Prevotella histicola inhibits neuroinflammation-induced activation of the Toll-like receptor 4 pathway by promoting the release of interleukin 10 from FoxP3+ Treg cells

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Research Article
Abstract

Background

Accumulating evidence indicates that intestinal microbiota not only influence development and behavior but also play important roles in the pathogenesis of neurodegenerative diseases. However, the relationships between gut bacteria and diseases of the nervous system remain to be fully explored. Microglial activation has been identified as an important factor affecting the progression of many degenerative diseases. Therefore, we aimed to investigate the effects of *Prevotella histicola* on neuroinflammation and associated mechanisms in an animal model of dopaminergic neuron death caused by microglia activation.

Methods

We used the Toll-like receptor 4 (TLR4) agonist lipopolysaccharide (LPS) to establish an animal model of dopaminergic neuron death induced by microglia activation. LPS was injected into the mouse substantia nigra, followed by gavage with *P. histicola*.

Results

*P. histicola* inhibited LPS-induced microglia activation by increasing the proportion of peripheral regulatory T cells (Treg), thereby increasing the secretion of interleukin (IL)-10, reducing dopaminergic neuron damage, stimulating activation of anti-inflammatory M2 type microglia, and improving exercise capacity. Interestingly, *P. histicola* treatment suppressed the expression of SUMO-specific protease 3 (SENP3), which plays an important role in LPS-stimulated inflammatory TLR4 signaling. *P. histicola* treatment also downregulated inflammatory factors including IL-1, IL-6, IL-12, and tumor necrosis factor-alpha (TNFα).

Conclusion

These results suggest that *P. histicola* inhibits the TLR4 inflammatory signaling pathway and release of inflammatory factors by regulating the release of IL-10 from FoxP3 + Treg cells in the spleen. Our findings may provide insight into a new therapeutic avenue for attenuating the progression of degenerative diseases involving microglial activation.

Background

Neurodegeneration, which refers to the progressive impairment and death of neurons, is implicated in neurodegenerative diseases such as Parkinson's disease (PD) and Alzheimer's disease (AD) [1]. The most significant pathological feature of PD is degeneration and death of dopaminergic neurons in the dense
part of the substantia nigra, which is particularly vulnerable to the effects of α-synuclein (α-Syn) aggregates (Lewy bodies in neuronal soma and Lewy neurites in neuronal processes) [2, 3]. High expression of α-Syn is a neurotoxic phenotype in the brain of patients with PD, and Lewy pathology can lead to direct proximity of activated microglia [3]. Microglia are the main immune cells in the central nervous system (CNS), and the inflammatory response not only affects the normal aging process of the human body but also plays an important role in the process of nervous system diseases [4].

Under physiological conditions, microglia have immune monitoring functions. When stimulated by pathogens, microglia change morphology from a branch-like shape to an amoeba-like shape, following which they develop the proinflammatory M1 phenotype, a common marker for which is inducible nitric oxide synthase (iNOS). M1 microglia release inflammatory cytokines such as IL-1β and TNF-α, as well as immunomodulatory cytokines such as IL-12 and IL-18. When exposed to cytokines such as IL-4, IL-10, and IL-13, microglia develop an anti-inflammatory M2 phenotype, which is commonly marked by arginase 1 (ARG1). M2 microglia release anti-inflammatory cytokines such as TGF-β and IL-10. This process is known as polarization [5].

Accumulating evidence indicates that microglia can intensify the process of neurodegenerative diseases by releasing inflammatory cytokines such as IL-1 and TNF-α, as well as toxic substances such as nitrogen oxides [6]. For example, studies conducted in PD model mice and positron emission tomography (PET) imaging studies involving human patients with PD have indicated that the development of PD is accompanied by activation of microglia and an increase in inflammatory factors [7, 8]. Notably, many recent studies have focused on the immune microenvironment of the nervous system to identify novel therapeutic approaches for the treatment of neurodegenerative diseases.

Clinical characteristics of PD include not only motor symptoms such as tremor at rest, rigidity, slowness or absence of voluntary movement, postural instability, and freezing, but also non-motor symptoms such as olfactory impairment, disturbance of emotion and cognitive disorders, sleep disorders, and gastrointestinal symptoms [9]. Gastrointestinal symptoms, such as constipation and gastroparesis, can be observed at all stages of PD and usually develop earlier than motor dysfunctions [10]. Many studies have shown that chronic low-grade inflammation mediated by dysbacteriosis is the basis of many age-related neurological disorders [3]. In addition, the fecal microbiological pattern differs between patients with PD and healthy controls [11], and a previous study noted that gut microbiota are required for α-Syn aggregation-induced motor deficits and gastrointestinal dysfunction in a mouse model of PD [2]. Similarly, in patients with PD, dysbiosis of the gut microbiota also appears earlier than α-Syn aggregation and Lewy body generation [12, 13]. These findings indicate that gut microbiota may be implicated in the pathogenesis of PD.

Accumulating evidence indicates a close link between gut microflora and CNS functions. Under normal conditions, most intestinal bacteria are restricted to the gut cavity or epithelial surface and perform their symbiotic functions [14]. However, under inflammatory conditions, signals associated with dysbiosis of the gut microbiota and immune disorders can pass to the brain through the gut–brain axis and cause
microglial activation [15]. Intestinal microflora participate in the two-way regulation of the enteric nervous system and CNS through a variety of channels, which form the intestinal flora–gut–brain axis to exert a major impact on the CNS. This axis regulates the functions of the immune, gastrointestinal, and nervous systems via close and complex connections [16]. The vagus nerve is the most direct route by which intestinal regulation of CNS activity can occur. Studies have demonstrated that materials from the intestine can be passed to the brain through the vagus nerve [17], and that PD occurs less frequently in patients who have undergone complete vagotomy [18]. On the other hand, some gut bacteria can also produce tryptophan metabolites to inhibit activation of microglia and proinflammatory cytokine production through aromatic hydrocarbon receptors [19]. The gut microflora is the main source of short-chain fatty acids (SCFAs). Studies have suggested that SCFAs such as butyrate can enhance the intestinal barrier and exert anti-inflammatory effects [20]. Notably, levels of anti-inflammatory bacteria that can produce butyrate are significantly decreased in patients with PD, while levels of proinflammatory bacteria are increased [21].

Lipopolysaccharide (LPS), a component of the outer cell wall of gram-negative bacteria, is composed of lipids and polysaccharides. LPS is a type of endotoxin that can not only produce cytokines and cause inflammatory responses but also induce inflammation of the CNS and lead to loss of dopaminergic (DA) neurons. Therefore, LPS injection into the mesencephalic substantia nigra is often used to model the pathogenesis of inflammation-mediated PD [22]. Our previous studies demonstrated that LPS injection into the mouse substantia nigra can cause death of a large number of DA neurons and induce clinical manifestations of PD, such as gait instability and decreased motor ability. The physiological function of LPS is reflected by activation of the Toll-like receptor 4 (TLR4) signaling pathway on the surface of the host cell membrane [23].

TLRs mediate inflammatory responses via two signaling pathways: NF-κB and mitogen-activated protein kinase (MAPK). Mitogen-activated protein kinase 4 (MKK4) and MKK7 can activate c-Jun N-terminal kinase (JNK) via phosphorylation of Thr-183 and Thr-185 [24]. JNK, a member of the MAPK family, is the downstream receptor of TLR signaling [25]. SUMOylation, a post-translational modification, can change the orientation, stability, and phosphorylation of substrate proteins and plays important roles in the innate immune response. SENP3 is a sensitive protein for oxidative stress and is mainly located in the nucleolus. Conditions of oxidative stress alter the subcellular localization and stability of SENP3, which then accumulates in the cytoplasm, following which the SUMO2/3 modification of the substrate is specifically removed [26]. Studies have suggested that the absence of SENP3 has a significant effect on the activation of TLR4 inflammatory signaling and the production of proinflammatory cytokines in macrophages in LPS-induced in vivo models [27]. In addition, MKK7 is the substrate of SENP3, and SENP3 can enhance the phosphorylation of JNK by removing the SUMO modification of MKK7. Phosphorylated JNK activates the expression of transcription factor activator protein 1 (AP-1) and leads to the release of proinflammatory factors, thereby activating the LPS-induced TLR4 signal pathway [27]. Our previous studies have shown that microglial-specific SENP3 knockout mice do not experienced significant death of DA neurons. Further, after knockdown of SENP3 in primary microglia using siRNA, the transcription and translation levels of various inflammatory factors decreased significantly under LPS
stimulation conditions. Therefore, SENP3 plays an important role in TLR4 inflammatory signaling stimulated by LPS.

Through the analysis of gut flora in patients with postmenopausal osteoporosis, we have found a new subspecies under *Prevotella*, which is a gram-negative anaerobic bacterium similar to *P. histicola strain JCM 15637*. Studies have suggested that the abundance of *Prevotella* in the stool of patients with PD reduced by 77.6%, and that there is a correlation between such decreases in abundance and postural disorders [28]. Treatment with *P. histicola* in encephalomyelitis model mice can reduce damage to the blood–brain barrier and intestinal permeability while inhibiting CNS inflammation [29]. In this study, we further investigated the relationship between gut microbiota and PD in LPS-induced PD model mice treated with intragastric infusions of *P. histicola*. Our findings suggest that *P. histicola* inhibits LPS-induced neuroinflammation via regulation of the TLR4 inflammatory signaling pathway and expression of inflammatory factors.

**Results**

**P. histicola treatment suppresses LPS-induced neuroinflammation and dopaminergic neuron loss in mice**

A mouse model of neuroinflammation and dopaminergic neuron loss was established by injecting LPS into the substantia nigra of the mouse midbrain, and the model mice were then treated with *P. histicola*. First, 8-week-old *C57BL/6* male mice were divided into four groups according to saline or LPS injection into their midbrain and treatment with *P. histicola* bacterial solution or the bacterial culture medium: normal saline + medium group (NS-M), normal saline + *P. histicola* group (NS-PH), LPS + medium group (LPS-M), and LPS + *P. histicola* group (LPS-PH). The mice were gavaged with *P. histicola* or medium on Day 3 after LPS injection. LPS injection in the midbrain activated the microglial TLR4 signaling pathway, resulting in neuroinflammation, a large loss of dopaminergic neurons, and clinical manifestations of PD diseased [16, 27], and the following tests were performed on the 14th day after modeling: open field test (Fig. 1a, 1b), footprint test (Fig. 1c, 1d), rotarod test (Fig. 1e), and tail suspension test (Fig. 1f). These tests were used to assess spontaneous movement, balance ability, and depression-like behavior, respectively. LPS injection altered performance in these tests, although deficits were significantly attenuated by *P. histicola* treatment (Fig. 1b, 1d, 1e, 1f). These results suggest that LPS injection into the midbrain substantia nigra of mice leads to diminished voluntary movement, gait instability, poor balance, and worsening depression, while *P. histicola* treatment ameliorates LPS-induced dyskinesia and depression.

[Figure 1 near here]

To examine the reasons for changes in mouse behavior, we evaluated the number of dopaminergic neurons in the substantia nigra via immunohistochemistry and western blotting using tyrosine hydroxylase (TH) antibody. Our findings indicated that treatment with *P. histicola* inhibited the decrease in
the number of dopaminergic neurons after LPS injection (Fig. 1g). We also examined the expression of the apoptosis marker activated caspase-3 (C-terminal caspase-3) using western blotting. C-terminal caspase-3 expression was increased by LPS stimulation but decreased by \textit{P. histicola} treatment (Fig. 1i,1j, 1k). LPS treatment decreased the number of TH-positive neurons and increased caspase-3 expression, and these changes were rescued by \textit{P. histicola} treatment.

\textbf{\textit{P. histicola} inhibits polarization of microglia towards M1 and promotes polarization towards M2}

Studies concerning the role of \textit{P. histicola} in the pathobiology of multiple sclerosis suggest that treatment with \textit{P. histicola} decreases CNS pathology and reduces microglia activation [30]. Therefore, we examined microglia phenotypes in the midbrain substantia nigra. LPS induced an increase in iNOS expression in microglia (Fig. 2a, 2b, 2f), and numbers of ARG1-positive microglia were increased following \textit{P. histicola} treatment (Fig. 2d, 2e, 2g). These results suggest that \textit{P. histicola} can change polarization of microglia from M1 to M2, thereby inhibiting neuroinflammation. However, the mechanisms by which this occurs remain unknown.

[Figure 2 near here]

\textit{P. histicola} treatment suppresses increases in SENP3 expression and activation of the microglial TLR4 signaling pathway in LPS-treated mice

To investigate the probable mechanisms by which \textit{P. histicola} inhibits CNS inflammation, we focused on the SENP3 and TLR4 signaling pathways. SENP3 can enhance the phosphorylation of JNK by removing the SUMO modification of MKK7, and phosphorylated JNK activates the expression of transcription factor activator protein 1 (AP-1) and leads to the release of proinflammatory factors; consequently, the TLR4 signaling pathway is activated by LPS [27]. Therefore, we examined levels of SENP3 expression and JNK phosphorylation in the substantia nigra. LPS treatment increased SENP3 expression, but \textit{P. histicola} inhibited this increase (Fig. 3a, 3b, 3c). Similarly, LPS treatment increased the level of JNK phosphorylation, which was also inhibited by \textit{P. histicola} treatment (Fig. 3d).

[Figure 3 near here]

We also examined the expression of downstream proinflammatory factors associated with the TLR4 inflammatory signaling pathway. RT-PCR was used to determine mRNA expression levels of IL-1\(\alpha\), IL-6, IL-12, and TNF-\(\alpha\) in the mouse substantia nigra (Fig. 3e, 3f, 3g, 3h, 3i), while enzyme-linked immunosorbent assays (ELISAs) were used to detect levels of IL-1\(\alpha\), IL -6, IL-12, and TNF-\(\alpha\) secretion in the serum (Fig. 3j). The results showed that after inhibiting SENP3 expression and activation of the TLR4 pathway, \textit{P. histicola} significantly inhibited the release and transcription of downstream inflammatory factors. Such reductions in these inflammatory factors may be essential for neuronal survival.

\textbf{SENP3 is a key protease in the activation of the TLR4 signaling pathway in microglia}
To verify the importance of SENP3 expression for the activation of the TLR4 signaling pathway, we performed overexpression (OE) and knockdown of SENP3 in a BV2 cell line. Overexpression and knockdown of SENP3 significantly affected the SUMOylation level of MKK7, the key protein of the TLR4 receptor pathway, and thus affected the phosphorylation level of downstream JNK. The protein levels of free M KK7 and SUMO2/3 were significantly increased upon SENP overexpression (Fig. 4a, 4c), suggesting that SENP3 promoted the de-SUMOylation of M KK7 and further activated the TLR4 signaling pathway. The protein levels of free M KK7 and SUMO2/3 were significantly decreased upon SENP3 knockdown, which inversely verified that SENP3 promotes de-SUMOylation of M KK7 (Fig. 4b, 4d). These results suggest that SENP3 expression is important for the activation of the TLR4 signaling pathway.

P. histicola upregulates the proportion of spleen Treg cells and promotes IL-10 release

When exposed to anti-inflammatory cytokines (such as IL-4, IL-10, and IL-13), microglia develop the anti-inflammatory M2 phenotype [5]. Therefore, we examined levels of IL-10 expression in the mouse serum and substantia nigra. LPS treatment led to a decrease in IL-10 expression (Fig. 5c, 5d); however, IL-10 mRNA and protein levels were significantly increased after P. histicola treatment (Fig. 5c, 5d). Regulatory T cells (Treg cells), which are important for maintaining immune tolerance, can regulate the immune system by proactively modulating the activation and proliferation of self-reactive T cells under physiological conditions [29]. P. histicola can lead to decreases in pro-inflammatory Th1 and Th17 cells, as well as increases in CD4+CD25+FoxP3+ Treg cells and tolerogenic dendritic cells, thereby increasing IL-10 expression [29]. Therefore, we also examined the proportion of spleen Treg cells using flow cytometry. The proportion of Treg cells was decreased following LPS treatment, and this decrease was rescued by P. histicola treatment (Fig. 5a, 5b). These results suggest that P. histicola can inhibit CNS inflammation by upregulating the proportion of spleen Treg cells and increasing the expression of peripheral anti-inflammatory cytokines.

IL-10 significantly inhibits LPS-induced increases in SENP3 expression and activation of the TLR4 signaling pathway

To verify the effect of peripheral anti-inflammatory cytokine expression on SENP3 expression and the TLR4 signaling pathway, microglia were cultured in vitro and stimulated with LPS. After 4 hours of stimulation, IL-10 was administered, and the culture was continued for more than 16 hours. The results indicated that IL-10 significantly inhibited LPS-induced activation of the TLR4 signaling pathway in microglia as well as de-SUMOylation of M KK7. IL-10 also significantly inhibited the increase in SENP3 expression (Fig. 6a, 6b, 6c). These suggest that, depending on the regulation of peripheral anti-inflammatory cytokine expression levels, P. histicola can inhibit LPS-induced increases in SENP3 expression and activation of TLR4 signaling by promoting IL-10 secretion.
**P. histicola treatment suppresses barrier destruction caused by neuroinflammation**

Recently, many studies involving patients with PD have demonstrated a correlation between barrier permeability and the progression/development of PD [31]. CNS inflammation can be transmitted to the intestine via three pathways: blood (based on significant increases in serum inflammatory factors in our previous experimental studies), endocrine, and nerve [32]. However, the manner in which *P. histicola* regulates peripheral levels of anti-inflammatory cytokine expression remains unclear. Therefore, we examined the permeability of different barriers along these pathways. *P. histicola* bacteria were supplemented into mice via gavage. Gut permeability is an indicator of its absorptive function and reflects the integrity of the gut barrier[30, 31]. We also observed the integrity of the blood–brain barrier. Our results indicated that the blood–brain barrier of mice that had received intragastric administration of *P. histicola* bacteria exhibited greater integrity than that in the bacterial negative control group after LPS injection. However, blood–brain barrier integrity remained worse in these mice than in the two groups injected with normal saline (Fig. 7a). This indicates that the activation of the TLR4 inflammatory signaling pathway after LPS injection is the reason for the continued destruction of the blood–brain barrier. However, intragastric administration of *P. histicola* inhibited activation of the TLR4 signaling pathway, thereby reducing the degree of damage to the blood–brain barrier.

Consequently, we also observed disruption of the intestinal barrier (Fig. 7b), and small intestinal villi of the duodenum and jejunum were significantly shortened in LPS-treated mice (Fig. 7c, 7d, 7e, 7f). At the same time, LPS-induced CNS inflammation exerted a major impact on the goblet cells of the intestine, and reduction of mucin may have also promoted damage to the intestinal barrier (Fig. 7g, 7h). However, *P. histicola* inhibited the above-mentioned damage to the intestinal barrier caused by LPS-induced CNS inflammation.

**P. histicola treatment suppresses increases in the intestinal expression of inflammatory substances caused by neuroinflammation**

Damage to the intestinal barrier is closely related to disruption of tight junctions [20, 31]. We found that *P. histicola* inhibited the destruction of jejunal epithelial tight junctions caused by CNS inflammation and maintained the expression of intestinal tight junction stabilizer proteins (Fig. 8a-f). At the same time, electron microscopy revealed that inflammation of the CNS resulted in the destruction of tight junctions in the small intestinal epithelium, while *P. histicola* inhibited such destabilization (Fig. 8g).
We then observed that large number of inflammatory cytokine (IL-1β)-positive cells were produced in the jejunum due to CNS inflammation (Fig. 9a, 9b). Levels of IL-1β and TNF-α transcription in the jejunum were also significantly increased by CNS inflammation (Fig. 9c, 9d, 9e). Therefore, we have reason to suspect that the significant increase in inflammatory factors in the intestine is due to the double disruption of the blood-brain barrier and the intestinal barrier.

SCFAs released by the gut microflora can enhance the intestinal barrier and inhibit inflammation [20]. We further investigated the metabolites of intestinal contents (Fig. 9f), observing that P. histicola treatment upregulated the expression of tetrahydrocannabinol, tryptophan, linolenic acid, thiazolidinedione, bexarotene, and other anti-neuroinflammatory substances (Fig. 9h) while downregulating the expression of neurotoxic substances such as styrene, indan, hypoxanthine, pantothenic acid, linoleic acid, lysophosphatidylcholine 14:1, and lysophosphatidylcholine 22:6 (Fig. 9g). This result suggests that P. histicola treatment can alter the composition of metabolites in the gut, which may explain its ability to inhibit disease in this model of neuroinflammation.

**Discussion**

In this study, we established Parkinsonism with dopaminergic neuron death due to TLR4 activation by injecting LPS into the substantia nigra of the mouse midbrain, and the model mice were then treated with P. histicola. On Day 14 after treatment, the mice were subjected to behavioral experiments to test their motor function. LPS injection led to altered mouse behaviors, such as weakened autonomous exercise ability, unstable gait, poor balance, and depression-like behavior (Fig. 1). Notably, these disorders were significantly improved by P. histicola treatment (Fig. 1). These results suggest that intragastric infusion of P. histicola can improve LPS-induced motor impairments and depression-like behavior in mice. The gut microflora is related to PD and its clinical phenotypes. The stool abundance of *Prevotella* is 77.6% lower in patients with PD than in healthy controls, and such decreases have been correlated with the development of posture disorders [28]. These findings are consistent with our motor behavior results in model mice. Moreover, a previous microflora study indicated that the abundance of *Prevotella* is reduced
in children with autism, suggesting a relationship between *Prevotella* and CNS diseases [33]. Consistently, our results indicated that *P. histicola* treatment significantly inhibited depression-like behavior (Fig. 1).

Loss of dopaminergic neurons is implicated in the motor dysfunctions characteristic of PD. Loss of DA neurons in the LPS + *P. histicola* group was significantly reduced when compared with that in the LPS + medium group (Fig. 1). Significant decreases in TH expression and increases in caspase-3 expression were observed in the LPS + medium group, although these changes were inhibited in the LPS + *P. histicola* group (Fig. 1). These findings indicate that *P. histicola* inhibits LPS-induced loss of DA neurons and thus attenuates decreases in motor ability in mice.

We further investigated changes in microglial phenotypes after LPS stimulation. Our findings indicated that *P. histicola* inhibited activation of M1 microglia (iNOS marker) and promoted activation of M2 microglia (ARG1 marker) (Fig. 2). When exposed to cytokines (such as IL-4, IL-10, and IL-13), microglia develop an anti-inflammatory M2 phenotype. Therefore, we further examined the expression of anti-inflammatory cytokines in the peripheral blood and midbrain. Transcription and secretion of IL-10 was significantly greater in the LPS + *P. histicola* group than in the other groups (Fig. 5). These findings indicate that *P. histicola* treatment promotes the production of anti-inflammatory M2 microglia in LPS-induced neuroinflammation.

To identify the probable mechanism by which *P. histicola* inhibits CNS inflammation, we focused on the SENP3 and TLR4 signaling pathways. SENP3 expression and JNK phosphorylation, which occur downstream of the TLR4 inflammatory pathway, were detected. *P. histicola* inhibited LPS-induced upregulation of SENP3 expression and JNK phosphorylation (Fig. 3, 4). Phosphorylated JNK activates the expression of transcription factor AP-1 and leads to the release of proinflammatory factors. Therefore, we also examined the expression of downstream proinflammatory factors associated with the TLR4 inflammatory signaling pathway. Our findings indicated that the TLR4 pathway was inhibited by *P. histicola* treatment, as demonstrated by reduced transcription and secretion of IL-1α, IL-6, IL-12, and TNF-α (Fig. 3). These findings suggest that *P. histicola* inhibits the activation of the TLR4 receptor signaling pathway. We then performed overexpression and knockdown of SENP3 in a BV2 cell line to verify the importance of SENP3 in the activation of the TLR4 signaling pathway. The expression of SENP3 significantly affected the SUMOylation level of MKK3 and thus affected the phosphorylation level of downstream JNK (Fig. 4). These results suggest that SENP3 plays important roles in the activation of the TLR4 signaling pathway, and that inhibition of TLR4 signaling by *P. histicola* is dependent on the inhibition of SENP3 expression. However, the mechanisms by which *P. histicola* inhibits the expression of SENP3 remain unclear.

*P. histicola* can lead to decreases in the expression of pro-inflammatory Th1 and Th17 cells as well as increases in the expression of CD4 + FoxP3 + Treg cells and tolerogenic dendritic cells, thereby increasing IL-10 expression [29]. Therefore, we also examined the proportion of spleen Treg cells suing flow cytometry. The proportion of Treg cells in the spleen was not affected by *P. histicola* in the mice injected
with saline (Fig. 5). However, the percentage of Treg cells decreased significantly in the LPS-injected group, but this decrease was significantly inhibited in the *P. histicola* group (Fig. 5). These results suggest that *P. histicola* can inhibit CNS inflammation by upregulating the proportion of spleen Treg cells and increasing peripheral levels of anti-inflammatory cytokine expression. Next, we examined the effect of peripheral anti-inflammatory cytokine (IL-10) expression on SENP3 expression and TLR4 signaling. IL-10 significantly inhibited LPS-induced activation of the TLR4 signaling pathway in microglia as well as de-SUMOylation of MKK7 while significantly inhibiting the increase in SENP3 expression (Fig. 6). These findings suggest that, depending on the regulation of peripheral anti-inflammatory cytokine expression levels, *P. histicola* can inhibit LPS-induced increases in SENP3 expression and activation of TLR4 signaling by promoting IL-10 secretion.

To understand how *P. histicola* performs anti-inflammatory functions in the CNS from the peripheral gut, Evans blue and FITC-dextran glycoside were used to examine blood–brain barrier and intestinal permeability in mice. *P. histicola* inhibited LPS-induced damage to the blood–brain barrier and the increase in intestinal permeability (Fig. 7). We also observed that LPS-induced neuroinflammation led to morphologic changes in the small intestine, such as significant shortening of intestinal villi, alterations in goblet cells, and increases in the proportion of IL-1β-positive cells in the jejunum (Fig. 7, 9). Especially in the jejunal epithelium, the expression of tight junction stabilizer proteins was decreased, and the morphology of tight junctions was altered (Fig. 8). These LPS-induced morphologic changes in the small intestine were rescued by treatment with *P. histicola* (Fig. 7, 8).

SCFAs can enhance the intestinal barrier and inhibit inflammation [20]. The metabolites of intestinal contents indicated that *P. histicola* treatment up-regulated the expression of anti-neural inflammatory substances, and down-regulated neurotoxic substances (Fig. 9). We also performed 16s RNA Sequencing to investigate the abundance of the gut microbiota. We observed that LPS-induced neuroinflammation led to an increase in the dispersion of gut microbiota, while *P. histicola* corrected this alteration (Fig. 10). This result suggests that *P. histicola* treatment can aid in inhibiting disease development in an LPS-induced model of neuroinflammation.

**Conclusions**

Our findings demonstrate that *P. histicola* treatment can attenuate disturbances in the gut microbiota, thereby altering the composition of intestinal metabolites and promoting the inhibition of neuroinflammation. Such treatment can also alleviate damage to the intestinal barrier due to CNS inflammation, preventing the invasion of foreign inflammatory factors. At the same time, our results suggest that *P. histicola* treatment stimulated spleen FoxP3+ Treg cells to secrete a large amount of IL-10 in the serum under the joint action of inflammation, which inhibited the activation of the microglial TLR4/SENP3/MKK7 signaling pathway caused by LPS injection. Inhibition of this pathway reduced neuroinflammation in the substantia nigra region of the midbrain, resulting in reduced loss of dopaminergic neurons. Therefore, our findings suggest that administration of *P. histicola* attenuated impairments in movement and depression-like behavior in LPS mice. Thus, *P. histicola* inhibits
neuroinflammation caused by activation of the TLR4 pathway by promoting the release of IL-10 from FoxP3 + Treg cells.

**Materials And Methods**

**Animal experiments**

The experimental animals used in this experiment were 8-week-old adult male C57BL/6 mice (purchased from Beijing Fox Company, clean grade), body weight (25 ± 3g). The animals were fed adaptively for 1 week. During the whole experimental process, the temperature of the animal rearing environment was controlled at 22 ± 3°C, the relative humidity was controlled at 55% ± 5%, the daily light time was 12 hours, and the water and food were free.

**Cultivation of P. histicola**

Under aseptic conditions, a culture tube filled with nitrogen containing 5% CO₂ was oven-dried under high-pressure for 30 s, following which it was covered with a latex plug to render it suitable for anaerobic cultures. The bacterial culture medium was prepared and placed into a sealed box with an anaerobic gas-generating bag, and anaerobic treatment was performed for 24 hours. The strains were implanted in an anaerobic culture tube, to which anaerobic culture medium had been added under aseptic operation, and the culture tube was then incubated at 37°C for 24 hours.

**Surgery**

After preparing the skin and local disinfection with iodophor, the skin of the mouse head was cut from the posterior to anterior sagittal line, the parietal bone was exposed, and the bregma was identified. The tip of a 2-µl microinjection needle was used to mark the bregma point as the coordinate zero points (0, 0, 0); the coordinates were then adjusted to 1.30 mm, -3.0 mm, and 0 mm and marked as such, following which holes were made using a skull drill. The coordinates were then adjusted to 1.30 mm, -3.0 mm, and −4.50 mm. A microinjection pump was used to inject 2 µl of LPS (2 µg/µl) or the same dose of saline as a control at an injection speed of 0.2 µL/min. After the injection, the injection needle was maintained in the brain for 10 minutes, following which it was slowly removed. Iodophor disinfection, fixation with dental cement, and skin suturing were performed.

**Open-field test**

The adapted mice were placed into an open field with a diameter of 0.5 m, and a Sony camera was used to record the activities of the experimental mice in the open field for 120 s. The camera was connected to XeyeAba3.2 software to automatically analyze the total movement. Distance, exercise time, static time, and average speed were recorded and analyzed for each mouse.

**Footprint analysis**
To assess motor function in the forelimbs and hindlimbs after establishing the PD model, footprint analysis was performed as described previously [34]. Briefly, after each forelimb and hindlimb was respectively brushed with red and blue nontoxic ink, the animals were required to run along a paper-lined runway (3 feet long, 3 inches wide) with a darkened box at the end. The gait of each mouse was measured and quantitatively analyzed.

**Rotarod performance**

To evaluate balance, grip strength, and motor coordination, the animals were placed on a rotary rod fatigue tester (Anhui Zhenghua Biological Instrument Equipment, YLS-10A) with six channels, which was set to 30 rpm over 5 min. One mouse was placed in each channel for testing (six at a time), and the mice were allowed to adapt to the rotating rod for 5 min. The time spent on the rod before falling was recorded for each mouse.

**Immunohistochemical staining**

After prefusion with 0.1 mol/L PBS followed by 4% paraformaldehyde (PFA), the brains were collected and immersed in 4% PFA for 24 h, following which they were transferred to 30% sucrose solution until immersion. Subsequently, the brains were cut into 30-µm-thick transverse and horizontal sections using a freezing microtome (Thermo, USA). For staining of brain tissue sections, the antigens were repaired for 6 min at 96°C using Sodium Citrate Antigen Retrieval Solution (Solarbio). Subsequently, the sections were treated with 3% hydrogen peroxide-methanol solution for 15 min at room temperature to block endogenous peroxidases, followed by overnight incubation with primary antibodies at 4°C. They were subsequently washed three times with PBS and were then incubated for 1 h at room temperature with appropriate secondary antibodies (1:1, 000, Invitrogen).

**Western blotting**

Substantia nigra tissues were lysed in ice-cold RIPA Buffer (P0013B, Beyotime) with 100 mM NaF, 100 mM Na3VO4, and 100 mM PMSF at 4°C for 30 min, and the sample was mixed with 2 × loading buffer. The proteins were boiled at 96°C for 5 min. The samples were separated using 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto nitrocellulose membranes (Life Sciences, USA). After blocking in 5% skim milk for 2 h, the membranes were incubated with different primary antibodies overnight at 4°C. Subsequently, the membranes were washed three times and were then incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies (1:5, 000, Pierce) for 2 h. ImageJ software was used to analyze the western blotting results.

**Reverse transcription-polymerase chain reaction (RT-PCR)**

Total RNA was extracted from the substantia nigra using TRIzol™ reagent (#15596026, Ambion) in accordance with the manufacturer’s instructions. RNA was reverse transcribed into cDNA using the SuperScript™ One-Step Reverse Transcription Kit (#10928-034, Invitrogen, CA, USA). After adding the corresponding parrot, PCR was performed on the cDNA. After the product was subjected to agarose gel
electrophoresis, the results were analyzed using ImageJ (Version 1.51n). We then measured transcription levels of IL-1α, IL-6, IL-12, and TNF-α mRNA using the following primers:

b-actin: 5′-3′: GACTGTGCCCATCTACGA
3′-5′: CCATCTCCTGCTCGAAG

IL-1β: 5′-3′: CGCTTGAGTCGGAAAGAATA
3′-5′: CTTCCCGTTGCTTGACGGTTG

TNF-α: 5′-3′: CGAGTGACAAGCCTGTAGC
3′-5′: TACTTGGGCAGATTGACCTCA

IL-6: 5′-3′: CCAAATCTAGTTTGCCGAGTAGATCT
3′-5′: AACAAGGATAGTTTGACGGTTG

IL-12: 5′-3′: AACCTCACCTGTGACAGGCT
3′-5′: CAAGTCCATGTTTCTTGCACC

Double-immunofluorescence staining

Frozen sections of mouse brain tissues were rinsed three times with PBS for 5 min each, following which they were incubated in citrate buffer at 95°C for 6 min for antigen retrieval and were then rinsed with PBS three times (5 min each time). These sections were incubated with primary antibodies overnight at 4°C and were then rinsed with PBS three times (5 min each time), followed by incubation with fluorescent secondary antibodies for 1 h at room temperature. After rinsing three times with PBS (5 min each time), these sections were mounted on slides with mounting medium and observed under a fluorescence microscope.

Enzyme-linked immunosorbent assay

Each sample was determined according to its own quantity, and multiple wells were used for each sample. The protocol was as follows: For blank wells/blank control wells without samples, biotin-labeled corresponding antibody, streptavidin-HRP, and chromogenic reagent A&B and stop solution were added, although other steps were the same. For standard wells, 50 µl standard and 50 µl streptavidin-HRP were added (biotin antibody was integrated in the standard, so was not added). For sample wells to be tested, 40 µl sample was added, followed by 10 µl of the corresponding primary antibody and 50 ul streptavidin Avidin-HRP, followed which the wells were covered with sealing film. Samples were subjected to gentle shaking and were incubated at 37°C for 60 min. Liquid preparation was performed by diluting the 30-fold concentrated washing liquid by 30-fold with distilled water before use. For washing, the sealing film was carefully removed, the liquid was discarded, and the samples were subjected to spin drying. Each well was filled with washing liquid, which was discarded after 30 s. This process was repeated five times, and samples were pat dry. Color development was achieved by adding 50 µl of developer A and 50 µl of
developer B to each well, which were shaken gently to mix. Samples were then incubated at 37°C for 15 min in the dark. To stop reactions, 50 µl stop solution was added to each well (at this time, color shifts from blue to yellow).

**Evans blue staining for investigating the permeability of the blood–brain barrier**

Evans blue was injected into the tail vein 1 min before the mice were sacrificed. After sacrifice, the brains were mashed in dimethylamine and incubated at 60°C overnight, and the supernatant was then collected on the next day to measure the absorbance of each well at a wavelength of 620 nm.

**Molecular fluorescence probe method for detecting intestinal permeability**

Mice were administered an intragastric injection of 10% FITC-dextran with a molecular weight of 4,000 kDa 4 h before sacrifice. Blood was taken from the orbit immediately after the mouse was sacrificed by cervical dislocation. After incubation at room temperature for 30 min, the blood samples were centrifuged at $1.6 \times 10^3$ rpm for 5 min at room temperature. The serum was collected and placed in a 96-well plate to detect the fluorescence intensity of each well at an excitation wavelength of 630 nm using a multifunctional microplate reader.

**Spleen Treg cell flow cytometry**

A total of 100 µl anticoagulant blood or $1–10 \times 10^6$ splenocytes were pipetted into test tubes, and 5 µl Anti-Mouse CD4, FITC, 5 µl Anti-Mouse CD25, and APC were then added to each tube. The tubes were vortexed to ensure thorough mixing and were incubated at room temperature for 15 min in the dark. Then, 2 mL of 1× FCM Lysing Solution (work solution) was added to each tube and pulse vortexed, followed by incubation at room temperature for 15 min in the dark. The tubes were centrifuged at 300–400 × $g$ for 5 min at room temperature, and the supernatant was discarded. The, 2 ml 1× Flow Cytometry Staining Buffer was added, and the tubes were pulse vortexed. After centrifugation at 300–400 × $g$ for 5 min at room temperature, the supernatant was discarded. Subsequently, 1 ml of Fixation/Permeabilization working solution was added, and the tubes were pulse vortexed. After incubation for 30–60 min at room temperature in the dark, without washing, 2 ml of 1× Permeabilization Buffer was added to each tube. The tube was centrifuged at 300–400 × $g$ for 5 min at room temperature, and the supernatant was then discarded. The pellet was resuspended in 100 µl of 1× Permeabilization Buffer (a typically residual volume after decanting). After incubation for 15 min at room temperature in the dark, without washing, 5 µl anti-mouse Foxp3 or 5 µl mouse IgG1 isotype control was added. After vortexing to ensure thorough mixing, the tube was incubated for at least 30 min at room temperature in the dark. After adding 2 ml of 1× Permeabilization Buffer to each tube and centrifugation at 300–400 × $g$ for 5 min at room temperature, the supernatant was discarded. Cells were resuspended in 500 µL of 1× Flow Cytometry Staining Buffer and analyzed on a flow cytometer.

**SENP3 knockdown**
All small interfering RNAs were purchased from MICRO-G BIOTECK company (Shanghai, China). According to the manufacture's protocol, BV2 microglia were seeded at 105 cells/well. 20 µM of siRNA was added to serum-free DMEM and then mixed with 5 µl of Lipo3000 transfection reagent. After incubation at room temperature for 15 min, the mixture was added to cells and cultured for 48 h.

siRNA-control: 5′-3′:UUCUCGAACGUGUCACGUTT,
3′-5′:ACGUGACACGUUCGGAGAATT

si-SENP3: 5′-3′:GGAUGCUGCUCUACUCAAAdTdT
3′-5′:UUUGAGUAGAGCAGCAUCCdTdT

**SENP3-containing plasmid transfection**

The SENP3 plasmids were got from Professor Jing Yi (Shanghai Jiaotong University, China). For transfection, microglia were grown in Dulbecco’s Modified Eagle’s Medium (Gibco), supplemented with 10% FBS (Gibco) and 1% penicillin/streptomycin (Gibco) in a 5% CO2 incubator at 37°C. Appropriate plasmids were transfected into the cells using Lipofectamine™ 3000 Transfection Reagent 4ul per 1ug of plasmid (L3000-015, Invitrogen) according to the manufacturer’s instructions.

**Establishment of IL-10-treated cell model**

BV-2 cells were evenly plated in 6-well plates at 1 million cells per well (cultured in 10% FBS in DMEM). 100 ng IL-10 was administered 1 hour after the administration of 100 ng per mL of LPS. The protein was extracted after an additional 3 hours.

**Histological of intestinal inflammation**

Upon sacrifice, proximal duodenal, jejunal, ileal and colon tissue were harvested and fixed in 10% phosphate-buffered formalin. These samples were embedded in paraffin, sectioned at 4 µm, and stained with H&E or PAS for light microscopy examination. The slides were reviewed in a blinded fashion by a pathologist and were assigned a histological score for intestinal inflammation.

**Transmission Electron Microscopy of tight junction**

Segments of proximal jejunum (2 cm each) were collected, fixed in 2.5% glutaraldehyde–2% paraformaldehyde solution (2 h; 4°C), rinsed in 0.1 mol/L cacodylate buffer, and then postfixed for 1 h at 4°C in 1% osmium tetroxide. Subsequently, tissue samples were dehydrated in graded ethanol and embedded in Epon-Araldite resin. Ultra-thin sections (70 nm) were obtained with an ultramicrotome system (Reichert O MU2) and collected on copper/palladium grids after staining with 4% uranyl acetate and 0.4% lead citrate. To evaluate changes in TJ morphology, the junctional regions of three randomly selected sections of longitudinally sectioned villi were examined for each specimen. Sections were examined with a Hitachi JEM1200-EX electron microscope. In the gut epithelial cells, open tight junctions
(TJs) appeared as a dilatation or a gap in the apical space of the junctional complex. Examinations were performed with a total of 6 TJs per specimen.

**Metabolomics data**

In a clean bench, the intestinal contents from the colon segment to the rectum segment of the euthanized mouse were taken. Send the contents to Lianlian Biotechnology Co., Ltd. for testing.

**Stool Sample Processing and DNA Extraction**

The fecal samples collected were frozen immediately in liquid nitrogen and stored at -80°C until analysis. Fecal genomic DNA was extracted from the fecal samples with the QIAamp® DNA Stool Mini Kit (Qiagen, Hilden, Germany), according to the manufacturer's protocol. The concentration and purity were detected through the Nanodrop, and the integrity was detected through regular 0.8% agarose gel electrophoresis.

**High throughput sequencing**

The bacterial genomic DNA was used as the template to amplify the V3–V4 hypervariable region of the 16S rRNA gene with the forward primer (5-CCTACGGGNGGCWGCAG-3) and the reverse primer (5-GACTACHVGGGTATCTAATCC-3). Each sample was independently amplified three times. Finally, the PCR products were checked by agarose gel electrophoresis, and the PCR products from the same sample were pooled. The pooled PCR product was used as a template, and the index PCR was performed by using index primers for adding the Illumina index to the library. The amplification products were checked using gel electrophoresis and were purified using the Agencourt AMPure XP Kit (Beckman Coulter, CA, USA). The purified products were the indexed in the 16S V3–V4 library. The library quality was assessed on the Qubit@2.0 Fluorometer (Thermo Scientific) and Agilent Bioanalyzer 2100 systems. Finally, the pooled library was sequenced on an Illumina MiSeq 250 Sequencer for generating 2×250 bp paired-end reads.

**Bioinformatics and statistical analysis**

The raw reads were quality filtered and merged with the following criteria: (1) Truncation of the raw reads at any site with an average quality score < 20, removal of reads contaminated by adapter and further removal of reads having less than 100 bp by TrimGalore, (2) The paired end reads are merged to tags by Fast Length Adjustment of Short reads (FLASH, v1.2.11), (3) Removal of reads with ambiguous base (N base) and reads with more than 6 bp of homopolymer by Mothur, (4) Removal of reads with low complexity to obtain clean reads for further bioinformatics analysis. The remaining unique reads were chimera checked compared with the gold.fa database (http://drive5.com/uchime/gold.fa) and clustered into operational taxonomic units (OTUs) by UPARSE with 97% similarity cutoff. All OTUs were classified based on Ribosomal Database Projet (RDP) Release9 201203 by Mothur. Rarefaction analysis and alpha diversities (including Shannon, Simpson and InvSimpson index) were analyzed by Mothur. Sample tree cluster by Bray-Curtis distance matrix and unweighted pair-group method with arithmetic means (UPGMA) and Jaccard principal coordinate analysis (PCoA) based on OTUs were performed by R Project (Vegan package, V3.3.1). Redundancy analysis (RDA) was analyzed by Canoco for Windows 4.5 (Microcomputer Power, NY, USA), which was assessed by MCPP with 499 random permutations.
Antibodies information

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Statistical analysis

All data were analyzed using Prism5 software. The experimental results are expressed as the mean ± standard deviation (X ± S), and all data presented represent results from at least three independent experiments. Student's unpaired two-tailed t-test or analyses of variance (ANOVAs) with pairwise comparisons were used to compare data among the groups. Statistical significance was defined as P < 0.05.

Declarations

Ethics approval and Consent to participate
The experimental protocol was established, according to the ethical guidelines of the Helsinki Declaration and was approved by the Ethics Committee of Wenzhou Medical University (Ethics number: wydw 2021-0316).

**Consent for publication**

Not applicable.

**Availability of data and materials**

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

**Conflicts of 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.

**Funding**

This study was supported by Natural Science of the Zhejiang Province Foundation of China [Grant number: LY20H090010].

**Authors' contributions**

ML was involved in project design and data analysis; DJJ, LSM, GYL, ZT, ZWH performed the major experimental work; ZSY, XZT and RYH wrote the paper; LQX CWG and ZP revised the manuscript.

**Acknowledgments**

The authors thank Professor Lei Chen from the Department of Orthopedics of the First Affiliated Hospital of Wenzhou Medical University for providing *Prevotella histicola*.

**References**


Figures
Figure 1

*P. histicola* treatment suppresses LPS-induced neuroinflammation and dopaminergic neuron loss in mice (n=6). (a) Movement trajectory diagram for the open-field experiment. (b) Statistical diagram for movement trajectory length in the open-field experiment. (c) Footprint experiment results. (d) Footstep distance in the footprint experiment. (e) Rotarod times. (f) Tail suspension times. (g) TH immunohistochemical staining in the mouse substantia nigra. (h) TH-positive cells in the mouse...
substantia nigra. (i) Western blotting results for TH and C-terminal caspase-3. (j) Gray values for TH western blotting. (k) Gray values for C-terminal caspase-3 western blotting. NS-M: normal saline + medium group; NS-PH: normal saline + *P. histicola* group; LPS-M: LPS + medium group; LPS-PH: LPS + *P. histicola* group. *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$, ns: not significant. LPS: lipopolysaccharide; TH: tyrosine hydroxylase.
*P. histicola* inhibits polarization of microglia towards M1 and promotes polarization towards M2 (n=4). (a) Results of Iba1 and iNOS dual-label immunofluorescence staining in each group. (b) Iba1 fluorescence intensity in each group. (c) iNOS fluorescence intensity in each group. (d) Results of Iba1 and ARG1 dual-label immunofluorescence staining in each group. (e) ARG1 fluorescence intensity in each group. (f) Western blotting and quantitative results for iNOS protein in each group. (g) Western blotting and quantitative results for ARG1 protein in each group; *: P< 0.05, **: P< 0.01, ***: P< 0.001, ns: not significant. iNOS: inducible nitric oxide synthetase; ARG1: arginase 1.
Figure 3

*P. histicola* treatment suppresses increases in SENP3 expression and activation of the TLR4 signaling pathway. (a) Immunofluorescence staining to detect dual labeling for Iba1 and SENP3 in the substantia nigra of each group. (b) SENP3 fluorescence intensity in each group; (c) Western blotting and gray values for SENP3 expression in the substantia nigra of each group. (d) JNK phosphorylation levels and gray values in the substantia nigra of each group. (e) Transcription levels of IL-1α, IL-6, IL-12, TNF-α in the substantia nigra of each group. (f-i) Gray values for IL-1α, IL-6, IL-12, and TNF-α transcription level. (j) Serum concentrations of IL-1α, IL-6, IL-12, and TNF-α; *: *P* < 0.05, **: *P* < 0.01, ***: *P* < 0.001, ns: not significant.

Figure 4
SENP3 is a key protease in the activation of the TLR4 signaling pathway. (a) Levels of MKK7, SUMO2/3, and p-JNK expression after SENP3 overexpression (Flag-SENP3-OE). (b) Levels of MKK7, SUMO2/3, and p-JNK expression after SENP3 knockdown and LPS or LPS+IL-10 treatment; (c) Gray values for SENP3 overexpression. (d) Gray values for SENP3 knockdown and LPS or LPS+IL-10 treatment. *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$, ns: not significant. LPS: lipopolysaccharide.

Figure 5

. histicola upregulates the proportion of spleen Treg cells and promotes IL-10 release. (a) Schematic diagram of CD4+ CD25+ FoxP3+ positive flow cytometry in the spleen for each group. (b) Results for CD4+ CD25+ FoxP3+ positive flow cytometry in each group. (c) Serum IL-10 concentration in each group. (d) IL-10 transcription level and gray values in the mice substantia nigra for each group; *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$, ns: not significant.
Figure 6

IL-10 significantly inhibits LPS-induced increases in SENP3 expression and activation of the TLR4 signaling pathway. (a) After LPS or LPS+IL-10 treatment of microglia in vitro, the expression levels of proteins in the TLR4 signaling pathway and microglial phenotype markers in each group. (b) Microglia immunofluorescence staining for Iba1, SENP3, and DAPI after LPS or LPS+IL-10 treatment in vitro. (c, d)
Gray values for Figure a; *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$, ns: not significant. LPS: lipopolysaccharide.

Figure 7

*P. histicola* treatment suppresses barrier destruction caused by neuroinflammation. (a, b) Blood-brain barrier and intestinal permeability in each group. (c, e) Hematoxylin and eosin staining results for the
duodenum and jejunum in each group. (d, f) Villus length in the duodenal and jejunal epithelium in each group. (g) PAS staining in each group. (h) Statistical results for PAS-positive staining. *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$, ns: not significant.

Figure 8
*P. histicola* treatment suppresses tight junction destruction caused by neuroinflammation. (a, b) Immunofluorescence staining for tight junction proteins occludin and zonula occludens 1 (ZO-1) in the jejunum of each group. (c) Immunofluorescence intensity for occludin. (d) Levels of occludin and ZO-1 expression in the jejunum of each group. (e, f) Statistics results for occludin and ZO-1. (g) Electron microscopy results for tight junctions in each group; *: \( P < 0.05 \), **: \( P < 0.01 \), ***: \( P < 0.001 \), ns: not significant.
Figure 9

*P. histicola* treatment suppresses the increase in intestinal inflammatory substances caused by neuroinflammation. (a) Results of IL-1β immunofluorescence staining in the jejunum of each group. (b) Statistical results for IL-1β positivity in the jejunum of each group. (c) Transcription levels of IL-1β and TNF-α in the jejunum of each group. (d, e) IL-1β and TNF-α transcription levels. (f) Metabolomic analysis of intestinal contents after *P. histicola* treatment in LPS-induced inflammatory model mice. (g) Statistical results for anti-inflammatory substances in the metabolomic analysis. (h) Statistical results for pro-inflammatory substances in the metabolomic analysis; *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$, ns: not significant.
Figure 10

*P. histicola* treatment corrects the destabilization of gut microbiota caused by neuroinflammation. (a) Absolute quantification heat map of various bacterial species obtained via 16sRNA sequencing of the intestinal contents in each group. (b) Differences in the intestinal flora in each group. (c) Correlation predictions for various bacteria. (d) The composition of the intestinal flora in each group.