Compromised immune status of patients with post-liver transplant biliary complications

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Immunosuppression (IS) is indispensable for liver transplant (LTx) patients to control the unwanted alloimmune responses, which are mainly mediated by T cells. However, antigen-independent homeostasis of T cells is vital for sustaining long-lived T cell-mediated immunity, and excessive IS may increase the risk of opportunistic infections and malignancies. Therefore, IS therapy for LTx patients should be tailored to the immune status of the individual patient.1,2 Biliary complications (BC), including biliary strictures with cholangitis, are the most common complications after LTx. However, the immunological characteristics especially T cell-mediated immunity of these patients are unknown yet.

Human T cells are heterogeneous with different subsets and functions according to the expression of CD45RA and CD62L. The cells include naive cells (Tn, CD45RA+CD62L+), stem cell memory T cells (Tscm, CD45RA+CD62L+CD95+), central-memory cells (Tcm, CD45RA-CD62L-), effector-memory cells (Tem, CD45RA-CD62L-), and terminally differentiated effector subsets (CD45RA-CD62L-). Pathogens are controlled more efficiently by memory than by naive T cells. Tscm are a specialized subset of memory T cells that differentiate directly from naive precursors. Tscm reconstitute the full diversity of memory T cells upon antigen priming and maintain their own pool size through self-renewal.2 However, the differentiation of human Tscm to Tcm or Tem, and their function in LTx patients are unclear.

In this study, 42 patients who underwent orthotopic LTx within one year in the First Affiliated Hospital of Xi’an Jiaotong University were included. Patients with post-LTx BC were mainly biliary stricture confirmed by magnetic resonance cholangiopancreatography (MRCP) within 1 year after LTx. Blood samples were collected next day after the confirmation of biliary stricture by MRCP. Age-matched patients with stable liver function and no BC within 1 year after LTx were put in the transplant group without BC. One Patient with rejection was excluded from the final analysis. Eighteen age-matched healthy volunteers were recruited to serve as healthy controls (HCs). This study was approved by the Institutional Review Board (No. 2019 G-213). All participants signed a written informed consent form to allow the research. Characteristics of the patients including age, gender, primary liver disease, liver enzyme levels, concentration of immunosuppressants, and immune cells count on the day for T cells analysis are listed [Supplementary Table 1, http://links.lww.com/CM9/A313]. To compare results between the two groups, Student’s unpaired t test was used for the normally distributed data, while Mann-Whitney U test was used for not normally distributed data. In BC group, the levels of aspartate aminotransferase and total bilirubin were significantly higher compared to the levels in patients without BC. Complete blood count data showed that patients with post-LTx BC had a decreased absolute number of white blood cells and neutrophils, with a slightly increased proportion of lymphocytes, but the absolute count of lymphocytes was similar. Three patients with infections in the BC group (18.75%) had C-reactive protein levels >10 mg/L on the day for T cell analysis.

We further focused on the T cell heterogeneity and function in LTx patients. Peripheral blood mononuclear cells (PBMCs) were isolated using Ficoll gradient centrifugation.

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Figure 1: Compromised T cell immunity in LTx patients with BC. (A) Decreased numbers of CD4+ T cells and increased numbers of CD8+ T cells in LTx patients with BC. (B) CD8+ T cells from LTx patients with BC showed less Tscm and more Tem. (C–H) Percentage of CD8+ Tscm was negatively correlated with percentage of CD8+ Tem, rather than with percentage of CD8+ Tcm, in LTx patients (with or without BC) and HCs. (I) MFI of PD-1, Tim-3, CD39, and CTLA-4 expression on T cell subsets. Data in each group in A, B, I were shown as median and interquartile range. BC group: n = 16, without BC group: n = 23, HCs group: n = 12. BC: Biliary complications; CTLA-4: Cytotoxic T-lymphocyte-associated protein 4; DNT: Double negative T cells; HCs: Healthy controls; LTx: Liver transplantation; MFI: Mean fluorescence intensity; PD-1: Programmed cell death protein 1; Tn: Naïve T cells; Tscm: Stem cell memory T cells; Tcm: Central-memory cells; Tem: Effector-memory cells; Temra: Terminally differentiated effector cells; Tim-3: T cell immunoglobulin and mucin domain 3.
PBMCs were either stained directly or stimulated with phorbol 12-myristate 13-acetate and ionomycin in the presence of brefeldin A for 5 h for cytokine detection by flow cytometry. The flow cytometry gating scheme for T cell subsets is shown in Supplementary Figure 1, http://links.lww.com/CM9/A312. Following LTx, patients with BC showed decreased numbers of CD4+ T cells and increased numbers of CD8+ T cells compared to the numbers in patients without BC [Figure 1A]. Significantly decreased numbers of Tn and Tscm, and increased numbers of Tem were observed in CD8+ T cells, but not in CD4+ effector T cells (Teff) or regulatory T cells (Tregs) [Figure 1B, Supplementary Figure 2A and 2B, http://links.lww.com/CM9/A312].

We also found a significant negative correlation between Tscm and Tem, rather than with Tcm, in both CD8+ and CD4+ T cells in HCs [Figure 1E and 1H, Supplementary Figure 2E and 2H, http://links.lww.com/CM9/A312]. The correlation was calculated by the Pearson correlation method using GraphPad Prism 6. These findings may suggest direct differentiation of Tscm to Tem. However, in LTx patients, a negative correlation between Tscm and Tem was observed in CD8+ T cells, but not in CD4+ T cells [Figure 1C, D, F, G, Supplementary Figure 2, http://links.lww.com/CM9/A312]. In LTx patients without BC, the value of the correlation coefficient between CD8+ Tscm and CD8+ Tem was similar to the value in HCs (−0.6318), while the value in LTx patients with BC was smaller (−0.5451) [Figure 1C–E].

To further investigate the function of CD4+ Teff, CD8+ T cells, and Tregs in LTx patients with BC, we performed cytokine secretion in CD4+ Teff and CD8+ T cells and CD39 and CTLA-4 expression on Tregs. [Figure 1I]. Higher expression of PD-1 and the weakened cytokine secretion capacity in CD4+ T cells [Supplementary Table 1, http://links.lww.com/CM9/A312]. T cell impairment was further supported by the higher expression of PD-1 and the weakened cytokine secretion capacity in CD4+ T cells. Interestingly, 87.5% of the patients with BC were under FK506 suppression, while 88% patients without BC were under cyclosporin A IS [Supplementary Table 1, http://links.lww.com/CM9/A313]. This might be noteworthy for a future study with large number of patients.

In summary, IS treatment of patients with post-LTx BC should be tailored more carefully with more precise immune monitoring of T cells differentiation.

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**Conflicts of interest**

None.

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