Transplant Immunology xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Transplant Immunology

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

A pilot study on the characteristics of circulating T follicular helper cells in liver transplant recipients

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ARTICLE INFO

Keywords:
T follicular helper cells
Liver transplantation
B cells

ABSTRACT

Circulating $CD4^+CXCR5^+$ T follicular helper cells (cTfh) have been demonstrated to be involved in B cell-mediated systemic autoimmune diseases and alloreactive responses following kidney transplantation; however, whether cTfh cells are involved in alloreactive responses after liver transplantation (LT) remains unclear. Our present study aimed to investigate the characteristics of cTfh, as well as $CXCR3^+CCR6^-$ Tfh1, $CXCR3^-CCR6^-$ Tfh2, and $CXCR3^-CCR6^+$ Tfh17 subsets in liver allograft recipients. A total of 30 liver transplant recipients were enrolled in this study. The frequencies of cTfh, Tfh1, Tfh2, and Tfh17 subsets, and interleukin (IL)-21-producing Tfh cells in the circulating blood were analyzed by flow cytometry. The capacity of cTfh cells to help B cells differentiate into plasmablasts was determined one day before and one month after LT. The results revealed that the frequency of cTfh cells remained unaltered before and after LT. However, the frequency of the cTfh subsets (e.g., Tfh1 and Tfh2 cells) and B cells were reduced one month after LT. Functionally, the capacity of Tfh cells to produce IL-21 was reduced one month after LT. In addition, cTfh cells exhibited the capacity to help B cells differentiate into plasmablasts in an IL-21-dependent manner in vitro, which was reduced after LT, despite the unaltered production of IgM and IgG by plasmablasts. Thus, our data suggest that cTfh cells may be involved in alloreactive responses following LT via helping B cells differentiate into plasmablasts and plasma cells.

1. Introduction

Follicular T helper (Tfh) cells are a heterogeneous population of $CD4^+$ T helper cells, which are active in secondary lymphoid organs. These Tfh cells are characterized by numerous surface markers, including CXC chemokine receptor 5 (CXCR 5), inducible costimulator (ICOS), programmed death 1(PD-1), and the transcription factor, B cell lymphoma 6 (Bcl-6) [1,2]. Differentiated Tfh cells also express interleukin (IL)-21, IL-21 receptor (IL-21R). IL-21 regulates the activation, proliferation, and survival of $CD4^+$ T cells and B cells, the functional activity of $CD8^+$ T cells and NK cells, as well as limits the differentiation of inducible regulatory T cells (Tregs) and counteracts their suppressive properties on effector T cells [3,4]. In addition, the IL-21R is expressed on a variety of immune cells, including T cells, B cells, NK cells, and dendritic cells (DCs), as well as non-immune cells (e.g. endothelial cells and epithelial cells) [3,5]. The binding of IL-21 to the IL-21R activates members of the JAK-family protein tyrosine kinases, and signal transducer and activator of transcription molecules [6]. Moreover, $CD4^+CXCR5^+$ Tfh cells are important for the survival of memory B cells and the differentiation of B cells into immunoglobulin (Ig)-producing plasmablasts or plasma cells [7]. Several studies have demonstrated that Tfh cells are elevated in the circulating blood of human and murine models of systemic autoimmune diseases, including autoimmune thyroid diseases [8] primary biliary cirrhosis [9], rheumatoid arthritis [10,11], systemic lupus erythematosus [12], primary Sjogren's syndrome [13], asthma [14], psoriasis vulgaris [15], Guillain-Barré syndrome [16], and autoimmune hepatitis [17,18]. Additionally, a recent study reported that Tfh cells are involved in B cell-mediated immune responses following kidney transplantation both in vitro and in vivo [19].

Human circulating $CD4^+CXCR5^+$ Tfh cells (cTfh) can be categorized into three distinct subsets with different capacities to regulate B

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https://doi.org/10.1016/j.trim.2018.01.003
Received 6 June 2017; Received in revised form 18 January 2018; Accepted 18 January 2018

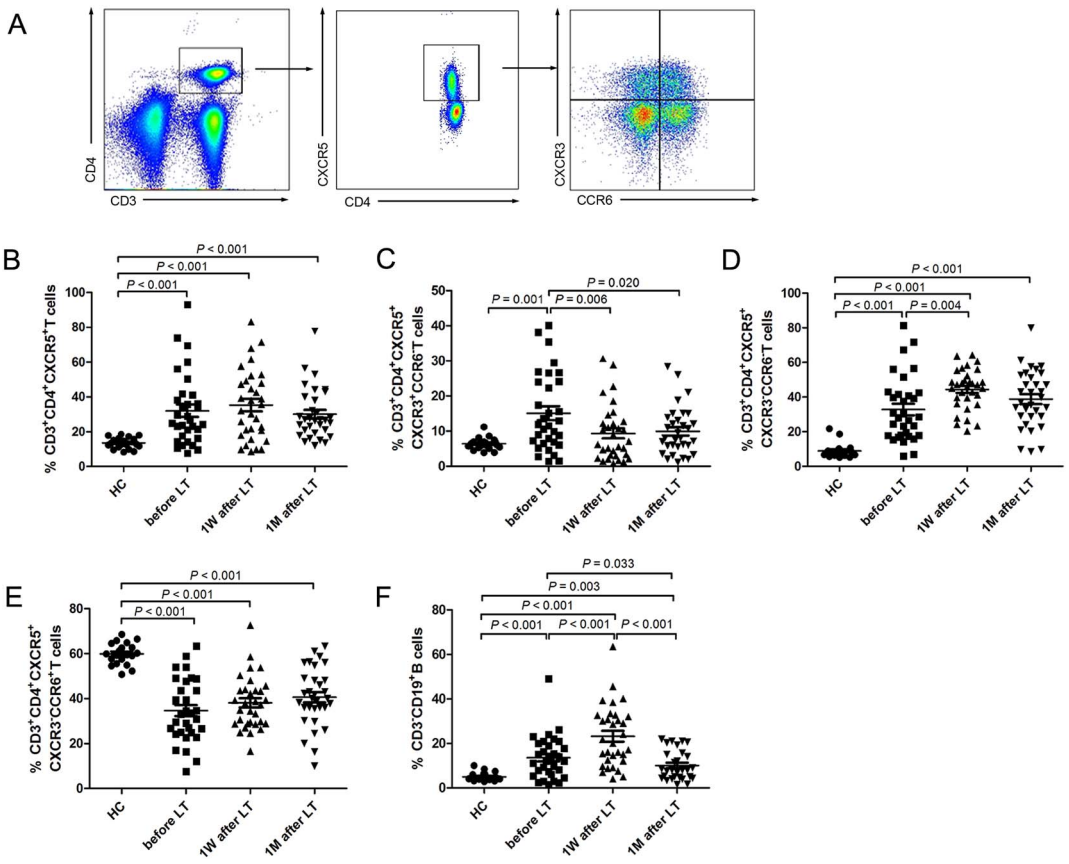

Fig. 1. The characteristics of circulating T follicular helper cell (cTfh) subsets in liver transplantation (LT) patients. (A) Representative cFfh subset profiles in the peripheral blood collected using fluorescence-activated cell sorting (FACS) before and after LT. (B) The frequencies of cTfh cells in patients before and after LT. (C) The frequency of the Tfh1 subset in patients before and after LT. (D) The frequency of the Tfh2 subset in patients before and after LT. (E) The frequency of the Tfh17 subset in patients before and after LT. (F) The frequency of B cells in patients before and after LT.

cell responses: 1) Tfh1 ($CXCR3^+CCR6^-$); 2) Tfh2 ($CXCR3^-CCR6^-$); and 3) Tfh17 ($CXCR3^-CCR6^+$) [20]. The Tfh2 and Tfh17 subsets, but not Tfh1, have been reported to induce naive B cells to produce Igs via the secretion of IL-21 [20]; however, whether cTfh or its subsets are involved in alloreactive responses following liver transplantation (LT) remains unclear.

2. Objective

In this study, we sought to examine the characteristics and function of cTfh in liver transplant recipients one day before and one month after LT.

3. Materials and methods

3.1. Patients

A total of 30 patients treated at our hospital and 20 age- and sex-matched healthy volunteers as controls were enrolled in this study. All patients received their first cadaveric LT with an identical or compatible blood-group graft. These patients received therapy with basiliximab (20 mg, intravenously [i.v.] during surgery), mycophenolate mofetil (starting dose of 0.75 g twice a day. The dose was subsequently adjusted according to the patient's condition), tacorlimus (aiming for predose concentrations of 8–13 ng/mL for months 0–3, 7–10 ng/mL for months 3–6, 6–8 ng/mL for months 6–12, approximately 5 ng/mL for months 12–24, and 3–5 ng/mL after 24 months), and prednisolone was tapered to 5 mg after month 1 and withdrawn after month 3. The patients were either hospitalized or followed up at Beijing 302 Hospital. Patients who suffered allograft rejection, primary disease recurrence and other complications during this study were excluded. The study protocol was approved by the institutional review board of our unit and written informed consent was obtained from each subject.

3.2. Flow cytometry

Peripheral blood mononuclear cells (PBMCs) were isolated from each subject one day before LT, as well as one week and one month after LT by density-gradient centrifugation using Ficoll-Hypaque (GE Healthcare, Uppsala, Sweden). PBMCs at a density of 10×10^5/tube were stained with the following fluorochrome-conjugated antibodies to characterize Tfh cells and the associated subsets, as well as B cells: brilliant violet 510 (BV510)-anti-CD3 and phycoerythrin (PE)-anti-CD19 (Biolegend, San Diego, CA, USA), peridinin chlorophyll protein (PerCP)-anti-CD4, Alexa Fluor 488-anti- CXCR5, allophycocyanin (APC)-anti-CXCR3 and BV421-anti-CCR6 (all from BD Bioscience, San Jose, CA, USA). PBMCs were stained at room temperature for 20 min. After washing with PBS, the cells were assayed using a FACSVerse (BD Bioscience). For the analysis of intracellular cytokine staining by flow cytometry, fresh whole blood samples were stimulated with PMA (500 ng/mL) and ionomycin (1 μg/mL) (Sigma-Aldrich, St. Louis, MO) at 37°C for 6 h. The cells were fixed and permeabilized with Foxp3 staining Buffer set (eBioscience, San Diego, CA) and stained with the following monoclonal antibodies: BV510-anti-CD3, PerCP-anti-CD4, Alexa Fluor 488-anti-CXCR5, PE-cy7-anti-IFN-γ, BV421-IL-17, PE-IL-21 (all from BD Bioscience, San Jose, CA, USA), and APC-anti-IL-4 (eBioscience). The cell populations were analyzed using FlowJo V10 software (Tristar Inc., San Carlos, CA, USA).