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Article

Dysbiosis of the gut microbiome may contribute to the pathogenesis of oral lichen planus through Treg dysregulation

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ABSTRACT

Oral lichen planus (OLP) is a chronic inflammatory disorder with autoimmune features and malignant transformation risk, lacking a definitive treatment, with $CD4^+$ T cells being pivotal in its pathogenesis. Dysbiosis, an imbalance in the microbiome, is linked to various autoimmune and inflammatory diseases, where $CD4^+$ T cells play a significant role. Given these insights, the development of OLP might be influenced by dysbiosis. This study investigates the association between dysbiosis and $CD4^+$ T cells in OLP. We collected stool and saliva samples from OLP patients, conducting 16S rRNA gene analysis and mass spectrometry, and assessed $CD4^+$ T cell characteristics in lesions through multiplex immunofluorescence and single-cell RNA sequencing. Peripheral blood samples were subjected to flow cytometry and cell culture assays. Results showed extensive gut dysbiosis in OLP patients, notably a reduction in short-chain fatty acid (SCFA)-producing bacteria essential for regulatory T cell (Treg) differentiation. While various $CD4^+$ T cell subsets, including Tregs, were present in tissues, these Tregs as unresponsive to specific antigens, showing reduced immunosuppressive molecule expression. The decline in SCFA-producing bacteria correlated with fewer activated Tregs in tissues and blood. These findings suggest that gut dysbiosis may contribute to OLP by impairing Treg regulation, influencing disease pathogenesis.

Abbreviations: B. longum, *Bifidobacterium longum*; *B. adolescentis*, *Bifidobacterium adolescentis*; CTLA-4, cytotoxic T-lymphocyte associated protein 4; *F. prausnitzii*, *Faecalibacterium prausnitzii*; HC, healthy control; IL, interleukin; LEfSe, linear discriminant analysis effect size; NSU, non-specific ulcer of oral mucosa; HK, hyperkeratosis; OED, oral epithelial dysplasia; OLP, oral lichen planus; OSCC, oral squamous cell carcinoma; PBMCs, peripheral blood mononuclear cells; PCoA, principal coordinate analysis; PERMANOVA, permutational analysis of variance; SCFA, short-chain fatty acid; scRNA-seq, single-cell RNA sequencing; Treg, regulatory T cell; TGF-β, transforming growth factor β.

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Introduction

Lichen planus is a chronic inflammatory condition affecting the skin. When it involves the mucous membranes, it is referred to as oral lichen planus (OLP). OLP is characterised by parakeratosis of the oral mucosa and band-like infiltration of T cells in the subepithelial layer. The disease is linked to cell-mediated immune dysfunction; some cases of OLP can progress to squamous cell carcinoma, highlighting the need for long-term monitoring.[1–3] One of the main challenges in managing OLP is that, despite its suspected autoimmune origins, the underlying aetiology remains unknown. This uncertainty has resulted in a lack of definitive treatments, although the disease is common and has the potential for malignant transformation.[4,5] Thus, a better understanding of OLP pathogenesis is essential for the development of more effective treatments.

The band-like infiltration of T cells in OLP suggests an association with immune dysfunction, and autoimmune mechanisms have been implicated in its pathogenesis. The predominant population in the subepithelial lymphocyte infiltrate is composed of cluster of differentiation $CD4^+$ T helper (Th) cells, which include Th1, Th2 and Th17 cells, as well as $CD8^+$ T cells.[6–9] Similar to other autoimmune diseases, there is evidence that regulatory T cells (Tregs) play a critical role in OLP pathogenesis.[10] Compared with samples from healthy controls (HCs), increased levels of Tregs have been found in OLP lesions and peripheral blood from affected patients, suggesting that the balance among various lymphocyte subtypes influences the clinical course of the disease. Studies of OLP lesions have revealed a negative correlation between Treg number and disease activity. Notably, the proportion of Tregs in peripheral blood significantly increased among patients who underwent immunosuppressive treatment.[11].

Despite ongoing research, the mechanisms underlying immune dysfunction, including factors that contribute to autoimmune diseases, remain unclear. However, several chronic immune-mediated conditions associated with immune dysfunction have been linked to alterations in the gut microbiome highlighting the importance of gut dysbiosis research.[12] The gut microbiome exists in close symbiosis with the host, providing essential metabolic molecules that influence various aspects of host physiology, including immune system maturation.[13] Advancements in next-generation sequencing technology have revealed that gut microbiome dysbiosis, characterised by the expansion or depletion of specific bacteria and their associated proteins and metabolic activities, is associated with numerous autoimmune diseases.[14–16] In conditions such as inflammatory bowel disease, rheumatoid arthritis, Kawasaki disease, and systemic sclerosis—where $CD4^+$ T cells have been implicated in the pathogenesis—microbiome analysis has provided insights into immune response distortion and corresponding pathogenesis. Considering that OLP affects the oral mucosa, which can be considered an extension of the gastrointestinal system, a potential relationship between dysbiosis and OLP pathogenesis has been suspected but remains unexplored. Several recent reports have suggested that dysbiosis in the oral/salivary microbiome of OLP patients supports the need to investigate the gut microbiome in these patients.[4,5].

We hypothesized that dysbiosis is associated with the pathogenesis of OLP and aimed to investigate the relationship between oral/gut microbiome and the pathogenesis of OLP, particularly from an immunological perspective. This study is the first to investigate gut and oral microbiome dysbiosis in samples of saliva and stool from corresponding OLP patients. We demonstrated a reduction in short-chain fatty acid (SCFA)-producing bacteria in the gut microbiome, which has been associated with impaired Treg differentiation.[17,18] Additionally, we examined all conventional $CD4^+$ T cell subsets in tissue lesions using single-cell RNA sequencing (scRNA-seq), T cell receptor (TCR) repertoire analysis, multiplex immunofluorescence (IF) staining, and flow cytometry. Our results showed that molecules associated with Treg activation and function were not upregulated in either tissue-infiltrating or circulating Tregs, consistent with the observed decrease in SCFA-producing bacteria. This comprehensive approach provides new insights concerning the connection between gut and oral microbiome dysbiosis and the functional alterations of Tregs in OLP tissue lesions, which may contribute to the observed disease symptoms.

Results

Comprehensive investigation of the relationships between immune cells and the gut and oral microbiomes

To understand these relationships of the oral and gut microbiomes with the pathogenesis of OLP, as well as their connection to immune cells, we performed 16S rRNA gene sequencing to identify bacteria in stool and saliva samples from 30 HCs and 33 OLP patients. Then, high-performance liquid chromatography–tandem mass spectrometry (HPLC-MS/MS) analysis was performed using stool samples from 6 HCs and 5 OLP patients. We also processed a biopsy sample from an OLP patient for scRNA-seq and integrated it with both our previous data and published scRNA-seq data from 9 OLP patients, 4 HCs, and 12 OSCC patients.[6,19-21] For validation, we performed multiplex IF staining and image quantification on tissue samples from 26 OLP patients, 9 HCs, 7 hyperkeratosis (HK) patients, 7 non-specific ulcer of oral mucosa (NSU) patients, 13 oral epithelial dysplasia (OED) patients, and 9 oral squamous cell carcinoma (OSCC) patients. Subsequently, we used flow cytometry to analyse peripheral blood mononuclear cells (PBMCs) isolated from blood samples of 10 HCs and 14 OLP patients. Finally, cell-culture assays by using sorted Treg were performed to investigate the impact of SCFAs co-culture on Tregs and evaluate the functionality of Tregs in OLP patients (Fig. 1A).

Altered gut microbial diversity between OLP and HC groups

It is widely accepted that changes in gut flora are associated with many diseases, including inflammatory bowel disease, rheumatoid arthritis, Kawasaki disease, and systemic sclerosis, and may play a role in the pathogenesis of those diseases. To determine whether gut microbial diversity differs between OLP patients and HCs, we initially calculated the alpha diversity of gut microbiome in each set of samples. Although no significant differences in Shannon and Pielou indices were evident between the OLP and HC groups, we found statistically significant differences in Faith's phylogenetic diversity (PD) and the number of observed features, suggesting variation in species' evolutionary relationships and feature richness (Fig. 1B). Next, we assessed the similarity of microbial communities between the OLP and HC groups via beta diversity analysis using the Bray–Curtis distance metric. Principal coordinate analysis (PCoA) was then conducted to visualise the sample distribution; samples were color-coded by group (Fig. 1C). Additionally, permutational analysis of variance (PERMANOVA) revealed a statistically significant difference between the OLP and HC groups. These diversity analyses demonstrated that gut microbial diversity in the OLP group was altered relative to the HC group.

Depletion of specific types of Bifidobacterium and Faecalibacterium prausnitzii in the OLP gut microbiome

Considering the observed differences in alpha and beta diversities, we analysed the gut microbiome composition in the OLP and HC groups at the species and genus levels using the linear discriminant analysis effect size (LEfSe) method. The analysis showed that the HC group had a higher abundance of *Bifidobacterium* at the genus level (Fig. 1D and Supplementary Fig. 1A). *Bifidobacterium*, a commensal bacterial genus, is known for its pro-homeostatic and anti-inflammatory immunomodulatory properties.[22,23] At the species level, 20 bacteria with relative abundances exceeding 1 % were considered predominant; *Faecalibacterium prausnitzii* C71358 (*F.prausnitzii* C71358), *Bifidobacterium adolescentis* (*B.adolescentis*), and *Bifidobacterium longum* (*B.longum*)

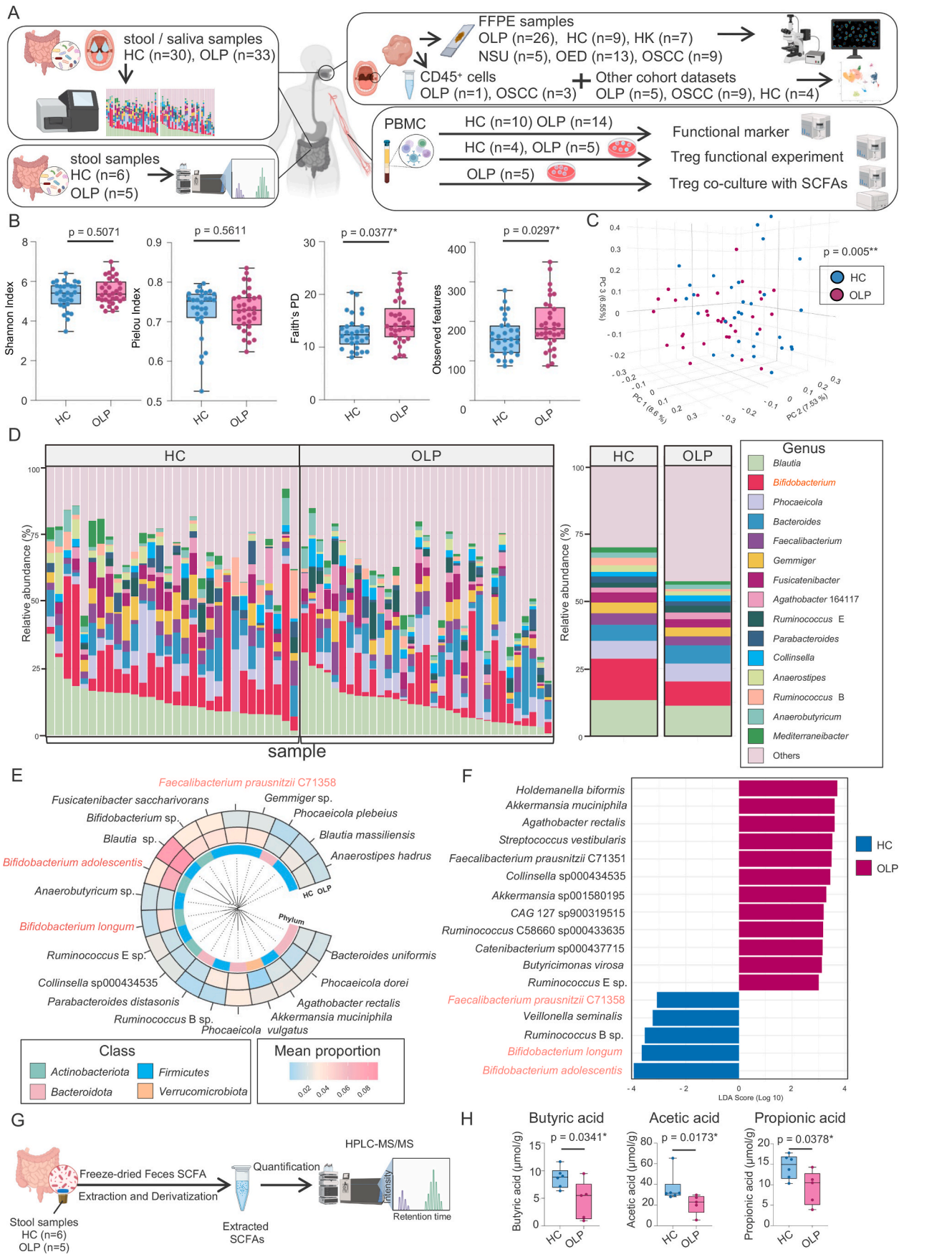

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Fig. 1. Altered gut microbiome diversity and depletion of SCFAs-producing bacteria in the oral lichen planus (OLP) gut microbiome. (A) Workflow schema. Gut and saliva samples from OLP patients and healthy controls (HCs) were used for 16S rRNA gene sequencing and high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS), tissue samples were used for multiplex immunofluorescence (IF) staining and single-cell RNA sequencing (scRNA-seq), and peripheral blood mononuclear cells (PBMCs) were used for in vitro experiments. (B) Alpha diversity indices of the gut microbiome. Faith's phylogenetic diversity (PD) and observed features values were significantly higher in the OLP group. $^{*}P < 0.05$ and $^{**}P < 0.01$ according to Mann–Whitney *U* test. (C) Principal coordinates analysis (PCoA) plot based on Bray–Curtis distance. Permutational analysis of variance (PERMANOVA) test shows a statistically significant difference between the two groups. (D) Relative abundances of gut microbes at the genus level. Left panels show individual compositions; right panels show the mean relative abundances for each group. (E) Relative abundances of gut microbes at the species level. Twenty species with ≥ 1 % relative abundance in all samples were ordered using a phylogenetic tree. (F) Linear discriminant analysis effect size (LEfSe) assessment at the species level. Only taxa with an LDA score (log10) > 3.0 are displayed. (G) Schematic illustration of quantification of SCFAs using HPLC-MS/MS. Stool samples were collected from OLP patients and HCs, and SCFAs were extracted and derivatized for quantification. (H) Comparisons of mass-specific concentration of SCFAs (short chain fatty acids). Butyric acid, acetic and propionic acid were quantified by HPLC-MS/MS. $^{*}P < 0.05$ and $^{**}P < 0.01$ according to Mann–Whitney *U* test or Student's *t*-test (two-tailed *t*-test). Each test was performed after applying the D'Agostino & Pearson test to determine and validate the presence of normal distribution.

exhibited higher abundances in the HC group than in the OLP group (Fig. 1E). Additionally, LEfSe confirmed that several bacteria, including *F. prausnitzii* C71358, *B. adolescentis*, and *B. longum*, were more prominent in the HC group (Fig. 1F, Supplementary Fig. 2A–C). Notably, some species of *Bifidobacterium*, as well as *F. prausnitzii*, are SCFA-producing gut bacteria; they also have been associated with the induction and differentiation of Tregs.[24–28] The depletion of these SCFA-producing bacteria in OLP patients suggests a link to Treg dysfunction, potentially contributing to weakened immune regulation. Although the presence of representative SCFA-producing bacteria has been found to decrease in OLP, it remained unclear whether the overall production of SCFAs was reduced. Thus, we quantified these SCFAs using HPLC-MS/MS, revealing that the total levels of butyrate, acetate, and propionate were significantly decreased in the stool of OLP patients compared to HCs (Fig. 1G and H, Supplementary Fig. S3).

Oral microbial community structure varied between OLP and HC groups

Similar to the gut microbiome, the relationship between OLP and the oral microbiome has not been thoroughly investigated. Because OLP is an immune-related disorder affecting the oral mucosa, we compared oral microbiome characteristics between OLP patients and HCs. Although there were no statistically significant differences in the alpha diversity of the oral microbiome between the OLP and HC groups—measured by the Shannon index, Pielou index, Faith's PD, and observed features (Fig. 2A)—beta diversity analysis revealed clear differences. Visualisation of sample coordinates using the Bray–Curtis distance metric and PCoA showed distinct clustering between the OLP and HC groups (Fig. 2B); PERMANOVA confirmed a statistically significant difference between the two groups. However, it remains unclear whether OLP influences the microbiome or the microbiome contributes to OLP onset. We also analysed the abundances of oral microbes at the genus and species levels (Fig. 2C, 2D and Supplementary Fig. 1B), and we performed LEfSe for species-level comparison between the two groups (Fig. 2E). No significant differences in the abundances of typical microbiome were present, and no high linear discriminant analysis scores were observed.

Tregs are the most dominant subset of infiltrating CD4^{+} T cells in OLP tissues

Given the potential association between SCFA-producing bacteria and Tregs, we conducted multiplex IF staining on OLP tissues to investigate the composition of CD4^{+} T cells in OLP lesions. In addition to Tregs, several subsets of conventional CD4^{+} T cells (e.g., Th1, Th2, Th17, T follicular helper cell, and CD4^{+} cytotoxic T lymphocytes) had infiltrated the lesions (Fig. 3A). Quantification revealed a statistically significant expansion of Tregs in OLP lesions compared with other CD4^{+} T cell subsets, in terms of density and frequency (Fig. 3B). It is established that Tregs exhibit various phenotypes. Upon examining the expression of representative markers in infiltrating Tregs, the majority unexpressed these markers, although some Tregs expressed GATA3 (Fig. 3C). Furthermore, the proportion of Tregs among CD4^{+} T cells in the lesional epithelium of OLP was significantly increased compared to the normal epithelium (HC) of the OLP tissue and HK (Fig. 3D). When evaluating these CD4^{+} T cell subsets, we noticed a relatively high proportion of marker-negative CD4^{+} T cells, particularly in HC. While leukocytes infiltrating tissues are generally regarded as activated, it is believed that a number of these may not be effector T cells that do not clearly express specific markers, or T cells possessing plasticity.[29].

Tregs exhibit a non-activated state and low clonal expansion in OLP lesions

ScRNA-seq and TCR repertoire analyses offer robust insights concerning tissue-infiltrating immune cells. To resolve the discrepancy in OLP, where SCFA-producing bacteria that promote functional Treg maturation are reduced, while substantial Treg infiltration is observed at the lesion site, we integrated our scRNA-seq data with previously published datasets. We analysed CD45^{+} immune cells from OLP tissue samples, OSCC tissue samples, and HC tissue samples. Various immune cell subsets were identified based on their marker genes across these groups (Fig. 4A and B). We then performed unsupervised clustering analysis of T cell subsets, which revealed distinct phenotypes such as Tregs, central memory T (T_{CM}) cells, effector memory T (T_{EM}) cells, and resident memory T (T_{RM}) cells (Fig. 4C and E). Compared with the OSCC and HC groups, the OLP group exhibited a greater proportion of infiltrating Tregs among the T cells (Fig. 4D). These Tregs expressed typical marker genes such as *FOXP3, CTLA4,* and *IL2RA* (Fig. 4E). Notably, the expression levels of *CTLA4, TGFB1,* and *IL2RA* in OLP Tregs were significantly downregulated relative to the high levels displayed by Tregs in OSCC but there was no significant difference compared to the Tregs in HC (Fig. 4F).[30] Although the proportion of Tregs was higher in OLP, their clonal expansion levels were lower than those of Tregs in OSCC (Fig. 4G and H). These results suggested that despite their large numbers, the Tregs infiltrating OLP lesions exhibit a non-activated state characterised by low clonal expansion and low expression of genes associated with immunosuppression, in contrast to OSCC involving Treg activation.

Increased infiltration of potentially non-activated Tregs in OLP lesions

To validate the results of our scRNA-seq analysis, we conducted multiplex IF staining on tissue samples obtained from patients with OLP, NSU and HK as a condition indicative of non-specific inflammation, OED demonstrating a degree of antigen-specific response, and OSCC characterized by significant Treg activation. The images showed a substantial infiltration of Tregs in the subepithelial regions (Fig. 5A). Compared with OED and OSCC, there were no significant differences in the proportion of Tregs among CD4^{+} T cells or in the density of Tregs in OLP (Fig. 5B). We then compared the expression patterns of several molecules associated with Treg immunosuppressive function across these diseases (Fig. 5C). The proportion of Tregs expressing interleukin (IL)-10 and TGF-β in OLP is significantly reduced compared to OSCC, and the

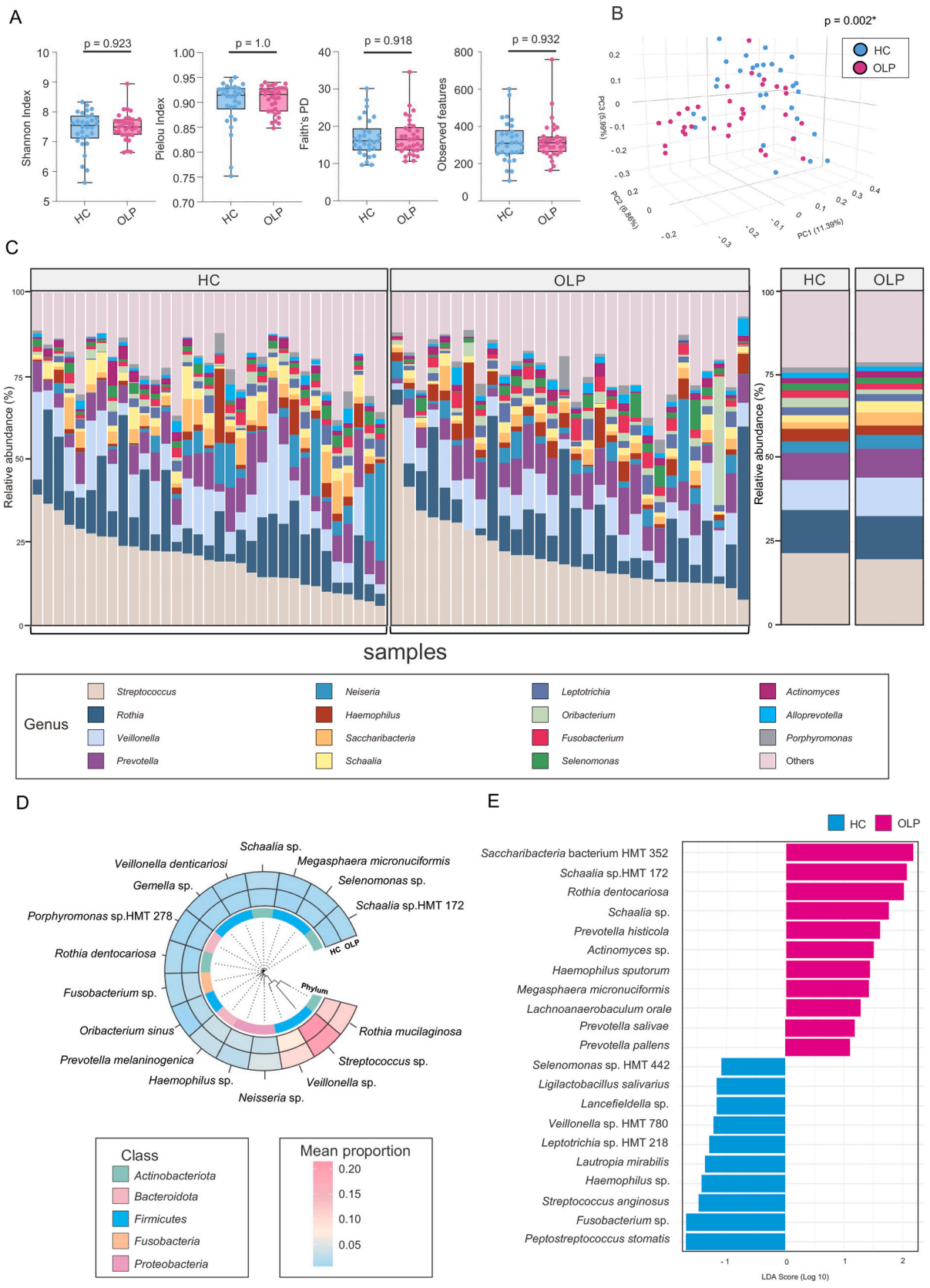

Fig. 2. The structure of oral microbiome from saliva varied between OLP and HC. (A) Alpha diversity indices of the oral microbiome. (B) PCoA plot based on Bray–Curtis distance. PERMANOVA test indicates a statistically significant difference between the OLP and HC groups. (C) Relative abundances of oral microbes at the genus level. Left panels show individual compositions; right panels show the mean relative abundances for each group. (D) Relative abundances of oral microbes at the species level. Sixteen species with ≥ 1 % relative abundance in all samples were ordered using a phylogenetic tree. (E) LEfSe assessment at the species level. Only taxa with an LDA score (log10) > 1.0 are displayed. *$P < 0.05$ and **$P < 0.01$ according to Mann–Whitney U test.

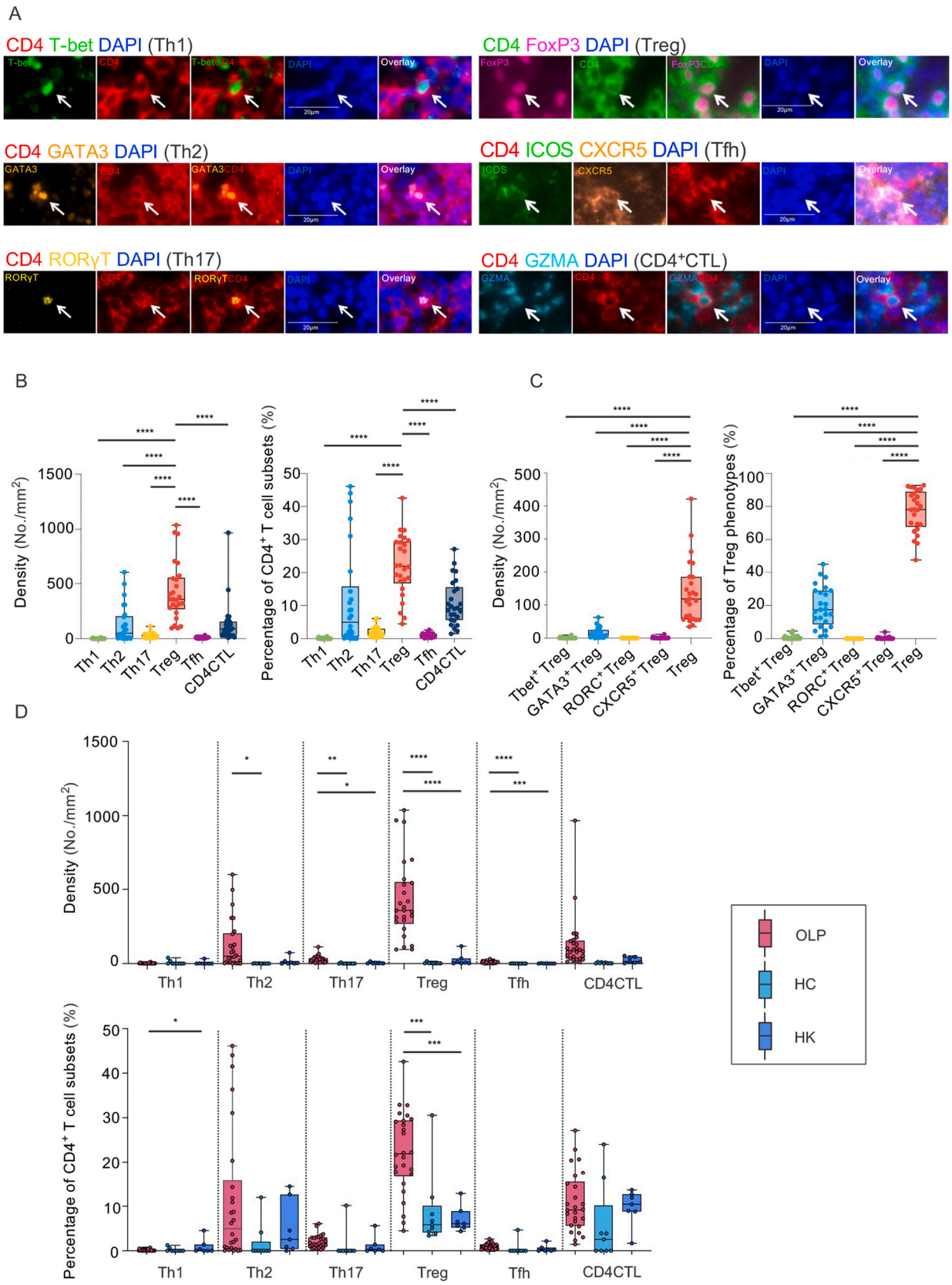

Fig. 3. Predominant infiltration of Tregs in OLP lesions. (A) Multiplex IF staining of $CD4^+$ T cell subsets in OLP lesions. White arrows indicate $CD4^+$ T cell subsets. Th1: T helper 1 cell; Th2: T helper 2 cell; Th17: T help 17 cell; Treg: regulatory T cell; Tfh: T follicular helper cell; $CD4^+$ CTL: cytotoxic T lymphocyte. Scale bars: 20 μm. (B) Density and proportion of $CD4^+$ T cell subsets among all $CD4^+$ T cells. (C) Density and proportion of distinct phenotypes of Tregs among Tregs. Each box represents the interquartile range; the line inside each box indicates the median. Symbols represent individual participants. $^{****}P < 0.0001$ according to Dunnett's multiple comparisons test. (D) Density and proportion of $CD4^+$ T cell subsets among $CD4^+$ T cells in OLP, HC and hyperkeratosis (HK). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, and $^{****}P < 0.0001$ according to Dunnett's multiple comparisons test.

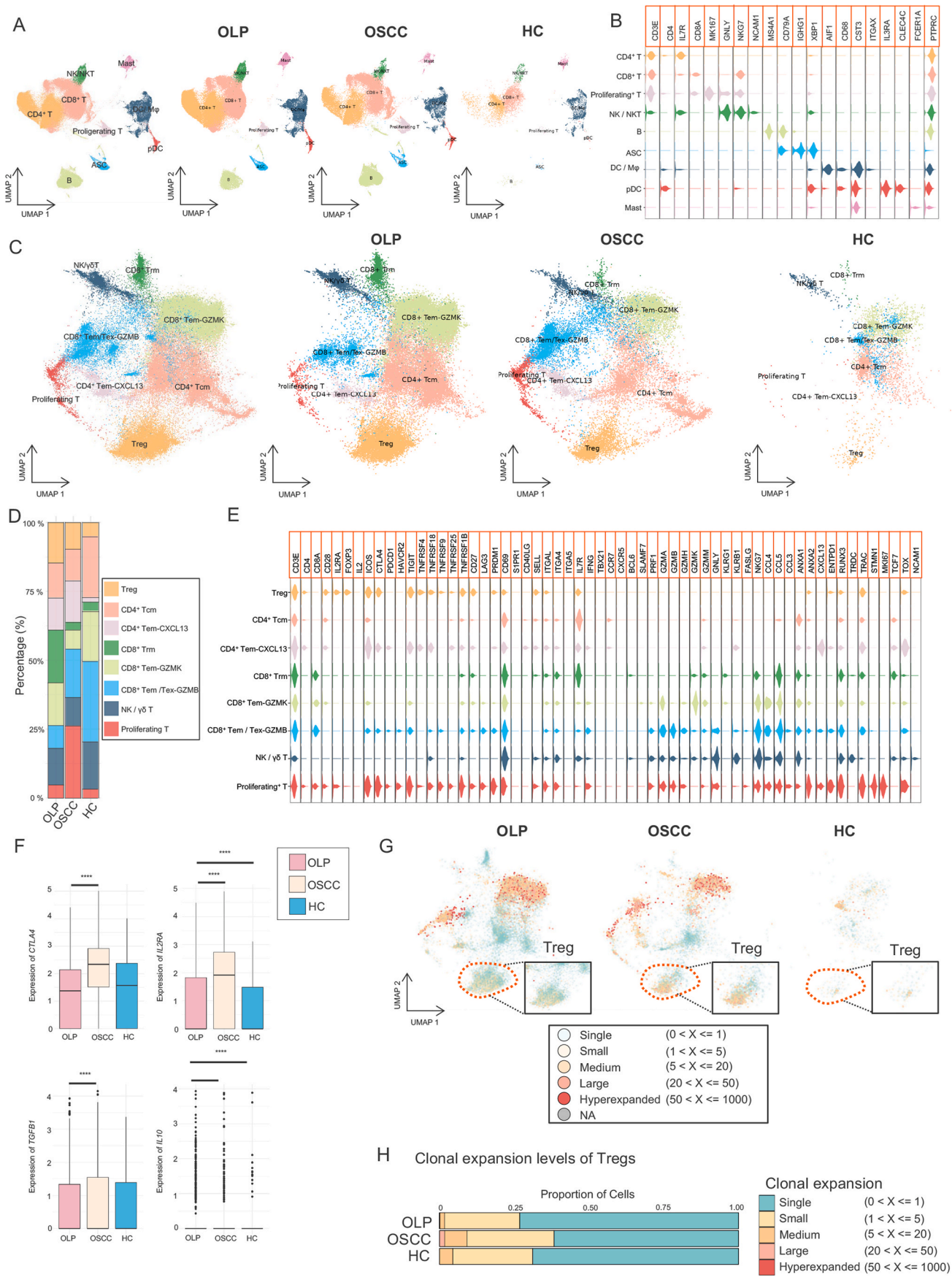

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Fig. 4. Limited clonal expansion and non-activated state of Tregs in OLP tissue lesions demonstrated by scRNA-seq. (A) Uniform manifold approximation and projection (UMAP) visualisation of infiltrating immune cells in OLP patients, oral squamous cell carcinoma (OSCC) patients and HCs. $CD4^+$ T: $CD4^+$ T cell; $CD8^+$ T; $CD8^+$ T cell; Proliferating T: proliferating T cell; NK/NKT: natural killer cell/natural killer T cell; B: B cell; ASC: antibody-secreting cell; pDC: plasmacytoid dendritic cell; Mast: mast cell; DC/Mφ: dendritic cell/macrophage. (B) The expression of marker genes for immune cells. (C) UMAP visualisation of T cells. Treg: regulatory T cell; $CD4^+$ Tcm: $CD4^+$ central memory T cell; $CD4^+$ Tem-CXCL13: CXCL13-expressing $CD4^+$ effector memory T cell; $CD8^+$ Trm: $CD8^+$ tissue-resident memory T cell; $CD8^+$ Tem-GZMK: GZMK-expressing $CD8^+$ effector memory T cell; $CD8^+$ Tem/Tex-GZMB: GZMB-expressing $CD8^+$ effector memory/exhausted T cell; NK/γδT: natural killer cell/γδ T cell. (D) Proportions of T cell subsets. (E) The expression of marker genes for distinct T cell subsets. (F) Comparisons of expression of *CTLA4, TGFB1, IL2RA*, and *IL10* in Tregs among OLP, HC, and OSCC. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, and $^{****}P < 0.0001$ according to Wilcoxon rank-sum test. (G) UMAP visualisation of gradient levels of clonal expansion for T cells. NA: αβ TCR sequences were not detected.

proportion of Tregs expressing CTLA-4 in OLP is also significantly reduced compared to OED (Fig. 5D). These findings suggest that parts of the Tregs in OLP lesions are not in an activated state.

Scfa-producing bacteria are associated with the expression of immunosuppressive molecules by Tregs in OLP lesions

As noted above, we suspected that the depletion of SCFA-producing bacteria in the gut microbiome, such as *F. prausnitzii* C71358, *B. adolescentis*, and *B. longum*, is linked to increased non-activated Treg in OLP. To explore this relationship, we conducted linear regression analysis of the abundances of these bacteria and all Tregs, as well as Tregs expressing immunosuppressive molecules including CTLA-4, TGF-β, and CD25 (Fig. 5E, F and Supplementary Fig. 4A and B). At the genus level, the abundance of *Bifidobacterium* was significantly positively correlated with the proportion of $CD25^+$ Tregs among $CD4^+$ T cells (Fig. 5E). Notably, regression analysis showed a significant positive correlation between the abundance of *F. prausnitzii* C71358 and the proportion of TGF-β^+ Tregs, as well as between the abundance of *B. longum* and the density of $CD25^+$ Tregs (Fig. 5F). These findings suggested that the reduced abundance of these SCFA-producing bacteria in the gut microbiome of OLP patients may be related to the non-activated state of Tregs in tissue lesions.

Circulating Tregs show reduced expression of CD25 and TGF-β in OLP patients

Considering the efficient recirculation of naïve and central memory T cells, which increases the probability that T cells will recognise specific antigens, changes in SCFA-producing bacteria within the gut also may affect circulating Tregs. To test this hypothesis, we used flow cytometry to assess circulating Tregs in the blood, as well as their patterns of immunosuppressive molecule expression. Tregs were identified as $CD3^+$ $CD4^+$ $FoxP3^+$ cells; the expression levels of CTLA-4, CD25, TGF-β, and IL-10 were measured in this population (Fig. 6A). Consistent with the results of scRNA-seq analysis and multiplex IF staining, the proportion of Tregs among circulating $CD4^+$ T cells was significantly greater in OLP patients than in HCs (Fig. 6B). However, the proportions of TGF-β^+ Tregs and $CD25^+$ Tregs within the Treg population were significantly lower in OLP patients than in HCs (Fig. 6C). This reduction in CD25 and TGF-β expression suggested that circulating Tregs in OLP patients are not activated because most Tregs in healthy individuals typically remain resting. We also performed linear regression analysis to examine the correlation between these Tregs and SCFA-producing bacteria (Fig. 6D and Supplementary Fig. 5A and 5B). The abundance of *F. prausnitzii* C71358 tended to be positively correlated with the proportion of IL-10^+ Tregs.

Circulating Tregs from OLP patients were less effective in suppressing T cell proliferation but SCFAs enhanced IL-10 and TGF-β production

To further validate the non-activated state of Tregs in OLP patients, we isolated Tregs and conventional T cells (Tconvs) from the PBMCs of both OLP patients and HCs (Supplemental Fig.6A). We performed an in vitro Treg suppression assay by co-culturing equal numbers of Tconvs with gradient-diluted Tregs and activators, and quantified the proportion of proliferating Tconvs under different co-culture conditions using flow cytometry (Fig. 6E, Supplemental Fig.6B). A significant difference between the OLP and HC groups was observed using two-way ANOVA, indicating that circulating Tregs from OLP patients were less effective in suppressing the proliferation of Tconvs, while there was no significant difference between the groups in the proliferation of Tconvs without Tregs added (Fig. 6F). Additionally, by adding appropriate concentrations of SCFAs, as well as IL-2 and activators to the Tregs from OLP patients and HCs, we assessed the membrane expression of CTLA-4 and the concentration of IL-10 and TGF-β in the culture supernatants (Fig. 6G, Supplemental Fig.6C). Compared to those without any added SCFAs, these Tregs from OLP patients significantly produced more IL-10 when treated with butyric acid and propionic acid, and more TGF-β when treated with acetic acid (Fig. 6H). Although Tregs from HCs also produced more TGF-β and IL-10 under the same conditions (Supplemental Fig. 7A), after calculating the ratios of these molecules in the SCFAs co-culture group relative to those in the activator-only group, it revealed that Tregs from OLP patients showed a greater increase in TGF-β and IL-10 production compared with those from HCs (Supplementary Fig. 7B).

Discussion

Although the exact cause of OLP remains unclear, immune dysregulation—particularly involving T cells—is suspected to play a substantial role.[31] No previous systematic investigation has included an analysis of the gut microbiome, but several publications have noted an association between OLP and specific bacteria. Recent researches have highlighted a link between OLP and oral microbial dysbiosis.[32–34] Several studies have identified bacterial species that exhibit increased or decreased abundance in OLP patients, offering valuable insights into the microbial changes associated with OLP and potentially contributing to the understanding of its pathogenesis. However, the mechanism by which these microbial alterations contribute to OLP pathogenesis remains an open question. Given the prominent T-cell infiltration and other immune abnormalities in OLP, systematic studies of the oral microbiome, gut microbiome, blood, and lesions are eagerly awaited.

Recent studies by Atarashi et al. and other groups have demonstrated that SCFAs, such as acetate, propionate, and butyrate, play crucial roles in various biological systems.[35] *Bifidobacterium* and *F. prausnitzii*, representative SCFA-producing bacteria, exhibited substantially lower proportions in the gut microbiome of OLP patients, suggesting that dysbiosis involving these bacteria contributes to OLP pathogenesis. Deficiencies in *Bifidobacterium* and *F. prausnitzii* also have been implicated in several autoimmune and autoinflammatory conditions, including rheumatoid arthritis, autoimmune myasthenia gravis, and encephalomyelitis.[22,36] Considering the autoimmune disease-like nature of OLP and the continuity between the gut and oral mucosa, it is reasonable to speculate that gut microbiome dysbiosis, characterised by alterations in specific bacteria, triggers the development of OLP. The HPLC-MS/MS analysis conducted alongside OLP microbiota analysis revealed a significant reduction in the total amount of each representative SCFAs in OLP patients, supporting these hypotheses.

Another important question is: how are SCFA-producing bacteria be involved in the pathogenesis of OLP? Given that SCFAs produced by gut

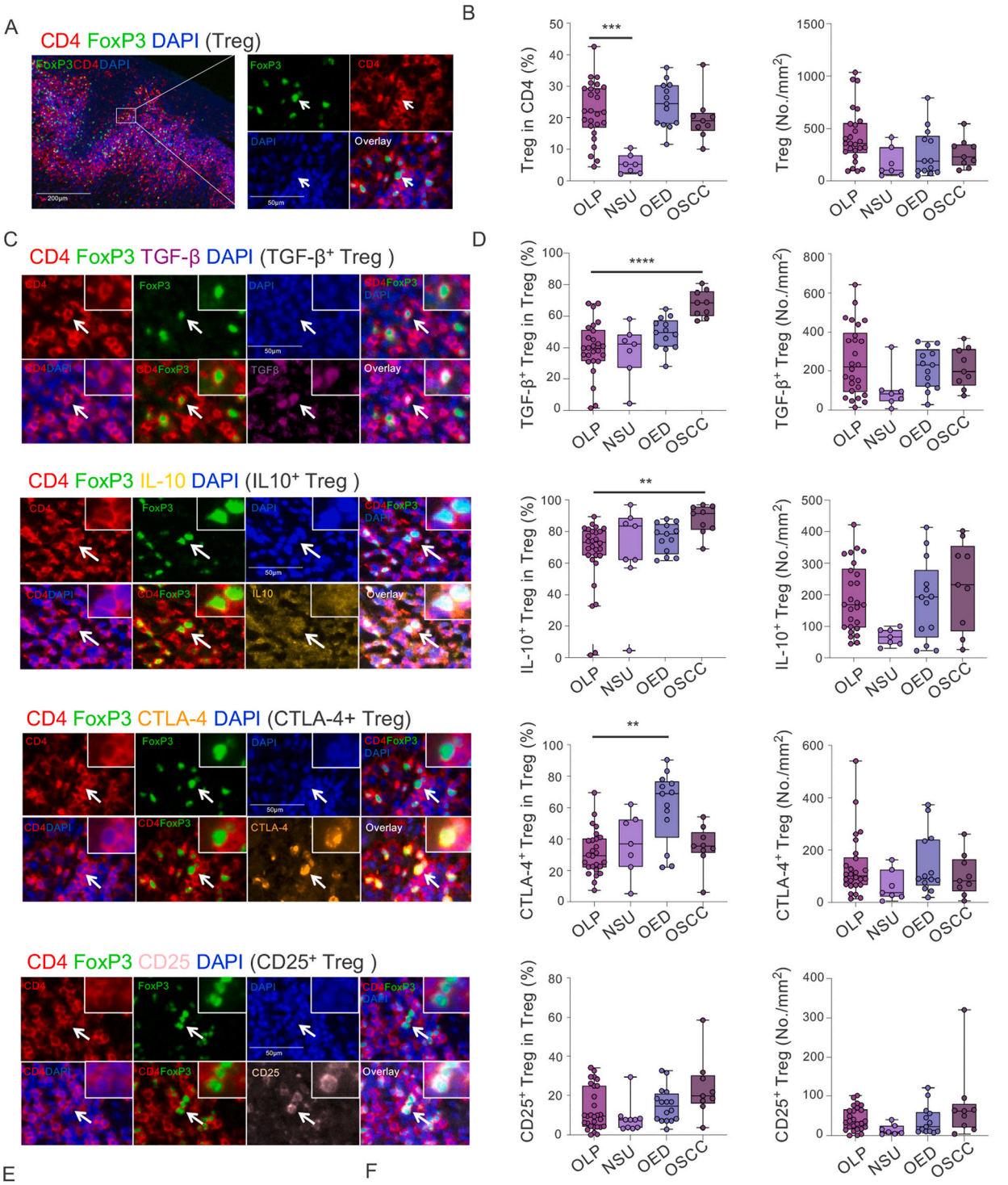

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Fig. 5. Low proportion of immunosuppressive molecule expressing Tregs within OLP tissue lesions and the correlation with SCFA-producing bacteria in matched samples. (A) Representative images of multiplex IF staining of Tregs in OLP tissue lesions. White arrows indicate Tregs. Scale bars: 200 μm (left) and 50 μm (right). (B) Comparisons of densities and percentages of Tregs within $CD4^+$ T cells in tissue lesions from patients with OLP, non-specific ulcer of oral mucosa (NSU), oral epithelial dysplasia (OED) and OSCC. ***P < 0.001, according to Kruskal–Wallis test (C) Representative images of multiplex IF staining of Tregs with expression of immunosuppressive molecules in OLP tissue lesions. White arrows indicate TGF-β^+ Tregs, IL-10^+ Tregs, CTLA-4^+ Tregs, and $CD25^+$ Tregs. Scale bar: 50 μm. (D) Comparisons of densities and percentages of CTLA-4^+ Tregs, TGF-β^+ Tregs, and $CD25^+$ Tregs within all Tregs. **P < 0.01 and ****P < 0.0001 according to Kruskal–Wallis test. (E, F) Correlations between the percentages of specific SCFA-producing bacteria and the densities or percentages of $CD25^+$ /TGF-β^+ Tregs in OLP tissue lesions were assessed by simple linear regression. (E) At the genus level, Bifidobacterium displayed a positive correlation with the percentage of $CD25^+$ Tregs. (F) At the species level, Faecalibacterium prausnitzii C71358 showed a positive correlation with the percentage of TGF-β^+ Tregs, and Bifidobacterium longum showed a positive correlation with the density of $CD25^+$ Tregs. Model significance was determined by the overall F-test, and r^2 is given.

bacteria play a pivotal role in the differentiation and induction of Tregs,[37,38] we hypothesised that Treg dysregulation occurs in OLP. Indeed, our co-culture study of Tregs and SCFAs highlighted the pivotal role of SCFAs in driving the expression of immunosuppressive molecules in Tregs, underscoring their essential function in Treg activation. We observed that the numbers of Tregs in tissue lesions and PBMCs from OLP patients were substantially higher compared with those numbers in HCs; Tregs were the predominant subset among all $CD4^+$ T cells. However, these Tregs lacked functional marker molecules such as TGF-β and IL-10, indicating a potential defect in their immunosuppressive function, which coincided with gut dysbiosis and the findings by co-culture study.[39–41] Tregs have two possible phenotypes: functional and non-suppressive, distinguished by the levels of several molecules expression, including FoxP3 and CD25.[42–44] Non-suppressive Tregs, which exhibit no immunosuppressive activity, have been observed in the contexts of cancer, autoimmune diseases, and infectious diseases. Intriguingly, a link between non-suppressive Tregs and SCFA-producing bacteria has been described.[26] The positive correlation between the abundance of SCFA-producing bacteria and functional Treg markers in our data highlights the importance of the microbiome in Treg differentiation. Furthermore, our functional assays revealed that the immunosuppressive capacity of Tregs sorted from OLP patients is diminished compared to that of HCs. This implies a relationship between non-activated Tregs and the aspect of prolonged chronic inflammation in the pathology of OLP, possibly resulting from impaired Treg differentiation.

Although there were relatively few differences in oral microbiome between HCs and OLP patients, pronounced gut microbiome dysbiosis was evident in OLP patients. The mechanism underlying oral microbiome dysbiosis remains unclear, but the oral microbiome may indirectly influence the gut microbiome, contributing to OLP development. Alternatively, the pain associated with OLP might hinder the maintenance of oral hygiene, thereby altering the oral microbiome. Considering that OLP lesions are predominantly infiltrated by T cells, which are typically activated in secondary lymphoid organs and then migrate to the lesion site via peripheral blood, it is unlikely that changes in the oral microbiome directly influence the differentiation of T cells, particularly Tregs. However, we observed a certain level of infiltration of GATA3-positive Tregs alongside conventional Tregs in OLP lesions in the present study. $GATA3^+$ Tregs increase in disease milieus with prolonged inflammatory responses and may induce further Treg accumulation. This suggests the potential of a synergistic effect between GATA3-positive Tregs and non-activated Tregs in provoking unique immune responses in OLP lesions. Examining the relationship between $GATA3^+$ Tregs and non-activated Tregs could be an intriguing topic for future research.[45,46].

In summary, our study provides the first evidence that dysbiosis involving both the gut and oral microbiome may play a role in the pathogenesis of OLP. Specifically, the depletion of SCFA-producing bacteria, such as *B. adolescentis*, *B. longum*, and *F. prausnitzii*, likely contributes to the pathogenesis of OLP by dysregulating Treg differentiation. A schematic overview of the proposed pathogenesis of OLP, based on our observations and hypotheses, is presented in Fig. 7. However, this study had some limitations. Due to the frequency of clinic visits and the challenges of sample collection (involving stool, saliva, lesional tissue, and PBMCs), the sample sizes were relatively small. Additionally, we used a limited approach to studying immune cell populations in blood and tissue lesions; the use of complementary methods would have strengthened the analysis. Furthermore, this study did not extensively focus on the functional roles of individual bacterial species. Future research should incorporate assembly-based taxonomic and functional characterisation, along with association and accessory gene set enrichment analysis. Despite these limitations, our results represent a step toward understanding the pathogenesis of OLP. Considering that SCFA depletion has been linked to various immune-related diseases, including systemic lupus erythematosus, systemic sclerosis, IgG4-related disease, and atopic dermatitis, the mechanisms proposed in this study may be relevant to a broader range of immune dysregulation disorders.[15,47-49] One key question remains: what causes the depletion of SCFA-producing bacteria? The “chicken or egg” dilemma persists—does dysbiosis precede the disease, or does the disease lead to dysbiosis? The findings of this study suggest that dysbiosis drives the progression of OLP, and we further speculate that it may also play a role in disease onset. Further studies in both humans and mice are needed to clarify the specific immunomodulatory pathways involved and to explore potential therapeutic applications. Probiotic therapy, which has shown promise in other diseases with few side effects, may be worth investigating as a treatment option for OLP.[50].

Methods

Patients

This study included 42 patients with OLP, 42 HCs (all volunteers), 7 patients with HK, 7 patients with non-specific NSU, 13 patients with OED, and 9 patients with oral OSCC. NSU and HK were employed as a disease control for general inflammation, OED was utilized to represent antigen-specific responses, and OSCC served as a disease control characterized by significant Treg activation, with each condition acting as a comparative model for OLP. All patients were diagnosed between 2014 and 2023 at the Department of Oral and Maxillofacial Surgery of Kyushu University Hospital; pathological diagnoses were confirmed by biopsy. A summary of the OLP patient information is provided in Supplementary Table 1.

Sample collection, library preparation, and 16S rRNA microbiome analysis

This analysis included 33 OLP patients from the Department of Oral and Maxillofacial Surgery at Kyushu University Hospital, as well as 30 HCs. Stool and saliva samples were collected. Patient information is summarised in Supplemental Table 2. Of the 33 OLP patients included in the microbiome analysis, 26, for whom tissue samples were available, were included in the tissue-based study. Detailed procedures for sample collection, preservation, library preparation, 16S rRNA gene sequencing, and microbiome data analysis are described in Supplementary Methods.

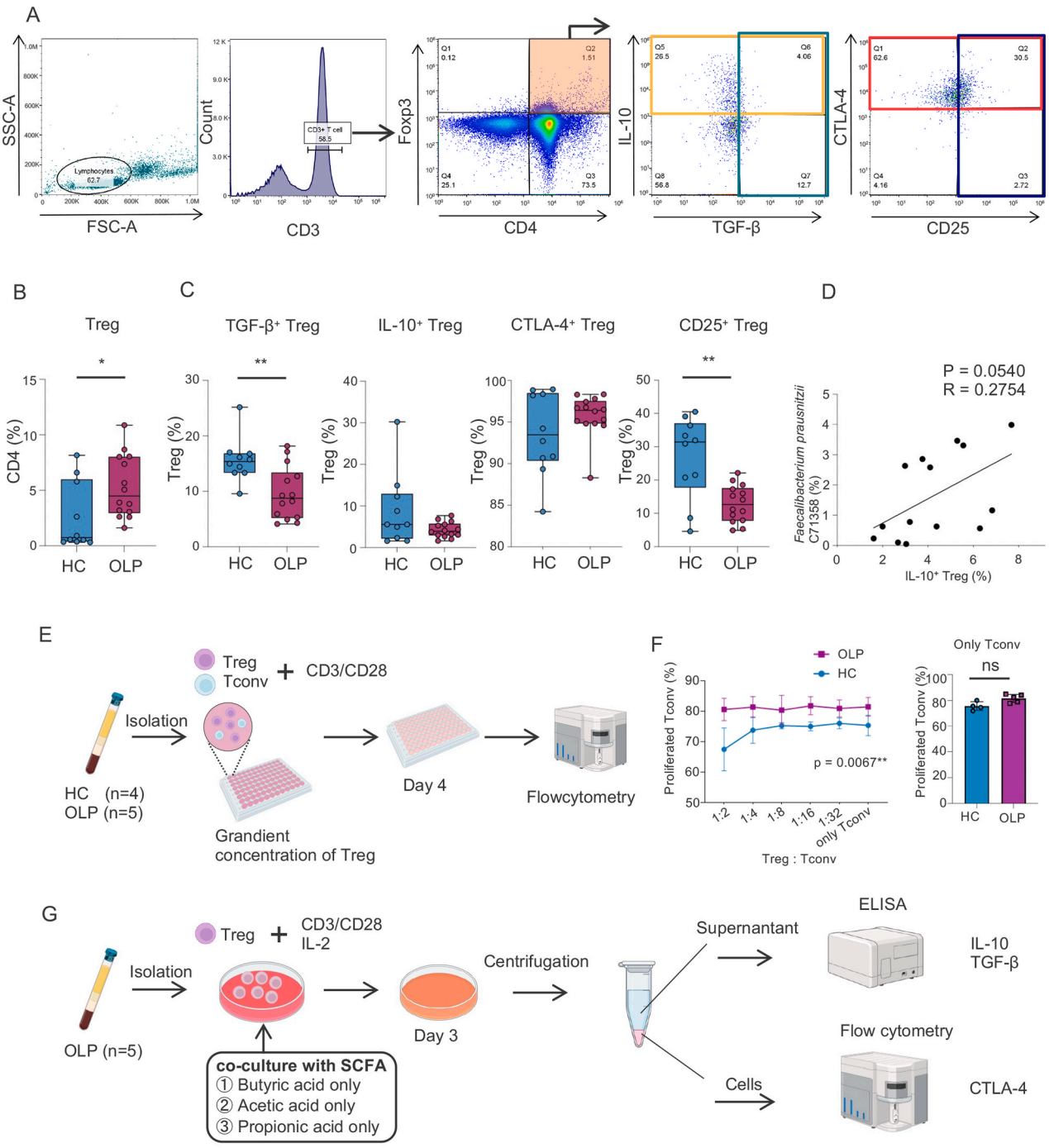

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Fig. 6. The non-activated state exhibited by circulating Tregs from of OLP. (A) Representative flow cytometry plots of Tregs and their immunosuppressive molecules. (B) Increased percentage of Tregs in OLP patients compared with HCs. *P < 0.05 according to Mann–Whitney *U* test. (C) Decreased percentages of TGF-β$^+$ Tregs and CD25$^+$ Tregs in OLP patients compared with HCs. **P < 0.01 according to Mann–Whitney *U* test. (D) Positive correlation between the percentage of Faecalibacterium prausnitzii C71358 and the percentage of IL-10$^+$ Tregs in OLP patients assessed by simple linear regression. P = 0.054, model significance was determined by the overall F-test, and r^2 is given. (E) Schematic illustration of the in vitro Treg suppression assay. Circulating Tregs and conventional CD4$^+$ T cells (Tconvs) were isolated from PBMCs, co-cultured and harvested after four days for analysis using flowcytometry. (F) Comparisons of proliferated Tconvs between OLP and HC. The ability of circulating Tregs from OLP patients to suppress Tconv proliferation is significantly lower than that from HC. **P < 0.01 according to two-way ANOVA (column factor); No significant differences were observed in the Tconv only group by Mann-Whitney test. (G) Schematic illustration of SCFA-treated Treg activation assay. Circulating Tregs from OLP patients were treated with SCFAs, harvested after three days, and analyzed by flowcytometry. The TGF-β and IL-10 levels in culture supernatants were quantified by ELISA. (H) Comparison of Treg functional molecules in Treg isolated from OLP patients with or without SCFAs treatment. Butyric acid and propionic acid promoted IL-10 secretion, and acetic acid and propionic acid promoted TGF-β secretion. *P < 0.05, and **P < 0.01 according to paired Student's *t*-test.

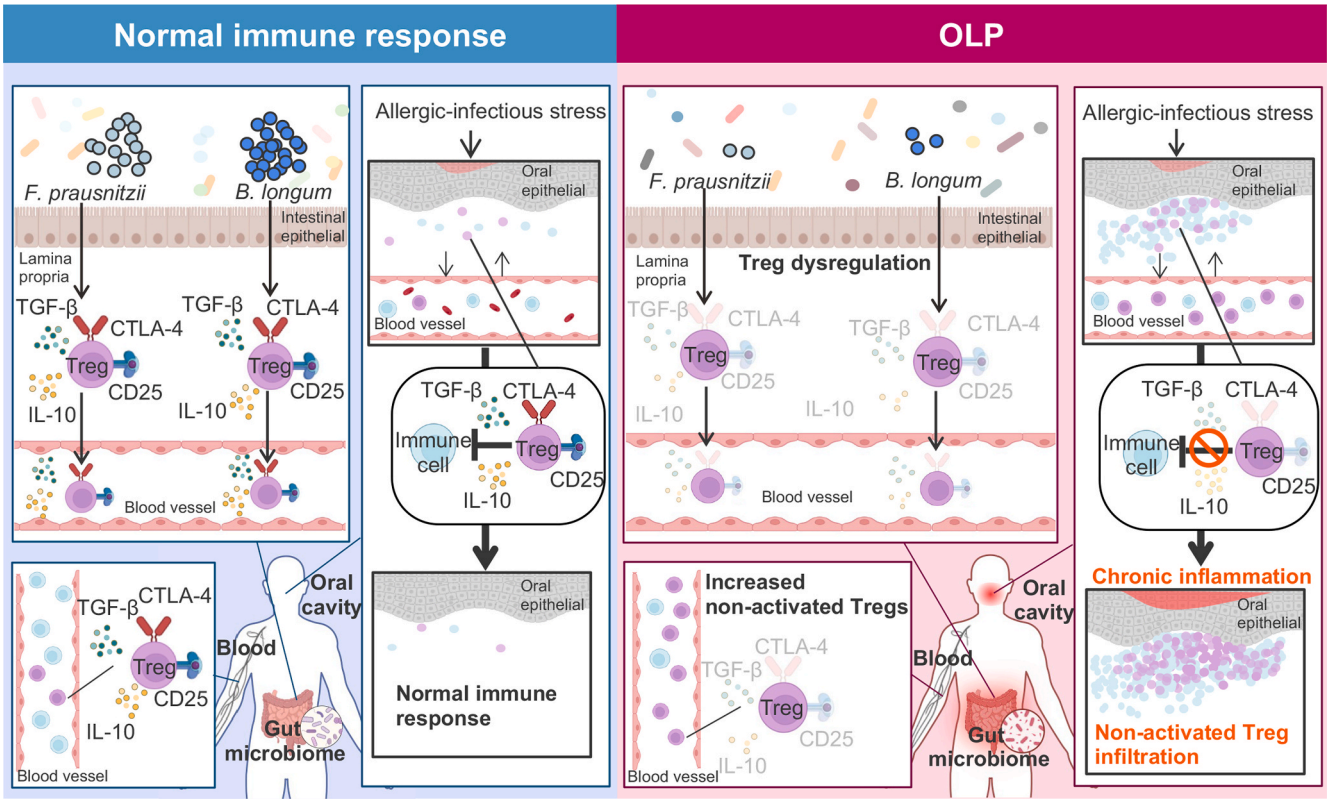

Fig. 7. Schematic model of dysbiosis and non-activated Treg expansion in OLP pathogenesis. Proposed schematic model of gut dysbiosis contributing to the pathogenesis of OLP. The left panel represents the normal immune response, where SCFAs produced by the intestinal bacteria maintain Treg activation ability and immunosuppressive function. Compared with the normal state, a decrease in the abundance of SCFA-producing bacteria, such as *Faecalibacterium prausnitzii* and *Bifidobacterium longum*, results in Treg dysregulation during OLP (right panel). These non-activated Tregs display an increased population in the bloodstream and are circulated throughout the body. Within the oral tissue, these non-activated Tregs fail to suppress the activation of other immune cells, resulting in chronic and harmful inflammatory responses.

High-performance liquid chromatography–tandem mass spectrometry (HPLC-MS/MS) analysis

Stool samples were collected from five OLP patients and six HCs. HPLC-MS/MS was performed as described in Supplementary Methods.

Multiplex IF staining and cell quantification

The protocols for multiplex IF staining and cell quantification are provided in Supplemental Methods. The following primary antibodies were diluented and used: anti-CD4 (1;500, ab133616, Abcam), anti-T-bet/Tbx21 (1:200, #13232, Cell Signaling), anti-GATA3 (1:200, ab199428, Abcam), anti-RORγT (1:100, 3208A, Biocare Medical), anti-FoxP3 (1:100, #98377, Cell Signaling), anti-CXCR5 (1:2000, ab254415, Abcam), anti-ICOS (1:200, #89601, Abcam), anti- Granzyme A (GZMA) (1:100, ab209205, Abcam), anti-TGF beta1 antibody (1:100, ab215715, Abcam), anti-IL10 (1:100, 60269–1-Ig, Proteintech), anti-CTLA4 (1:400, ab237712, Abcam), and anti-IL-2 Receptor alpha (1:200, ab1289555, Abcam). Lymphocyte subsets were identified by the following criteria: CD4$^+$ T-bet$^+$ for Th1 cells; CD4$^+$ GATA3$^+$ for Th2 cells; CD4$^+$ RORγT$^+$ for Th17 cells; CD4$^+$ FoxP3$^+$ for Tregs; CD4$^+$ ICOS$^+$ CXCR5$^+$ for T follicular helper (Tfh) cells; CD4$^+$ Granzyme A$^+$ for CD4$^+$ cytotoxic T lymphocytes; TGF-β, IL-10, CTLA-4 and CD25 for Tregs expressing immunosuppressive molecules.

Library preparation, sequencing for scRNA-seq, and data analysis

A fresh tissue sample of an OLP lesion was collected from the buccal mucosa of an affected patient during surgical resection. Tissue homogenisation, library construction, and sequencing were conducted as described in the online Supplemental Methods. Our dataset was integrated with several published datasets, which included tissues from nine

OLP patients, four HCs, and 12 OSCC patients.[6,19-21] Quality control, data integration, gene expression analysis, and TCR repertoire analysis are described in Supplemental Methods.

Leukocyte phenotypes were subsequently assigned to these clusters based on the following marker genes: *CD3E, CD4, IL7R* for $CD4^+$ T cell; *CD3E, CD8A* for $CD8^+$ T cell; *CD3E, MKI67* for Proliferating T cell; *NCAM1, NKG7, GZMB* for NK/NK T cell; *CD79A, MS4A1* for B cell; *CD79A, CD38, XBP1* for ASCs; *AIF1, ITGAX, CD68, CST3* for dendritic cell (DC) and macrophage (Mφ); *CD4, CLEC4C, IL3RA* for plasmacytoid dendritic cell (pDC); *CST3, FCER1A, CPA3* for mast cell. T cell subsets were assigned based on the following criteria: *FOXP3, IL2RA* for regulatory T cell; *SELL, CCR7* for central memory T cell (T_{CM}); *ICOS, CXCL13, CD4* for $CD4^+$ effector memory T cell (T_{EM}); *GZMB, GZMK, CCL4, CCL5, CD8A* for $CD8^+$ T_{EM}; *CTLA4, PDCD1* for $CD8^+$ exhausted T cell (T_{EX}); *CD3E, TRDC* for γδ T.

Isolation of peripheral blood mononuclear cells

Peripheral blood samples were collected from 25 OLP patients and 11 HCs. PBMC isolation was performed as described in Supplementary Methods.

Isolation of regulatory and conventional T cells

Peripheral blood samples were collected from 11 patients diagnosed with OLP and four healthy volunteers. Treg and conventional T cells isolation are described in Supplementary Methods.

In vitro Treg suppression assay

We performed in vitro Treg suppression assay with five patients diagnosed with OLP and four healthy volunteers. Detailed methods are described in Supplementary Methods.

Scfa-treated Treg activation assay

We performed SCFA-treated Treg activation assay with five patients diagnosed with OLP and five healthy volunteers. Detailed methods are described in Supplementary Methods.

Flow cytometry

Flow cytometry methods are described in Supplemental Methods.

Enzyme-Linked Immunosorbent assay

Enzyme-Linked Immunosorbent Assay methods are described in Supplemental Methods.

Contributors

SY, ST, SA, and YG performed the 16S rRNA sequencing analysis. SY, HC, JS, TS, HN, and MY performed tissue analyses using multiplex immunofluorescence and quantified cellular infiltrates. HC and YL contributed to single-cell RNA sequencing and T cell repertoire analyses. SY, HC, HK, and YH performed flow cytometry analyses. YH was responsible for performing experiments involving HPLC-MS/MS, analysing the HPLC-MS/MS data, and drafting the manuscript of the HPLC-MS/MS experiment. YK, YM, MM, TK, YO, and SK provided and collected the samples. SY, HC, and NK wrote the manuscript, with input from all authors. The study was conceived by NK, KK MM, and SK. NK and MM were responsible for overall execution of the study.

Patient and public involvement

Patients and/or the public were not involved in the design, conduct, reporting, or dissemination plans of this research.

Patient consent for publication

Consent was obtained directly from patients.

Provenance and peer review

Not commissioned; externally peer reviewed.

Data availability statement

The data used in this study are available from the corresponding author upon request.

CRediT authorship contribution statement

Shiho Yokomizo: Writing – review & editing, Visualization, Investigation, Formal analysis, Data curation. **Naoki Kaneko:** Writing – review & editing, Writing – original draft, Supervision, Funding acquisition, Conceptualization. **Hu Chen:** Writing – review & editing, Visualization, Validation, Formal analysis, Data curation. **Lijing Yan:** Writing – review & editing, Visualization, Formal analysis. **Shoji Tsuji:** Writing – review & editing, Methodology, Formal analysis. **Shohei Akagawa:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Junsei Sameshima:** Writing – review & editing, Validation, Data curation. **Tomoki Sueyoshi:** Writing – review & editing, Validation, Data curation. **Haruki Nagano:** Writing – review & editing, Validation, Data curation. **Yuka Miyahara:** Writing – review & editing, Resources, Data curation. **Yasuhisa Kamikaseda:** Writing – review & editing, Resources, Funding acquisition. **Hajime Kido:** Writing – review & editing, Data curation. **Yoshikazu Hayashi:** Writing – review & editing, Methodology, Data curation. **Masaki Yamauchi:** Writing – review & editing, Funding acquisition. **Tamotsu Kiyoshima:** Writing – review & editing, Resources, Data curation. **Yuichi Goto:** Writing – review & editing, Methodology, Formal analysis. **Yukiko Ohyama:** Writing – review & editing, Resources, Methodology. **Kazunari Kaneko:** Writing – review & editing, Conceptualization. **Masafumi Moriyama:** Writing – review & editing, Supervision, Conceptualization. **Shintaro Kawano:** Writing – review & editing, Supervision, Resources, Conceptualization.

Ethics approval

This research protocol was approved by the Ethics Committee of Kyushu University, Japan (IRB serial numbers: 21108–2, 2021–192, 23115–00). Written informed consent was obtained from all patients.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence

the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mucimm.2025.05.009.

References

1. Howell FV. Oral mucous membrane lesions. Pathologic features. *Calif Med*. 1964;100:186–191.
2. Villa TG, Sanchez-Perez A, Sieiro C. Oral lichen planus: a microbiologist point of view. *Int Microbiol*. 2021;24:275–289.
3. Oujdad S, Ben YI. Malignant transformation of oral lichen planus: a case report. *Cureus*. 2023;15, e38432.
4. Andabak-Rogulj A, Vindiš E, Aleksijević LH, et al. Different treatment modalities of oral lichen planus-a narrative review. *Dentistry J*. 2023;11.
5. Deng X, Wang Y, Jiang L, Li J, Chen Q. Updates on immunological mechanistic insights and targeting of the oral lichen planus microenvironment. *Front Immunol*. 2022;13, 1023213.
6. Qing M, Yang D, Shang Q, et al. CD8(+) tissue-resident memory T cells induce oral lichen planus erosion via cytokine network. *Elife*. 2023;12.
7. Wang Y, Xue N, Wang Z, Zeng X, Ji N, Chen Q. Targeting Th17 cells: a promising strategy to treat oral mucosal inflammatory diseases. *Front Immunol*. 2023;14, 1236856.
8. Yamauchi M, Moriyama M, Hayashida JN, et al. Myeloid dendritic cells stimulated by thymic stromal lymphopoietin promote Th2 immune responses and the pathogenesis of oral lichen planus. *PLoS One*. 2017;12, e0173017.
9. Miyahara Y, Chen H, Moriyama M, et al. Toll-like receptor 9-positive plasmacytoid dendritic cells promote Th17 immune responses in oral lichen planus stimulated by epithelium-derived cathepsin K. *Sci Rep*. 2023;13:19320.
10. Liu W, Zhang Q, Bao Z, Shen X. A potential reciprocal emergence of regulatory T cells in oral lichen planus and pemphigus vulgaris: a meta-analysis. *J Dental Sci*. 2023;18:437–442.
11. Tao XA, Xia J, Chen XB, et al. FOXP3 T regulatory cells in lesions of oral lichen planus correlated with disease activity. *Oral Dis*. 2010;16:76–82.
12. Donald K, Finlay BB. Early-life interactions between the microbiota and immune system: impact on immune system development and atopic disease. *Nat Rev Immunol*. 2023;23:735–748.
13. Skelly AN, Sato Y, Kearney S, Honda K. Mining the microbiota for microbial and metabolite-based immunotherapies. *Nat Rev Immunol*. 2019;19:305–323.
14. Teramoto Y, Akagawa S, Hori SI, Tsuji S, Higasa K, Kaneko K. Dysbiosis of the gut microbiota as a susceptibility factor for Kawasaki disease. *Front Immunol*. 2023;14, 1268453.
15. Plichta DR, Somani J, Pichaud M, et al. Congruent microbiome signatures in fibrosis-prone autoimmune diseases: IgG4-related disease and systemic sclerosis. *Genome Med*. 2021;13:35.
16. Kaneko K, Akagawa S, Akagawa Y, Kimata T, Tsuji S. Our evolving understanding of Kawasaki disease pathogenesis: role of the gut Microbiota. *Front Immunol*. 2020;11:1616.
17. Furusawa Y, Obata Y, Fukuda S, et al. Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. *Nature*. 2013;504:446–450.
18. Takahashi D, Hoshina N, Kabumoto Y, et al. Microbiota-derived butyrate limits the autoimmune response by promoting the differentiation of follicular regulatory T cells. *EBioMedicine*. 2020;58, 102913.
19. Li Q, Wang F, Shi Y, et al. Single-cell immune profiling reveals immune responses in oral lichen planus. *Front Immunol*. 2023;14, 1182732.
20. Chen J, Yang J, Li H, et al. Single-cell transcriptomics reveal the intratumoral landscape of infiltrated T-cell subpopulations in oral squamous cell carcinoma. *Mol Oncol*. 2021;15:866–886.
21. Peng Y, Xiao L, Rong H, et al. Single-cell profiling of tumor-infiltrating TCF1/TCF7(+) T cells reveals a T lymphocyte subset associated with tertiary lymphoid structures/organs and a superior prognosis in oral cancer. *Oral Oncol*. 2021;119, 105348.
22. Gavzy SJ, Kensiski A, Lee ZL, Mongodin EF, Ma B, Bromberg JS. Bifidobacterium mechanisms of immune modulation and tolerance. *Gut Microbes*. 2023;15, 2291164.
23. Lim HJ, Shin HS. Antimicrobial and immunomodulatory effects of Bifidobacterium strains: a review. *J Microbiol Biotechnol*. 2020;30:1793–1800.
24. Sun S, Luo L, Liang W, et al. Bifidobacterium alters the gut microbiota and modulates the functional metabolism of T regulatory cells in the context of immune checkpoint blockade. *Proc Natl Acad Sci U S A*. 2020;117:27509–27515.
25. Mandelbaum N, Zhang L, Carasso S, et al. Extracellular vesicles of the gram-positive gut symbiont Bifidobacterium longum induce immune-modulatory, anti-inflammatory effects. *NPJ Biofilms Microbiomes*. 2023;9:30.
26. Qiu X, Zhang M, Yang X, Hong N, Yu C. Faecalibacterium prausnitzii upregulates regulatory T cells and anti-inflammatory cytokines in treating TNBS-induced colitis. *J Crohns Colitis*. 2013;7. e558 e568.
27. Mohebali N, Weigel M, Hain T, et al. Faecalibacterium prausnitzii, bacteroides faecis and roseburia intestinalis attenuate clinical symptoms of experimental colitis by regulating Treg/Th17 cell balance and intestinal barrier integrity. *Biomed Pharmacother*. 2023;167, 115568.
28. Zhou L, Zhang M, Wang Y, et al. Faecalibacterium prausnitzii produces butyrate to maintain Th17/Treg balance and to ameliorate colorectal colitis by inhibiting histone deacetylase 1. *Inflamm Bowel Dis*. 2018;24:1926–1940.
29. Zhu J, Paul WE. Heterogeneity and plasticity of T helper cells. *Cell Res*. 2010;20:4–12.
30. Chen H, Sameshima J, Yokomizo S, et al. Expansion of CD4+ cytotoxic T lymphocytes with specific gene expression patterns may contribute to suppression of tumor immunity in oral squamous cell carcinoma: single-cell analysis and in vitro experiments. *Front Immunol*. 2023;14, 1305783.
31. Lavanya N, Jayanthi P, Rao UK, Ranganathan K. Oral lichen planus: an update on pathogenesis and treatment. *J Oral Maxillofacial Pathol : JOMFP*. 2011;15:127–132.
32. Baek K, Choi Y. The microbiology of oral lichen planus: is microbial infection the cause of oral lichen planus? *Mol Oral Microbiol*. 2018;33:22–28.
33. Yu FY, Wang QQ, Li M, et al. Dysbiosis of saliva microbiome in patients with oral lichen planus. *BMC Microbiol*. 2020;20:75.
34. Wang K, Lu W, Tu Q, et al. Preliminary analysis of salivary microbiome and their potential roles in oral lichen planus. *Sci Rep*. 2016;6:22943.
35. Deleu S, Machiels K, Raes J, Verbeke K, Vermeire S. Short chain fatty acids and its producing organisms: an overlooked therapy for IBD? *EBioMed*. 2021;66, 103293.
36. Moon J, Lee AR, Kim H, et al. Faecalibacterium prausnitzii alleviates inflammatory arthritis and regulates IL-17 production, short chain fatty acids, and the intestinal microbial flora in experimental mouse model for rheumatoid arthritis. *Arthritis Res Ther*. 2023;25:130.
37. Atarashi K, Tanoue T, Shima T, et al. Induction of colonic regulatory T cells by indigenous clostridium species. *Science (New York, NY)*. 2011;331:337–341.
38. Hu M, Alashkar Alhamwe B, Santner-Nanan B, et al. Short-chain fatty acids augment differentiation and function of human induced regulatory T cells. *Int J Mol Sci*. 2022;23.
39. Evans HM, Schultz DF, Boiman AJ, McKell MC, Qualls JE, Deepe Jr GS. Restraint of Fumarate accrual by HIF-1alpha preserves miR-27a-mediated limitation of interleukin 10 during infection of macrophages by Histoplasma capsulatum. *MBio*. 2021;12, e0271021.
40. Zhang B, Zhang X, Tang FL, Zhu LP, Liu Y, Lipsky PE. Clinical significance of increased CD4+CD25-Foxp3+ T cells in patients with new-onset systemic lupus erythematosus. *Ann Rheum Dis*. 2008;67:1037–1040.
41. Miyara M, Yoshioka Y, Kitoh A, et al. Functional delineation and differentiation dynamics of human CD4+ T cells expressing the FoxP3 transcription factor. *Immunity*. 2009;30:899–911.
42. Saito T, Nishikawa H, Wada H, et al. Two FOXP3(+)CD4(+) T cell subpopulations distinctly control the prognosis of colorectal cancers. *Nat Med*. 2016;22:679–684.
43. Arruvito L, Sabatté J, Pandolfi J, et al. Analysis of suppressor and non-suppressor FOXP3+ T cells in HIV-1-infected patients. *PLoS One*. 2012;7, e52580.
44. Horwitz DA. Identity of mysterious CD4+CD25-Foxp3+ cells in SLE. *Arthritis Res Ther*. 2010;12:101.
45. Choi J, Kim BR, Akuzum B, Chang L, Lee JY, Kwon HK. T(REG)king from gut to brain: the control of regulatory T cells along the gut-brain Axis. *Front Immunol*. 2022;13, 916066.
46. Wang Y, Su MA, Wan YY. An essential role of the transcription factor GATA-3 for the function of regulatory T cells. *Immunity*. 2011;35:337–348.
47. Pan Q, Guo F, Huang Y, et al. Gut Microbiota dysbiosis in systemic lupus erythematosus: novel insights into mechanisms and promising therapeutic strategies. *Front Immunol*. 2021;12, 799788.
48. He H, Xu H, Xu J, et al. Sodium butyrate ameliorates gut Microbiota dysbiosis in lupus-like mice. *Frontiers in Nutrition*. 2020;7, 604283.
49. Nylund L, Nermes M, Isolauri E, Salminen S, de Vos WM, Satokari R. Severity of atopic disease inversely correlates with intestinal microbiota diversity and butyrate-producing bacteria. *Allergy*. 2015;70:241–244.
50. Ochoa-Repáraz J, Magori K, Kasper LH. The chicken or the egg dilemma: intestinal dysbiosis in multiple sclerosis. *Annals Translational Med*. 2017;5:145.