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

Hong Lei[1], Min Tian[2], Xiao-Gang Zhang[2], Lie-Su Meng[3], Wen-Hua Zhu[3], Xue-Min Liu[2], Meng-Zhou Wang[1], Tao Wang[1], Peng-Kang Chang[1], Huan Chen[1], Bo Wang[2], Rong-Qian Wu[1], Yi Lyu[1,2]

[1]National Local Joint Engineering Research Center for Precision Surgery and Regenerative Medicine, The First Affiliated Hospital of Xi'an Jiaotong University, Xi'an, Shaanxi 710061, China;
[2]Department of Hepatobiliary Surgery, The First Affiliated Hospital of Xi'an Jiaotong University, Xi'an, Shaanxi 710061, China;
[3]Department of Biochemistry and Molecular Biology, School of Basic Medical Sciences, Xi'an Jiaotong University, Xi'an, Shaanxi 710061, China.

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] 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 naïve 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 naïve T cells. Tscm are a specialized subset of memory T cells that differentiate directly from naïve 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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Correspondence to: Dr. Yi Lyu, National Local Joint Engineering Research Center for Precision Surgery & Regenerative Medicine, The First Affiliated Hospital of Xi'an Jiaotong University, Xi'an, Shaanxi 710061, China
E-Mail: luyi169@126.com

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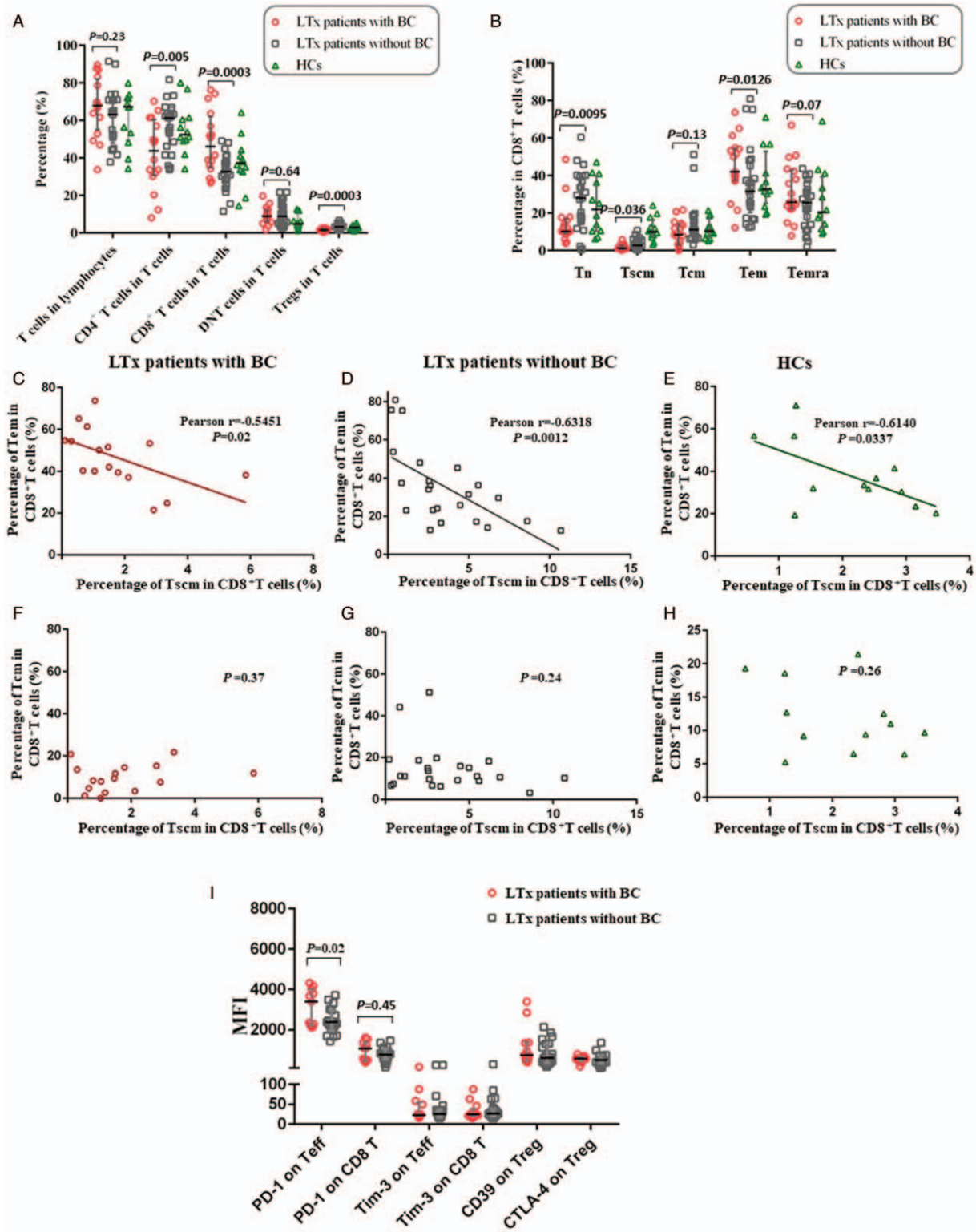

Figure 1: Compromised T cell immunity in LTx patients with BC. (A) Decreased numbers of $CD4^+$ T cells and increased numbers of $CD8^+$ T cells in LTx patients with BC. (B) $CD8^+$ T cells from LTx patients with BC showed less Tscm and more Tem. (C–H) Percentage of $CD8^+$ Tscm was negatively correlated 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, the expression of the co-inhibitory receptors programmed cell death protein 1 (PD-1) and T cell immunoglobulin and mucin domain-3 together with CD39 and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) was evaluated [Figure 1I]. Higher expression of PD-1 was observed in LTx patients with BC on $CD4^+$ Teff, indicating the Teff exhaustion. $CD4^+$ Teff and $CD8^+$ T cells from LTx patients with BC also showed decreased interferon-γ or interleukin-2 production [Supplementary Figure 2I and 2J, http://links.lww.com/CM9/A312]. However, we did not find significant differences in CD39 and CTLA-4 expression on Tregs.

Regarding the differentiation of Tscm to memory T cells, some researchers support the initial differentiation of Tscm to Tcm, then to Tem, upon antigen priming,[3] while others believe that Tscm first differentiates to form effector T cells, with some becoming long-lived memory cells.[4-6] Our results support the direct differentiation to Tem. Importantly, the decreased correlation coefficient between $CD8^+$ Tscm and $CD8^+$ Tem in LTx patients with BC may indicate the decelerated differentiation of Tscm to Tem. Similarly, impairment of this differentiation may be more severe in $CD4^+$ T cells in LTx patients, as evidenced by the lack of negative correlation between $CD4^+$ Tscm and $CD4^+$ Tem [Supplementary Figure 2, 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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