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## **Identification and comparison of functional microbiomes affecting immune homeostasis in long-term stable and tolerant patients after liver transplantation**

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**Introduction:** In this study, we aimed to identify the gut microbial balance and functional microbiomes affecting immune homeostasis in the long-term post-liver transplant (LT) and tolerant patients.

**Methods:** A total of 27 long-term LT patients and 20 healthy volunteers were consecutively enrolled in our study. Of 27 included LT patients, 22 patients ingested immunosuppressants (long-term post-LT group) and the other five were tolerant patients without immunosuppressants (tolerance group). Included LT patients had normal liver function without history of rejection and underwent LT more than 5 years ago. Healthy controls have no medical diseases including metabolic and alcoholic disease. The frequency of regulatory T (Treg) and T helper 17 (Th17) cells and cytokines in the blood were analyzed by flow cytometry and multiplex cytokine assay. Moreover, the diversity and composition of fecal microbiomes were analyzed by 16S rRNA sequencing.

**Results:** The mean age of LT patients was 63.0 years and the mean time from LT was 13.2 years. The gut microbiome of the long-term post-LT group showed lower alpha-diversity ( $P < 0.05$ ) with distinct overall microbial composition ( $P = 0.001$ ) compared to healthy controls. Among the 11 distinct bacterial genera in abundance, Faecalibacterium was the most decreased in the long-term post-LT group (Figure 1A). The long-term post-LT group also demonstrated a decrease in Treg with an increase in Th17 cells, recovered by administration of *F. prausnitzii* and butyric acid in in vitro analysis. Moreover, in tolerant patients, Faecalibacterium was marginally increased, coupled with an increase in Treg cells, compared to the long-term post-LT group (Figure 1B).

**Conclusion:** The long-term post-LT patients showed a decrease in functional microbiomes represented as Faecalibacterium affecting immune homeostasis, which were recovered in tolerant patients.