human Thy/Liv transplantation.

2.5. Immunofluorescence staining and flow cytometry (FCM)

Lymphocytes of peripheral blood (PB), spleens, LNs and thymocytes were incubated with 2.4G2 to block FcRs and then incubated with an optimal concentration of mAbs for 30 min at 4 °C in the dark. Cells were washed three times, resuspended by FCM buffer (PBS with 0.1% BSA and 0.1% NaN_3) and assayed using a FASCalibur flow cytometry (Becton Dickinson, CA). In some experiments, non-viable cells were excluded using the vital nucleic acid stain propidium iodide (PI). The data were analyzed with CellQuest software.

For the intracellular staining, cells were incubated with PE-CY5-PRA-T4 and FITC-M-A251 mAbs firstly. After washing, these cells were fixed and stained with anti-human CTLA-4 or Foxp3, according to the instruction provided by the manufacturer (eBioscience, San Diego, CA).

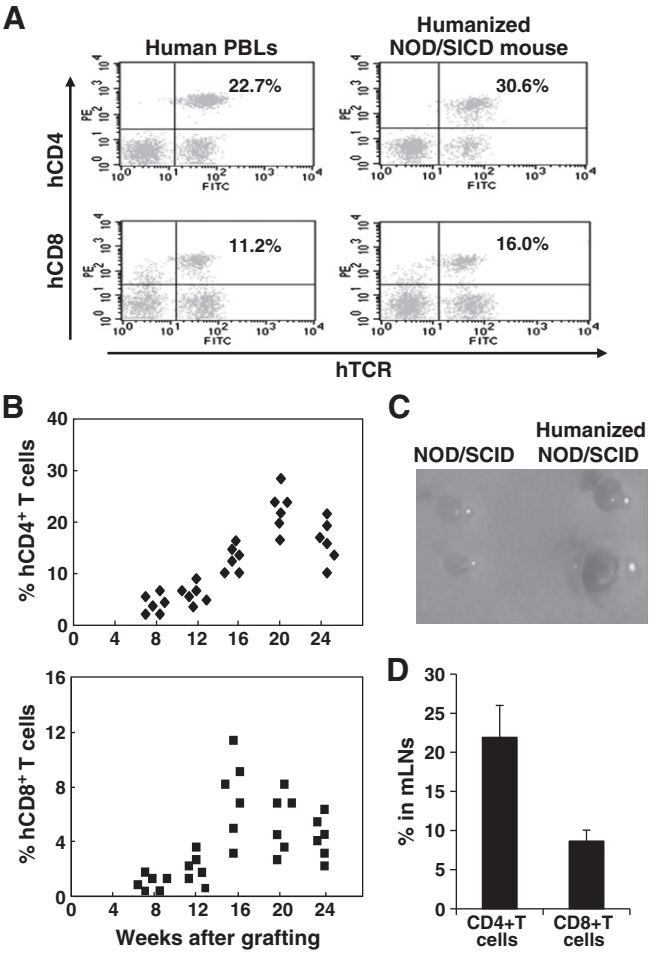

Fig. 1. Efficient population of human $CD4^+$ and $CD8^+$ T cells in the periphery of NOD/SCID mice after grafting with fetal human Thy/Liv tissue and $CD34^+$ cells. NOD/SCID mice were received 2Gy whole body irradiation and then grafted with fetal human Thy/Liv tissue in the kidney capsules and an i.v. injection of human $CD34^+$ cells. The percentages of human $CD4^+$ and $CD8^+$ T cells in the periphery were determined by FCM as described in Materials and methods. (A) One representative of the lymphocytes stained with anti-human CD4 or CD8 and TCR mAbs. Numbers in the dot plot indicate the percentage of $CD4^+TCR^+$ T cells or $CD8^+TCR^+$ T cells. (B) The percentages of human $CD4^+TCR^+$ T cells or $CD8^+TCR^+$ T cells in PBLs of humanized NOD/SCID mice at different time points after thymic grafting. Six grafted mice were followed for about 6 months. (C) The sizes of mLNs in control and humanized NOD/SCID mice 6 months after thymic grafting. (D) The percentages of human $CD4^{\pm}TCR^{\pm}$ T cells or $CD8^{\pm}TCR^{\pm}$ T cells in mLNs of humanized NOD/SCID mice 4 months after thymic grafting. No detectable human T cells in control NOD/SCID mice. Results were shown as mean ± SD(n = 5). The data presented were one representative of two experiments with identical results.