Gut microbiota derived immunological signature in metabolic alcohol associated liver disease

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Introduction

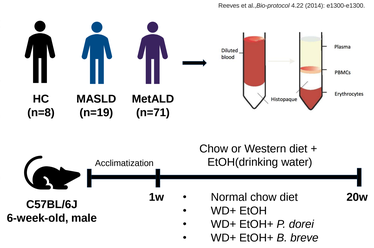
Liver has been known as an immune organ rich in innate immune cells and exposed to nutrients and endotoxins derived from the gut microbiome. Mucosal-associated invariant T (MAIT) cells are activated and initiate immune responses when their T cell receptor recognizes the riboflavin metabolite (5-OP-RU) derived from bacteria, presented by MR1, under co-stimulatory signals from specific cytokines or toll-like receptors.

Aim

Although MAIT cells play a central role in various diseases, studies on the gut microbiome related to the function of MAIT cells are still lacking in metabolic alcohol associated liver disease (MetALD). We aimed to demonstrate the role of *Phocaeicola dorei* (with high riboflavin secretion) and *Bifidobacterium breve* (with low riboflavin secretion) in the MetALD model.

Method

A total of 98 human blood samples [healthy control: n = 8, metabolic dysfunction-associated steatotic liver disease (MASLD): n = 19 and MetALD (hepatitis: n = 16 and cirrhosis: n = 55)] were collected for flow cytometry analysis. Six-week-old male C57BL/6J mice were divided into four groups [n = 9 /normal control, n = 12 /group; Western diet (WD)+EtOH, WD+EtOH+*P. dorei*, WD+EtOH+*B. breve* (10^9 CFU/ 100ul by oral gavage 3 times a week for 20 weeks)].



Results

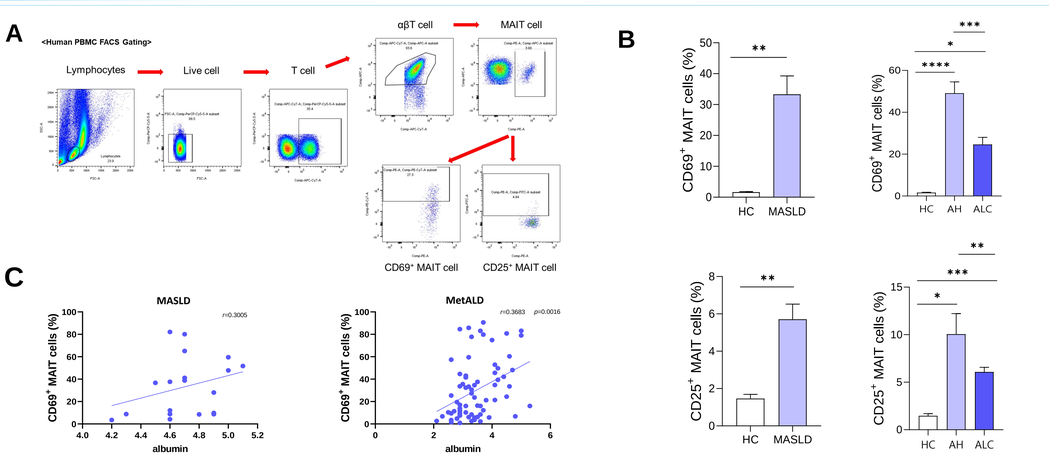


Figure 1. (A) Flow cytometry gating strategy to identify MAIT cells. (B) Flow cytometry analysis of MAIT cell activation isolated from PBMs of healthy controls, MASLD, and MetALD patients. (C) Correlation between biomarkers in blood and MAIT cell activation in liver disease patients.

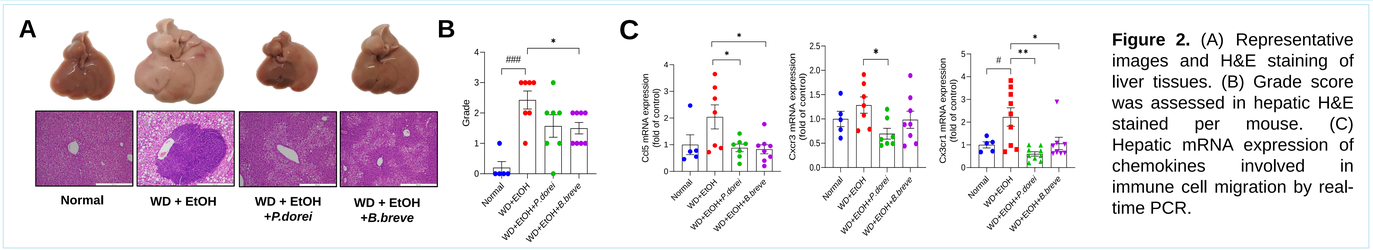


Figure 2. (A) Representative images and H&E staining of liver tissues. (B) Grade score was assessed in hepatic H&E stained per mouse. (C) Hepatic mRNA expression of chemokines involved in immune cell migration by real-time PCR.

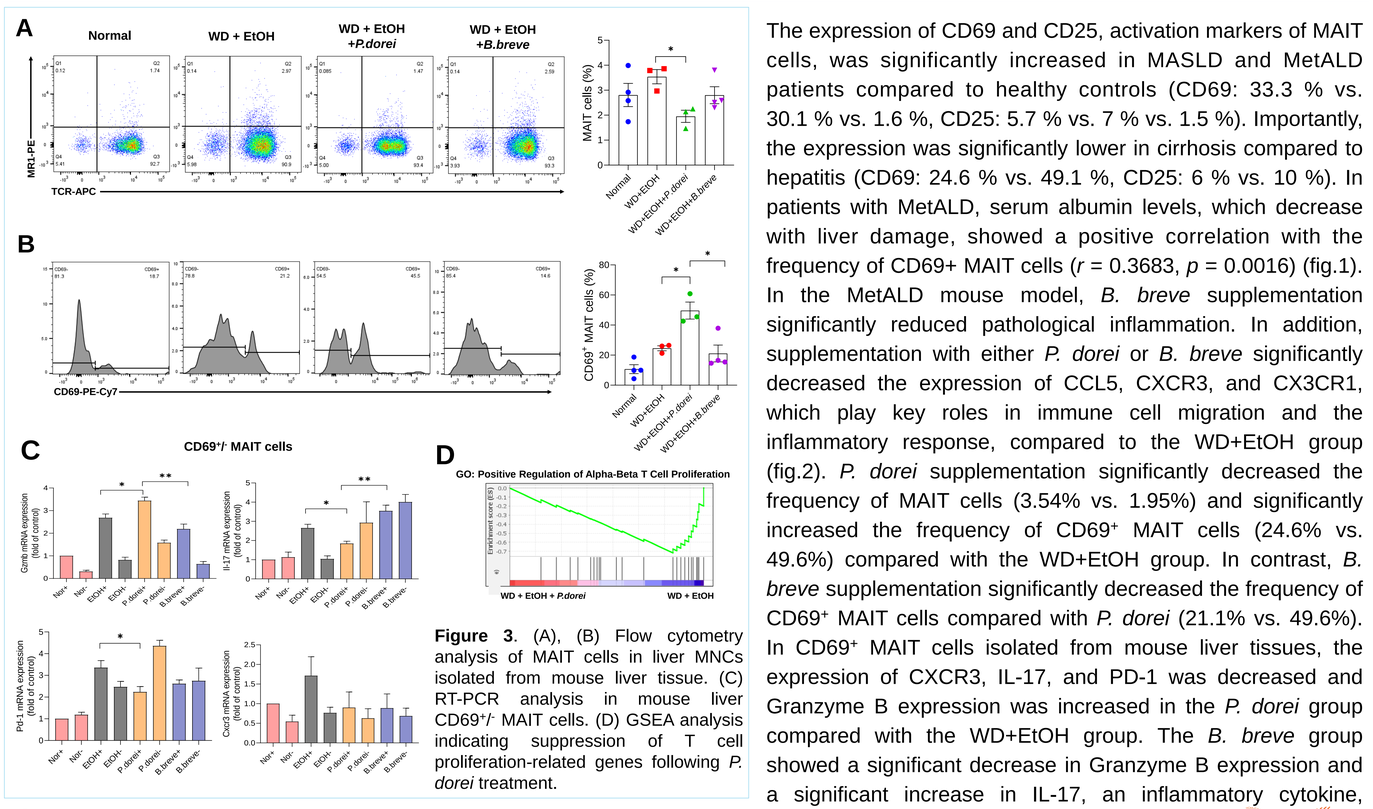


Figure 3. (A), (B) Flow cytometry analysis of MAIT cells in liver MNCs isolated from mouse liver tissue. (C) RT-PCR analysis in mouse liver CD69$^{+/-}$ MAIT cells. (D) GSEA analysis indicating suppression of T cell proliferation-related genes following *P. dorei* treatment.

The expression of CD69 and CD25, activation markers of MAIT cells, was significantly increased in MASLD and MetALD patients compared to healthy controls (CD69: 33.3 % vs. 30.1 % vs. 1.6 %, CD25: 5.7 % vs. 7 % vs. 1.5 %). Importantly, the expression was significantly lower in cirrhosis compared to hepatitis (CD69: 24.6 % vs. 49.1 %, CD25: 6 % vs. 10 %). In patients with MetALD, serum albumin levels, which decrease with liver damage, showed a positive correlation with the frequency of CD69+ MAIT cells (r = 0.3683, p = 0.0016) (fig.1). In the MetALD mouse model, *B. breve* supplementation significantly reduced pathological inflammation. In addition, supplementation with either *P. dorei* or *B. breve* significantly decreased the expression of CCL5, CXCR3, and CX3CR1, which play key roles in immune cell migration and the inflammatory response, compared to the WD+EtOH group (fig.2). *P. dorei* supplementation significantly decreased the frequency of MAIT cells (3.54% vs. 1.95%) and significantly increased the frequency of CD69$^+$ MAIT cells (24.6% vs. 49.6%) compared with the WD+EtOH group. In contrast, *B. breve* supplementation significantly decreased the frequency of CD69$^+$ MAIT cells compared with *P. dorei* (21.1% vs. 49.6%). In CD69$^+$ MAIT cells isolated from mouse liver tissues, the expression of CXCR3, IL-17, and PD-1 was decreased and Granzyme B expression was increased in the *P. dorei* group compared with the WD+EtOH group. The *B. breve* group showed a significant decrease in Granzyme B expression and a significant increase in IL-17, an inflammatory cytokine, compared to the *P. dorei* group GSEA showed that *P. dorei* treatment suppressed gene sets associated with the proliferation of alpha-beta T cells, suggesting an immunoregulatory effect. (fig.3).

Conclusions

P.dorei suppresses inflammatory responses, alleviates liver damage, and contributes to regulating immune balance in the liver through activation of MAIT cells.

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Metabolism, alcohol and toxicity
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