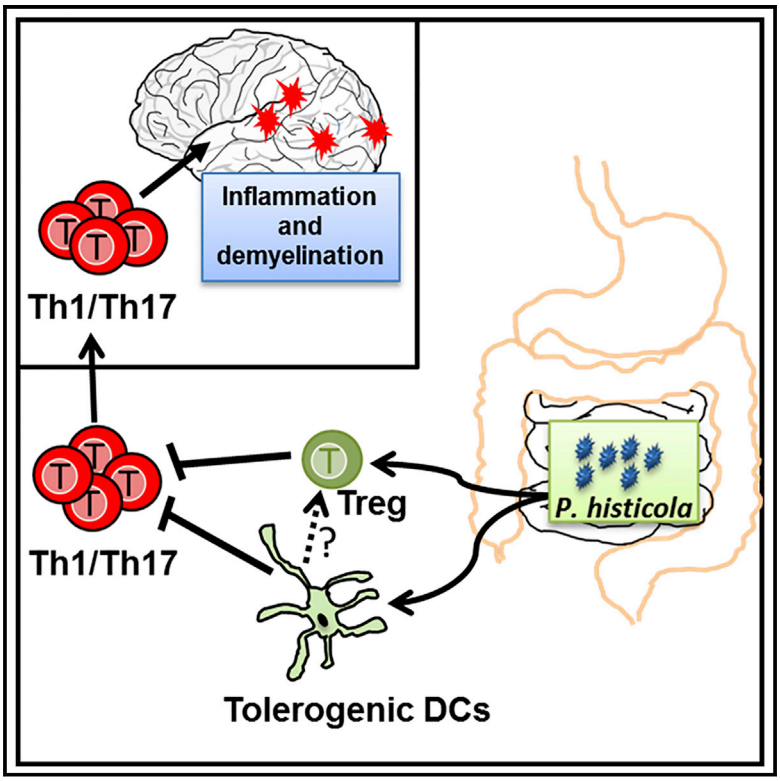
Cell Reports

Report

Human Gut-Derived Commensal Bacteria Suppress CNS Inflammatory and Demyelinating Disease

Graphical Abstract



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In Brief

Mangalam et al. show that *P. histicola* can suppress autoimmune disease in a preclinical model of multiple sclerosis. Thus, gut bacteria have the potential to be used as a therapy in human diseases.

Highlights

- A human gut bacterium has potential as a therapy for multiple sclerosis (MS)
- *P. histicola* can suppress disease in a preclinical animal model of MS
- *P. histicola* suppresses disease by inducing $CD4^+$ $FoxP3^+$ regulatory T cells

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Human Gut-Derived Commensal Bacteria Suppress CNS Inflammatory and Demyelinating Disease

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SUMMARY

The human gut is colonized by a large number of microorganisms (~10^{13} bacteria) that support various physiologic functions. A perturbation in the healthy gut microbiome might lead to the development of inflammatory diseases, such as multiple sclerosis (MS). Therefore, gut commensals might provide promising therapeutic options for treating MS and other diseases. We report the identification of human gut-derived commensal bacteria, *Prevotella histicola*, which can suppress experimental autoimmune encephalomyelitis (EAE) in a human leukocyte antigen (HLA) class II transgenic mouse model. *P. histicola* suppresses disease through the modulation of systemic immune responses. *P. histicola* challenge led to a decrease in pro-inflammatory Th1 and Th17 cells and an increase in the frequencies of $CD4^+FoxP3^+$ regulatory T cells, tolerogenic dendritic cells, and suppressive macrophages. Our study provides evidence that the administration of gut commensals may regulate a systemic immune response and may, therefore, have a possible role in treatment strategies for MS.

INTRODUCTION

The human gut is colonized by a large number of microorganisms (~10^{13} bacteria) that support various physiologic functions (Sender et al., 2016). Recent research envisages humans as holobionts having evolved with our microbiome, with the latter playing an important role in maintaining human health (Charbonneau et al., 2016; Honda and Littman, 2016). The increased prevalence of inflammatory diseases in developed countries has been attributed to an altered gut microbiome that is characteristically linked with the disease state. Therefore, gut commensals capable of restoring the healthy microbiome could be a promising therapy for treating inflammatory diseases such as multiple sclerosis (MS). Although several therapies for MS are available, none cure the disease, and many are not well tolerated.

The hypothesis that MS is an autoimmune disease caused by myelin-specific CD4 T cells comes from experimental autoimmune encephalomyelitis (EAE), an animal model of MS (Gold et al., 2000). The CD4 T cell repertoire is selected in humans by human leukocyte antigen (HLA) class II molecules, and MS patients show increased frequency of particular HLA class II haplotypes, such as HLA-DR2/DQ6, DR3/DQ2, and DR4/DQ8 (Dyment et al., 2005; Zivadinov et al., 2007). We have used transgenic mice expressing human class II genes and lacking endogenous class II genes to identify disease-susceptible and disease-resistant class II alleles (Luckey et al., 2011; Mangalam et al., 2008). Transgenic mice expressing HLA-DR3 and DQ8 genes (HLA-DR3.DQ8) develop EAE and have severe brain and spinal cord pathology (Mangalam et al., 2009; Mangalam et al., 2004).

During recent decades, the incidence of autoimmune diseases in developed countries has increased steadily (Bach, 2002; Okada et al., 2010). Numerous hypotheses have been suggested to explain this phenomenon, including alterations of the gut microbiome due to decreased exposure to parasites, antibiotics, western diet, and other environmental factors (Rook, 2012). Additionally, western diets are associated with an abundance of the *Bacteroides* enterotype, whereas the *Prevotella* enterotype is more prevalent in persons with a carbohydrate-rich, high-fiber, agrarian diet (Wu et al., 2011).

As certain commensal bacteria residing in the intestine might have immunomodulatory properties, we cultured proximal small bowel biopsies from celiac disease patients to isolate and characterize gut commensals with the ability to modulate immune response (Marietta et al., 2016). We isolated strains of *Prevotella histicola*, *Prevotella melaninogenica*, and *Capnocytophaga sputigena* and then investigated the ability of these anaerobic,

Gram-negative *Bacteroidetes* commensals to modulate proteolipid protein $(PLP)_{91–110}$-induced EAE in HLA-DR3.DQ8 transgenic mice. Here, we report that a strain of *P. histicola* can suppress or prevent disease in EAE. We further show that *P. histicola* suppresses disease through downregulation of proinflammatory Th1/Th17 response and induction of regulatory $CD4^+FoxP3^+$ regulatory T cells (Tregs).

RESULTS

Treatment with *P. histicola* Suppressed $PLP_{91–110}$-Induced EAE in HLA-DR3.DQ8 Transgenic Mice

Previously, we showed that double-transgenic mice expressing HLA-DR3.DQ8 develop EAE with CNS pathology (Mangalam et al., 2009). Therefore, we tested the immunomodulatory capabilities of *P. histicola*, *P. melaninogenica*, and *C. sputigena* in HLA-DR3.DQ8 transgenic mice (see Figure S1A). Among the groups tested with the three bacteria strains, the *P. histicola*-treated group had higher levels of interleukin (IL)-10 and transforming growth factor β (TGF-β), compared to medium or *C. sputigena*-treated mice. Mice treated with *P. melaninogenica* had higher levels of IL-10 but no change in the levels of TGF-β or tumor necrosis factor alpha (TNF-α). Next, we tested whether these commensals modulated EAE in HLA-DR3.DQ8 transgenic mice.

EAE was induced in HLA-DR3.DQ8 transgenic mice (Mangalam et al., 2009), and 7 days post-immunization, animals were challenged with 1×10^9 colony-forming units (CFUs) per milliliter of bacteria or medium (Lavasani et al., 2010). Mice were gavaged every other day with *P. histicola*, *P. melaninogenica*, *C. sputigena*, *Escherichia coli* (control-derived from mouse intestine), or medium alone and monitored for disease incidence and severity. *P. histicola*-treated mice had a reduced incidence of disease, with only 5 of 20 mice (25%) developing EAE, as compared with 100% EAE incidence (20/20) in medium-fed mice ($p < 0.005$) (Figures 1A and 1B; Table 1). Challenge with *P. melaninogenica* had a mild suppressive effect, but the cumulative disease score was not different from that of control groups. In contrast, all mice in the *C. sputigena*-treated and *E. coli*-treated groups developed disease. Because *P. histicola*-challenged mice had a less cumulative disease (13.6 ± 18.9 versus 76.75 ± 7.7; $p < 0.001$) (Figure 1B), and not *P. melaninogenica*-challenged mice, we focused on *P. histicola* for later experiments. Further, disease onset was delayed in *P. histicola*-challenged mice compared with medium-challenged mice (17.5 ± 0.3 days versus 10.6 ± 0.2 days; $p < 0.005$) (Table 1). To address whether colonization with *P. histicola* alone can modulate the disease, we depleted microbial flora of mice using broad-spectrum antibiotics for 3 weeks (Rakoff-Nahoum et al., 2004) and gavaged with medium or *P. histicola*. The *P. histicola*-challenged group developed milder EAE, compared to the medium-treated group (see Figure S1B). These data suggest that *P. histicola* alone can modulate EAE in HLA-DR3.DQ8 mice.

P. histicola challenge did not cause any pathology in the upper gut (see Figure S1C). To investigate which part of the gut *P. histicola* colonizes, naive mice (8–12 weeks old) were treated with either medium or *P. histicola*. Utilizing qPCR and *Prevotella*-specific primers, we observed higher colonization in the stomach and jejunum/ileum (see Figure S1D). Thus, our data suggest that *P. histicola* might colonize the upper gut of HLA-DR3.DQ8 transgenic mice.

Disease-Suppressive Effects of *P. histicola* Required Viable Bacteria

To determine whether *P. histicola* suppression of disease required whole bacteria or bacteria-derived soluble factors, we investigated the ability of cell-free *P. histicola* culture supernatant (PH-CS) to suppress EAE. The PH-CS-treated group had a disease incidence of 67% (10/15), compared with a 27% incidence rate in the *P.* histicola-challenged group (see Table S1). Mice challenged with PH-CS had a greater risk of developing EAE than mice given live *P. histicola* (hazard ratio, 3.3; 95% confidence interval [CI], 1.03–10.5; $p = 0.04$), indicating that PH-CS had a modest effect on disease incidence (Figure S2). The risk of EAE development was significantly lower in the live-bacterium group (hazard ratio, 7.7; 95% CI, 2.5–24; $p = 0.005$). We tested live *P. histicola* in doses that ranged from 1×10^7 to 1×10^9 CFUs/mL and observed a dose-dependent effect with optimal suppression at 1×10^8 CFUs/mL (see Table S2).

Treatment with *P. histicola* Reduced Inflammation and Demyelination in the CNS

Analysis of CNS tissues from medium-challenged HLA-DR3.DQ8 transgenic mice showed severe inflammation and demyelination in the brain and spinal cord compared to the *P. histicola*-challenged groups (Figure 1C). Quantitative analysis of spinal cord inflammation and demyelination showed that *P. histicola*-challenged animals had fewer regions with inflammation and demyelination (Figure 1D). Groups receiving other bacteria (*E. coli* and *C. sputigena*) had severe CNS inflammation and demyelination, similar to the medium-challenged group. Thus, treatment with *P. histicola* reduced CNS inflammation and demyelination, compared to the medium-only group.

P. histicola Administration Downregulated $PLP_{91–110}$-Specific T Cell and Cytokine Response

To determine the effect of *P. histicola* on antigen-specific T cell responses, we isolated splenocytes from mice given bacteria or medium and stimulated with the $PLP_{91–110}$ peptide. *P. histicola*-challenged mice had less antigen-specific T cell response compared with the medium-challenged group or the *E. coli*-challenged group (Figure 2A). Splenocytes from *P. histicola*-challenged mice produced less proinflammatory cytokines IL-17 and interferon (IFN)-γ after stimulation with $PLP_{91–110}$, whereas the anti-inflammatory cytokine IL-10 was increased (Figure 2B). Levels of tumor necrosis factor (TNF)-α were similar among different groups (Figure 2B). Animals given control bacteria (*E. coli*) showed cytokine levels similar to that of the medium-only group.

P. histicola Treatment Reduced the Blood-Brain Barrier (BBB) and Gut Permeability and Downregulated CNS Inflammation

The *P. histicola*-challenged group had reduced BBB permeability, as measured by fluorescein isothiocyanate (FITC)-

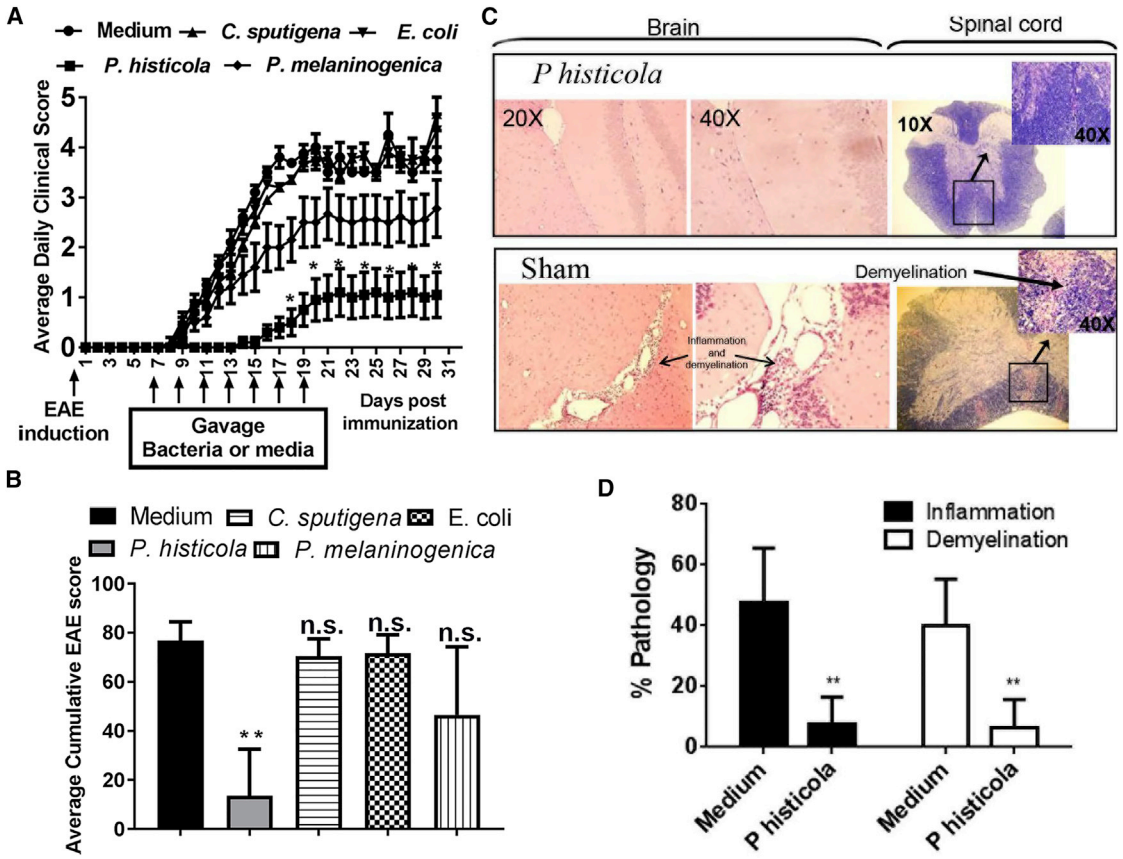

Figure 1. Effect of Various Human Commensals on PLP_{91-110}-Induced EAE in HLA-DR3.DQ8 Transgenic Mice

Animals were immunized with PLP_{91-110} peptide and treated with either bacteria or medium starting at day 7 post-immunization and every other day, for a total of seven doses.

(A) Only *P. histicola*-treated mice had late disease onset and lower disease incidence. No significant effect was observed in the groups receiving *P. melaninogenica*, *C. sputigena*, or *E. coli*. Animals were scored daily for disease, and the daily mean disease score for each group is plotted. Error bars represent SEM.

(B) *P. histicola*-treated animals had a lower average cumulative disease score compared with medium-treated animals. Error bars represent SD.

(C) Mice were sacrificed on day 30 post-immunization, and the brain and spinal cord were examined by histopathology. Representative photomicrographs show mild to severe inflammation and demyelination in medium-treated mice compared with *P. histicola*-treated mice.

(D) Quantitative analysis of spinal cord pathology similarly showed increased inflammation and demyelination in medium-treated groups compared with the *P. histicola*-treated group. Error bars represent SD.

The data in (A) and (B) represent average cumulative scores of three experiments performed at different times with n = 10 mice per group. The data in (C) and (D) represent one of three experiments performed at different time points (n = 5 mice per group). *$p \leq 0.05$; **$p \leq 0.005$; n.s., not significant when compared to the medium-treated group.

albumin, compared with the medium-only group (Figure 2C). Mice with EAE also had increased gut permeability, compared to the medium-treated group. However, challenge with *P. histicola* restored the gut permeability to pre-EAE levels (Figure 2D). Groups receiving *E. coli* had increased permeability of both BBB and gut, which was similar to that of the medium-treated group (Figures 2C and 2D). CNS tissue from the *P. histicola*-treated mice had reduced cellular infiltration, compared with the control groups (Figure 2E), with lower levels of total CD4 T cells as well as IFN-γ- and IL-17-expressing CD4 T cells, compared with the medium- or *E. coli*-treated groups (Figures 2F–2H). Thus, *P. histicola* decreased BBB permeability and reduced the frequency of pro-inflammatory Th1 and Th17 cells in the CNS.

P. histicola Suppresses EAE through an Increase in $CD4^+FoxP3^+$Tregs

To identify the cell population(s) responsible for the disease-protective effect, we characterized the cellular profile of bacterially challenged and untreated animals. *Prevotella*-challenged mice had an increased frequency of $CD4^+FoxP3^+$ Tregs in the spleen and mesenteric lymph nodes (MLNs) (Figures 3A and 3B). To test whether *P. histicola* could directly induce a Treg population, naive HLA-DR3.DQ8 transgenic mice were challenged with 1×10^8 CFUs/mL *P. histicola* or medium on alternate days for seven doses. *P. histicola*-treated animals had higher levels of $CD4^+CD25^+FoxP3^+$ Tregs in splenocytes, MLNs, and cervical lymph nodes (CLNs), compared with the medium-treated group (see Figure S3). $CD4^+CD25^+$ Tregs isolated from

Table 1. Effect of Commensal Bacteria on PLP_{91-110}-Induced EAE in HLA-DR3.DQ8 Transgenic Mice

Treatment	Disease Incidence (%)	Disease Free, Mean Days ± SE[a]	Hazard Ratio (95% CI)	p Value
Medium fed	20/20 (100)	10.6 ± 0.2	1 (reference)	
P. histicola	5/20 (25)	17.5 ± 0.3	0.03 (0.01–0.09)	<0.0001
C. sputigena	20/20 (100)	11.4 ± 0.4	0.6 (0.3–1.2)	0.15
P. melaninogenica	16/20 (80)	12.5 ± 0.4	0.2 (0.1–0.5)	<0.0009
E. coli	20/20 (100)	11 ± 0.3	0.8 (0.4–0.6)	0.47

[a]Estimated by the log-rank test.

P. histicola-challenged animals also showed higher suppression of PLP_{91-110}-specific $CD4^+$ T effector cells compared with the Tregs isolated from naive, medium-treated, or *E. coli*-treated mice (Figure 3C). Therefore, *P. histicola* increased regulatory $CD4^+$ T cell numbers and also enhanced their suppressive function.

P. histicola Challenge Increased the Frequency and Activity of Tolerogenic Dendritic Cells (DCs)

Next, we analyzed levels of $CD103^+CD11c^+$ tolerogenic DCs in MLNs, and in splenocytes, among different treatment groups. *P. histicola*-treated animals had increased levels of $CD103^+CD11c^+$ DCs in the spleen and MLNs, compared with the medium-treated group (Figure 3D). $CD11c^+$ cells from *P. histicola*-treated animals had lower antigen presentation capacity, compared with $CD11c^+$ DCs isolated from naive, medium-treated, or *E. coli*-treated mice (Figure 3E). Lipopolysaccharide-stimulated $CD11c^+$ DCs from medium-treated mice produced high levels of IL-23 and low levels of IL-10, whereas *P. histicola*-treated animals produced low levels of IL-23 and high levels of IL-10 (Figure 3F). $CD11c^+$ DCs isolated from the *E. coli*-treated group behaved similarly to the medium-treated group (Figure 3F). We also observed that *P. histicola* challenge led to the induction of $CD11b^+Gr\text{-}1^{med}$ macrophages (Figure S4A) and that $CD11b^+$ cells from the *P. histicola*-challenged group had reduced antigen presentation capacity, compared to the medium-treated group (Figure S4B). Macrophages from *P. histicola*-treated animals produced higher IL-10 and lower IL-12 (Figure S4C), compared with medium-treated or *E. coli*-treated groups. Thus, *P. histicola*-treated animals had an increased frequency of tolerogenic antigen-presenting cells and decreased antigen presentation capacity compared with medium-treated animals.

Splenocytes from *P. histicola*-Treated Animals Suppressed PLP_{91-110}-Induced EAE

Next, we tested whether adoptive transfer of immune cells from *P. histicola*-treated mice would ameliorate EAE. We transferred splenocytes (1×10^7) from PLP_{91-110} EAE-immunized animals treated with either *P. histicola* or medium into different mice that had been previously immunized with PLP_{91-110} 5 days earlier (Mangalam et al., 2012). The group receiving splenocytes from *P. histicola*-treated animals had lower disease incidence and severity, as compared with the untreated mice or those receiving splenocytes from medium-treated animals (Figure S5). Thus, our data indicated that the disease-suppressive effect of *P. histicola* was transferable.

P. histicola Challenge Caused a Shift in the Composition of the Gut Microbiota

Finally, to investigate whether treatment with *P. histicola* can alter gut microbiota composition, fecal samples were collected from pre- (naive) and post-immunized (EAE) mice receiving either medium or *P. histicola*. Mice with EAE had a distinct fecal microbiota, compared to naive mice (Figure 4A); however, challenge with *P. histicola* shifted gut microbiota composition closer to that of pre-immunized (naive) mice. Naive mice had a higher relative abundance of certain genera such as *Prevotella*, *Lactobacillus*, and *Sutterella*, which were reduced in the EAE group receiving medium. However, mice induced for EAE and treated with *P. histicola* had increased abundance of *Prevotella*, *Lactobacillus*, and *Sutterella* (Figures 4B and 4C). Thus, *P. histicola* challenge restored gut microbiota to the pre-immunized state.

DISCUSSION

We have identified a commensal bacterium, *P. histicola*, from the human upper gastrointestinal tract that had a systemic suppressive effect distant from the small intestine. *P. histicola* inhibited the development of EAE in HLA-DR3.DQ8 transgenic mice, a preclinical model of MS. We observed that *P. histicola* treatment markedly attenuated inflammation and demyelination and reduced BBB permeability, compared to medium or control bacteria. We demonstrated that *P. histicola* suppressed disease by downregulating pro-inflammatory cytokines IFNγ and IL-17, by inducing $CD4^+CD25^+FoxP3^+$ Tregs, tolerogenic DCs, and suppressive macrophages. These observations suggest that *P. histicola* monotherapy is effective when administered to HLA transgenic mice, a model of the chronic disease. Disease protection induced by *P. histicola* was transferable into other animals; this, therefore, supports our hypothesis that *P. histicola* protects the mice from EAE by modulating the systemic immune response. Our study shows that a human commensal from the upper gastrointestinal tract possesses potent disease-protective characteristics and provides proof of principle that human commensal gut bacteria protect against neuroinflammation.

Recent studies have indicated that the ecosystem that is composed of human commensal bacteria (i.e., the microbiome) is a new frontier of biologic discovery with great therapeutic potential. Examples of bacterial therapeutics for ameliorating EAE include *Bacteroides fragilis* (Round and Mazmanian, 2010; Surana and Kasper, 2012) and mixtures of *Lactobacillus* strains (Lavasani et al., 2010). The disease-protective effects of *B. fragilis* have been attributed to its production of polysaccharide A (PSA) (Ochoa-Repáraz et al., 2010). However, live

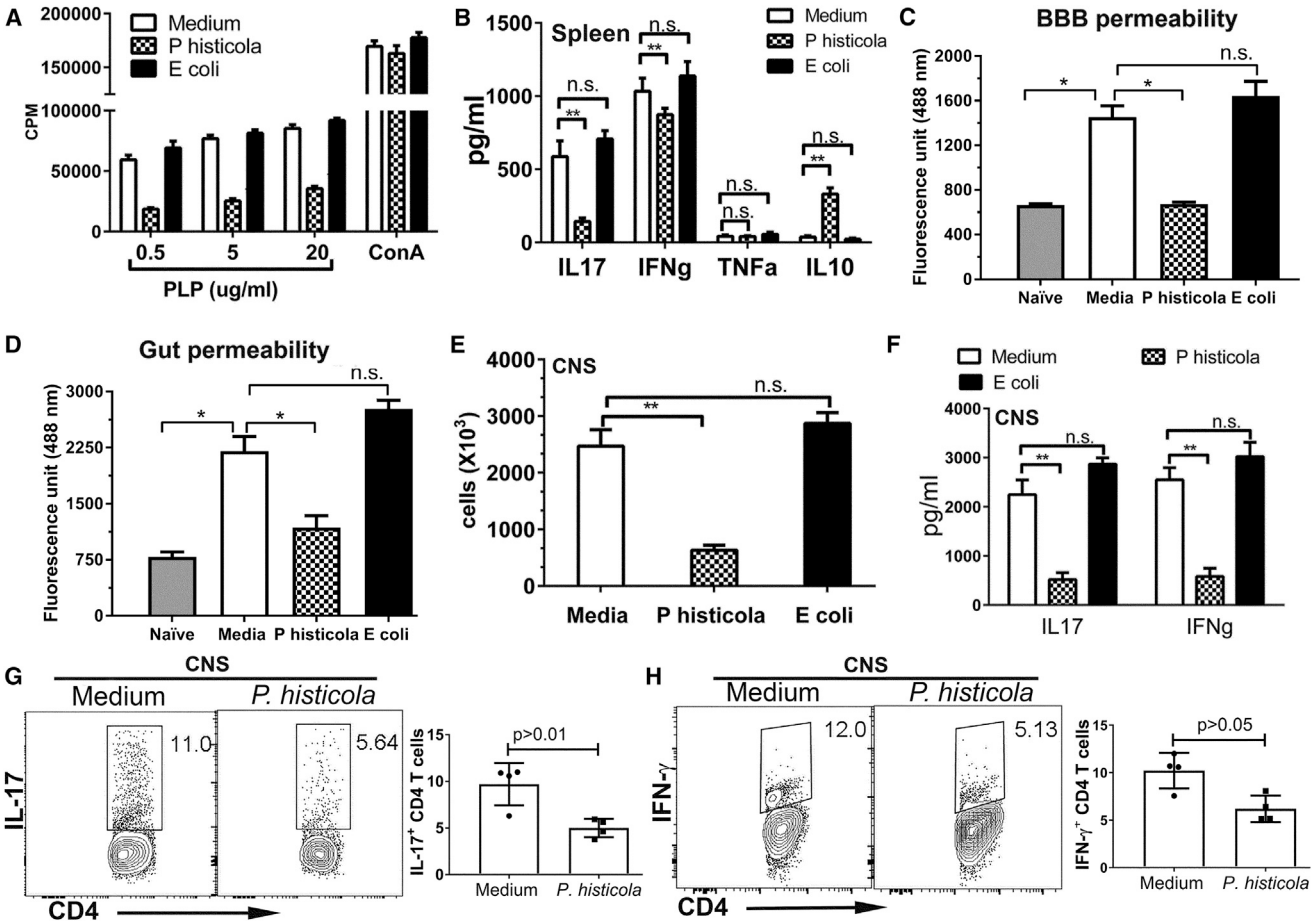

Figure 2. *P. histicola*-Treated Mice Show Decreased T Cell Recall Response, CNS Inflammation, and Cytokine Levels

(A) *P. histicola*-treated animals had a reduced T cell proliferative response to the $PLP_{91–110}$ peptide *in vitro*, compared with the medium- and *E. coli*-treated groups. Response to concanavalin A was similar among three groups. The data presented are the mean counts per minute ± SD.

(B) Splenocytes stimulated with antigen from *P. histicola*-treated mice had reduced levels of inflammatory cytokines IL-17 and IFN-γ and increased levels of anti-inflammatory cytokine IL-10, compared with medium-treated mice. The data presented are the average of two independent experiments (n = 4 mice per group).

(C and D) Medium-treated HLA-DR3.DQ8 transgenic mice had compromised BBB permeability (C) and gut permeability (D). Challenge with *P. histicola* restored BBB permeability (C) as well gut permeability to pre-EAE levels.

(E) Cellular infiltration into the CNS was reduced in *P. histicola*-treated mice, compared with medium- or *E. coli*-treated mice.

(F–H) CNS inflammatory cells from the *P. histicola*-treated group had reduced levels of inflammatory cytokines IL-17 and IFN-γ as measured by ELISA (F) and flow cytometry (G and H, respectively), compared with the medium-treated group.

Error bars represent SD. The data represent two separate experiments performed in triplicate (n = 5 mice per group). *$p \leq 0.05$; **$p \leq 0.005$; n.s., not significant, when compared to the medium-treated group.

P. histicola was needed for maximal disease protection in our study, which suggests that the interaction of the living bacteria with the gut mucosal immune systems may be required. The ability of *P. histicola* to suppress EAE in mice with depleted gut flora suggests a direct role for *P. histicola* in disease suppression. However, we cannot rule out potential additional indirect effects through modulation of other microbes, because *P. histicola* treatment was also associated with a shift in gut microbiota to more closely resemble that of a healthy mouse, with an increase in the relative abundance of *Prevotella*, *Sutterella*, and *Lactobacillus* at the genus level. Although both *Prevotella* and *Sutterella* genera had been shown to be increased in relapsing-remitting MS (RRMS) patients on disease-modifying treatments (Jangi et al., 2016), it is currently unclear whether the species of *Prevotella* (i.e., *P. histicola*) used in our study is part of the observed alteration of *Prevotella* at the genus levels in patients with MS. A previous study has shown that *B. fragilis*, a gut commensal, can influence host behavior by modulation of gut microbes (Hsiao et al., 2013). Thus, it is possible that *P. histicola*-induced changes in gut microbiome might also contribute to *P. histicola*'s disease-protective effect either directly, indirectly, or both.

P. histicola challenge was associated with elevated levels of IL-10, $CD4^+FoxP3^+$ Tregs, tolerogenic DCs, and suppressive macrophages. The disease-protective effect of other human commensals such as *B. fragilis* and a mixture of *Lactobacillus* species in EAE has been attributed to their ability to induce $CD4^+FoxP3^+$ Tregs and IL-10 (Lavasani et al., 2010; Ochoa-Repáraz et al., 2010). A role of tolerogenic DCs observed in our study

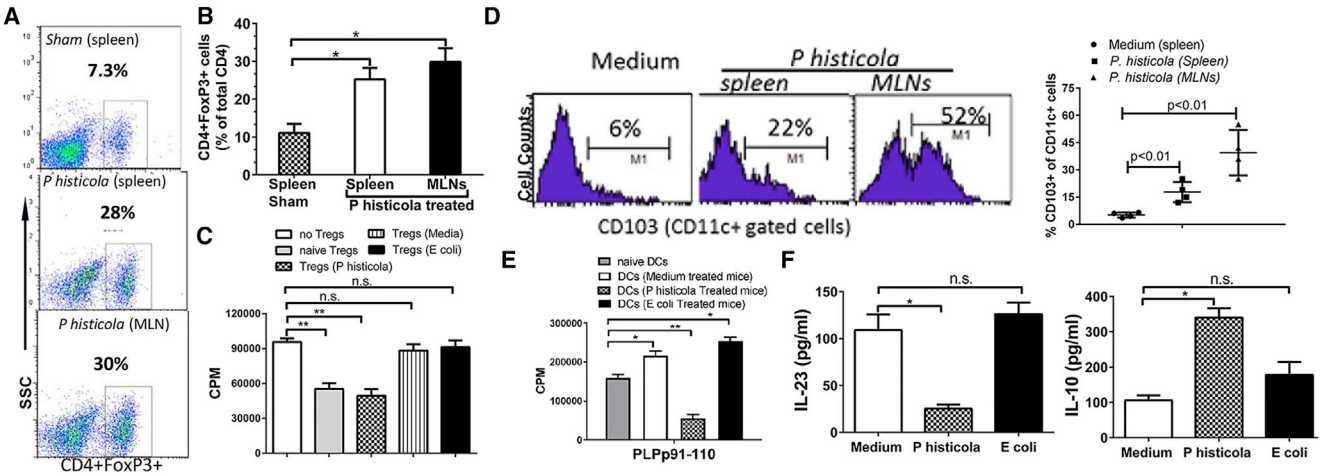

Figure 3. *P. histicola* Challenge Increased the Frequency and Activity of CD4$^+$FoxP3$^+$ Regulatory T Cells and Tolerogenic DCs

(A) *P. histicola*-treated mice had an increased frequency of CD4$^+$FoxP3$^+$ Tregs, compared with the medium-treated group or *E. coli*-treated group. Numbers in scatterplots indicate the percentage of positive cells.

(B) Histogram plot of Treg frequency in the *P. histicola*-treated and untreated groups. The data presented are the average of two independent experiments (n = 4 mice per group).

(C) CD4$^+$CD25$^+$ Tregs from *P. histicola*-treated mice had increased suppressive activity, compared with the medium-treated group or the *E. coli*-treated group.

(D) The *P. histicola*-treated group had increased levels of CD103$^+$CD11c$^+$ DCs in the spleen and MLNs, compared with the medium-treated group. Numbers in the histogram indicate the percentage of CD103$^+$ cells gated from the CD11c$^+$ population. Data are from one of three experiments performed at different times.

(E) Co-culture of CD11c$^+$ cells from the *P. histicola*-treated group with PLP-specific CD4 T cells resulted in reduced proliferation, compared to the medium-treated group or the naive group. The data are presented as the mean counts per minute (CPM) ± SD and are the average of two independent experiments (n = 3 mice per group).

(F) CD11c$^+$ DCs from the *P. histicola*-treated group produced low IL-23 and high IL-10, compared with the medium-treated group. The data are presented as the mean CPM ± SD and are the average of two independent experiments (n = 3 mice per group).

(B–F) Error bars represent SD.

*p $\leq$ 0.05; **p $\leq$ 0.005; n.s., not significant.

is consistent with earlier studies (Ochoa-Repáraz et al., 2010) showing a role for CD11c$^+$ DCs in suppressing disease in a PSA-treated EAE model. *P. histicola* increased levels of tolerogenic CD103$^+$ DCs, producing high levels of IL-10 and low levels of IL-23.

Both IL-17 and IFN-γ are the major pro-inflammatory cytokines associated with the pathology of MS. *P. histicola* treatment suppressed myelin antigen-specific T cell recall response and reduced IL-17 and IFN-γ in the periphery, as well as in CNS. Thus, *P. histicola* might mediate its effect through the downregulation of pro-inflammatory cells of both Th1 and Th17 phenotypes. Once inflammatory cells are activated in the periphery, their movement across the BBB and into the CNS is an essential step for initiating inflammation and demyelination in the brain and spinal cord. Reduced BBB permeability and milder pathology in the brain and spinal cord from *P. histicola*-treated mice suggests that *P. histicola* mediates its suppressive effect through modulation of inflammatory cells trafficking to the CNS.

Our study demonstrates that *P. histicola* can suppress EAE in mice; however, it is currently unclear whether *P. histicola* supplementation will be effective as a treatment for MS. Interestingly, the genus *Prevotella* is reduced in RRMS patients, compared with healthy controls (Chen et al., 2016; Miyake et al., 2015), and increased in RRMS patients on disease-modifying treatments (Jangi et al., 2016). Thus, human MS studies suggest that individual, or a combination of, *Prevotella* strains might have immunomodulatory properties; however, it is unknown whether these changes at the genus level correlate with changes in *P. histicola* or some other species of *Prevotella.*

In summary, we report the association of a human commensal bacterium with disease-protective abilities in an animal model of MS. Our data indicate that *P. histicola*, a human commensal, has immunomodulatory and anti-inflammatory capabilities that suppress PLP$_{91-110}$-induced EAE in HLA-DR3.DQ8 transgenic mice. The disease protection was due to the ability of *P. histicola* to induce regulatory and suppressive immune subsets. Our future studies will investigate whether *P. histicola* modulates human immune cells directly or via its interaction with intestinal epithelial cells. This work demonstrates that *P. histicola* can manipulate the systemic immunity and organ-specific disease far away from its localization in the gut.

EXPERIMENTAL PROCEDURES

Mice

HLA-DR3.DQ8 double-transgenic (DQ8 [DQA1*0103, DQB1*0302]-DR3 [DRB1*0301]) mice lacking major histocompatibility complex (MHC) class II genes (AE$^{-/-}$) were generated as previously described (Das et al., 2000; Mangalam et al., 2009) and are referred to as HLA-DR3.DQ8 transgenic mice in the text. Mice of both sexes were studied. All mice were bred and maintained in the pathogen-free Immunogenetics Mouse Colony of the Mayo Clinic (Rochester, MN) and the University of Iowa in accordance with NIH and institutional guidelines. All experiments were approved by the Institutional Animal Care and Use Committee at the Mayo Clinic, Rochester, MN, and the University of Iowa, Iowa City.

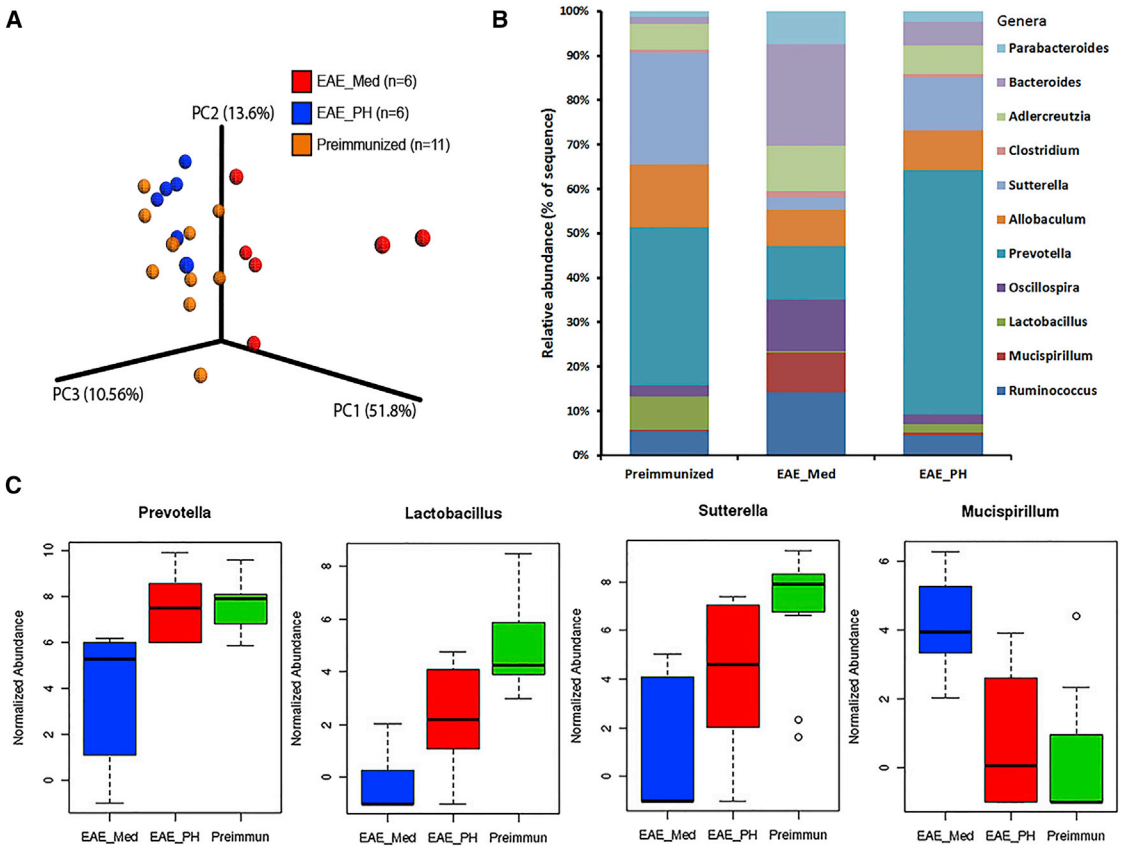

Figure 4. *P. histicola* Challenge Caused a Shift in the Composition of the Gut Microbiota

(A) HLA-DR3.DQ8 transgenic mice with EAE had a distinct gut microbiome profile compared to those from pre-immunized (naive mice). The *P. histicola*-treated group had a gut microbiota profile similar to that of the pre-immunized (naive mice) group. Weighted UniFrac-based 3D PCoA plot based on all OTUs from fecal samples of mice from the pre-immunized (n = 6), medium-treated EAE group (n = 11), and the *P. histicola*–treated group (n = 6).

(B) Relative abundance of gut microbiota at the genus levels in pre-immunized (naive mice), EAE mice treated with medium, and EAE mice treated with *P. histicola*.

(C) Boxplot showing a normalized relative abundance of bacteria (genus-level profile) between groups. Mice with EAE had loss of certain genera, especially *Prevotella*, *Sutterella*, and *Lactobacillus*, compared to pre-immunized (naive) mice. *P. histicola* challenge restored the abundance of *Prevotella*, *Sutterella*, and *Lactobacillus*. The difference between groups was analyzed using one-way ANOVA (Kruskal-Wallis rank-sum test) and false-discovery-rate-adjusted $p < 0.05$. The colored box represents middle 50% of the distribution with top and bottom horizontal lines representing maximum and minimum abundance levels of particular bacteria.

Isolation and Culture of Bacteria

Prevotella and similar anaerobic Gram-negative *Bacteroidetes* bacteria were isolated from the duodenum of treated celiac disease patients, and their identity was confirmed using 16S rRNA-specific PCR followed by sequencing, as well as whole-genome sequencing, as described previously (Marietta et al., 2016).

Flow Cytometry

Expression of HLA-DR and HLA-DQ molecules on peripheral blood leukocytes, lymph node cells, and splenocytes were analyzed by flow cytometry. Additional details are provided in the Supplemental Experimental Procedures.

Disease Induction and Scoring

For disease induction, 8- to 12-week-old transgenic mice were immunized subcutaneously in both flanks with 100 μg of $PLP_{91–110}$. Disease severity was scored as described previously (Mangalam et al., 2009); additional details are provided in the Supplemental Experimental Procedures.

Treatment of Animals with Commensal Bacteria

HLA-DR3.DQ8 transgenic mice were treated with *P. histicola*, *P. melaninogenica*, *C. sputigena*, and mouse-specific *E. coli* or media by oral gavage, starting 7 days after immunization. Animals were gavage-fed on alternate days with 1×10^8 CFUs in 100 μL of culture medium or medium alone, for a total of seven doses. Mice were evaluated for disease incidence, duration, and severity for 4 weeks after immunization.

T Cell Proliferation and Cytokine Assay

T cell recall response was measured in splenocytes and lymph nodes from immunized mice using standard thymidine incorporation methods (Das et al., 2000).

Gut Flora Depletion and Colonization with *Prevotella*

Gut flora was depleted by giving a broad-spectrum antibiotic cocktail (0.5 g/L vancomycin, 1 g/L neomycin sulfate, 1 g/L metronidazole, 1 g/L ampicillin) in drinking water as described previously (Rakoff-Nahoum et al., 2004). After 3 weeks of antibiotic treatment, animals were placed on sterile water for 3 days before being challenged with *P. histicola* (10^8 CFUs) or medium on alternate days, for the total of seven doses. One week after the last dose, EAE was induced, and the disease was monitored (Mangalam et al., 2009).

Isolation of Tregs, DCs, and Macrophages

Tregs, DCs, and macrophages were isolated from splenocytes using commercial cell isolation kits (Miltenyi Biotec, San Diego, CA). Additional details are provided in the Supplemental Experimental Procedures.

In Vitro Functional Analysis of Tregs, DCs, and Macrophages

Suppressive abilities of $CD4^+CD25^+$ Tregs isolated from *P. histicola*-treated or medium-treated HLA-DR3.DQ8 transgenic mice were analyzed by coculturing Tregs (5×10^4 cells per well) with $PLP_{91–110}$-specific $CD4^+$ T cells (5×10^4 cells per well) in the presence of irradiated antigen-presenting cells (APCs) loaded with antigen. Additional details are provided in the Supplemental Experimental Procedures. Lipopolysaccharides from *E. coli* 026:B6 (Sigma-Aldrich, St. Louis, MO) were used to stimulate macrophages and DCs.

Adoptive Transfer of Splenocytes

The splenocytes were collected from EAE-immunized animals treated with either *P. histicola* or medium and transferred (1×10^7 cells per mouse) intravenously into $PLP_{91–110}$-immunized HLA-DR3.DQ8 transgenic mice 5 days after immunization. The timing for adoptive transfer studies was based on our previous study (Mangalam et al., 2012).

Microbiome Analysis

Fecal pellets were collected from pre- and post-immunized mice. Microbial DNA extraction, 16S amplicon preparation (V3–V5 region), and sequencing were done as described previously (Chen et al., 2016). R1.fastq and R2.fastq reads were merged using Paired-End reAd mergeR (PEAR) (Zhang et al., 2014), merged reads were converted to FASTA, and merged FASTA sequences were processed by Cloud Virtual Resource (CloVR) (Angiuoli et al., 2011) to form operational taxonomic units (OTUs) at 97% similarity; and histograms were generated using METAGENassist (Arndt et al., 2012).

Pathology

Brain and spinal cord sections were analyzed for inflammation and demyelination after staining with modified H&E, as described previously (Mangalam et al., 2009). Additional details are provided in the Supplemental Experimental Procedures.

Statistical Analysis

Differences in proliferation or cytokine levels between groups was assessed by a one-way ANOVA with multiple comparisons of the means when more than two groups were analyzed or by Student's t test when only two groups were analyzed if their data were normally distributed. The Kaplan-Meier method was used to estimate the probability of survival. The log-rank test was used to compare outcomes of different treatment groups. Hazard ratios (HRs) for developing disease (and 95% CIs) were calculated from the estimated coefficients in Cox proportional hazards regression models. All statistical analyses were performed with GraphPad Prism 6 (GraphPad Software, La Jolla, CA) or SAS version 9.3 (SAS Institute, Cary, NC). A significance level of 0.05 was used.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, five figures, and two tables and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2017.07.031.

AUTHOR CONTRIBUTIONS

A.M. conceptualized the study, designed and performed the experiments, wrote the manuscript, and gave final approval of the manuscript to be published; S.K.S, D.L, M.K., N.L., and J.J. performed the experiments; E.M. helped with experimental design and performed experiments; R.S.C. and R.S. performed statistical analysis. K.G.-C. performed intestinal pathology. R.P. helped with experimental designs and manuscript writing; M.R. performed histological scoring, helped with experimental design, and manuscript editing. C.D. and V.T. helped with the study design and interpretation of the data; J.M. conceptualized the study and edited and approved the final version of the manuscript. All authors commented on the manuscript.

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