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Effect of *Inonotus obliquus* polysaccharide on composition of the intestinal flora in mice with acute endometritis

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Abstract

Inonotus obliquus Polysaccharide (*IOP*) is a large molecule extracted from *Inonotus obliqus*, a medicinal fungus, which has a wide range of biological activities and has been shown to be associated with inflammation. The purpose of this study is to investigate whether IOP can help to reduce acute endometritis by regulating intestinal flora. We observed pathological changes in mice with endometritis following treatment with IOP and evaluated changes in the levels of interleukin-6 (IL-6), interleukin-1β (IL-1β) and tumor necrosis factor α (TNF-α), and further studied the effects of IOP on the intestinal flora of endometritis mice using 16S rRNA high-throughput sequencing. The results showed that IOP improved the condition of uterine tissues and reduced the release of pro-inflammatory cytokines. Meanwhile, the 16S rRNA sequencing results showed that IOP could regulate the changes in intestinal microflora at the level of genera, possibly by changing the relative abundance of some genera.

Introduction

Endometritis is an infectious uterine disease that is closely related to infertility [1, 2]. According to histological criteria, endometritis can be divided into acute, chronic and fibrosis subtypes. The histological features of acute endometritis are congenital and intrahepatic polynuclear infiltration [1]. The cause of acute endometritis is bacterial infection [3], including *Escherichia coli*, *Staphylococcus aureus*, or *Streptococcus Lipopolysacchride* (LSP) in the cell wall of Gram-negative bacteria plays an important role in inflammation [4–6].

There are rich interactions between microorganisms and hosts [7]. Under normal conditions, large numbers of bacteria form a microbial barrier to protect the intestinal tract in order

to maintain gastrointestinal stability and resist the invasion of pathogenic bacteria [8]. Generally speaking, the host and enteroviruses exist in a dynamic equilibrium, which if disrupted can lead to various diseases. There is growing evidence that disequilibrium of intestinal flora can contribute to the development of diabetes [9], joint inflammation [10], hepatitis [11], and neuroinflammation [12]. In addition, some studies have shown that an imbalance of enteroviruses can lead to increased estrogen [13], which is closely related to inflammation [14]. Therefore, the regulation of estrogen by intestinal flora may be related to the development of endometritis in the uterus.

Natural polysaccharides are associated with a wide range of biological effects and can provide therapeutic value by directly affecting metabolism *in vivo*, the polysaccharides concentrate symbiotic bacteria to form a biological barrier to protect the host from pathogens [15, 16]. In addition, they can change the composition of rose organisms such as *Glycyrrhiza Uralensis* Fisch Polysaccharides (*GCP*) that affect can inhibit tumor growth *in vivo* [17]. Pumpkin polysaccharide (*PP*) reduces the pathogenesis of type II diabetes in rats by regulating intestinal flora [18]. *Inonotus obliqus* is an edible and medicinal fungi that grows in frosty conditions in Asia and Europe and has been used as folk medicine in Russia to treat tumors and stomach ulcers. Furthermore, it has also been used to treat and prevent cancer, diabetes, cerebrovascular diseases and other diseases in Europe [19–21]. The polysaccharide components of *Inonotus obliqus* have an extensive range of biological characteristics such as the anti-inflammatory, anti-oxidative and anti-viral activity [22–24]. Previous research has found that *Inonotus obliquus* polysaccharide (*IOP*) may improve chronic pancreatitis (*CP*) in mice and promote the intestinal flora at the same time [25]. However, the potential role of IOP for the treatment of endometritis has not been studied, and the correlation between endometritis and intestinal flora has not been confirmed.

To investigate the relationship between endometritis and intestinal flora, pathological changes in mice with endometritis treated with IOP and the levels of IL-6, IL-1β and TNF-α were observed. We also performed an additional study to analyze the effect of IOP on intestinal flora in mice with endometritis using 16S rRNA high-throughput sequencing.

Materials and methods

Materials

Inonotus obliqus was provided by the Veterinary medicine laboratory of People's Friendship University of Russia. Phosphate buffered saline (PBS) was purchased from Beijing Labgic Technology Co. (Beijing, China), and lipopolysacchride (LPS) was purchased from Sigmal-Aldrich (USA).

The extraction of IOP

IOP was extracted according to traditional methods using hot water extraction followed by centrifugation [26]. Briefly, The IOP was crushed and the powder was degreased with petroleum ether. After degreasing, the residue is dried at low temperature. Heat the defatted residue and boil it with distilled water, repeat three times. The filtrates were combined, the solvent was recovered by reduced pressure, and 1% trichloroacetic acid was added to precipitate the protein. After centrifugation, the filtrate is concentrated into a liquid extract form. After precipitation with absolute ethanol, it was placed at 0°C overnight. The alcohol precipitation solution is centrifuged by a high-speed centrifuge to obtain a crude polysaccharide precipitate. The precipitate is washed 2–3 times with a small amount of absolute ethanol to obtain a crude polysaccharide product.

Experimental processing

The animal study protocol was approved by the Animal Care Office of Chengdu Normal University, Chengdu, China and complied with the ARRIVE guidelines and followed the National Institutes of Health guide for the care and use of Laboratory animals.

SPF BALB/C female mice were purchased from Dossy Experimental Animals Co. (Chengdu, China). The mice were housed in a settled environment (temperature: 25±3˚C, humidity: 75±5%) with adequate food and water. The mice were exposed to light for 12h each day. The mice were randomly divided into four groups with twelve mice in: Control group; LPS group; LPS+IOP group. To avoid infection by other bacteria, mice were pretreated with streptomycin [27]. A murine model of LPS-induced endometritis was established as previously reported [28]. LPS group injected with 20ul LPS (3mg/ml) in vivo [29, 30]. The control group were injected with the same amount of PBS, After 3 h, the LPS+IOP group were given IOP orally (150 mg/kg) [30]. The mice were monitored every hour for temperature, vaginal bleeding or death. During this process, none of the mice exhibited morbidity. After 9 h, the mice were euthanized, and uterine and colorectal tissues were collected and stored at -80˚C.

Histopathology analysis

Uterine tissue was fixed by paraformaldehyde followed by trimming, dehydration, and embedding in paraffin. The 5 μm thick slices were dyed using hematoxylin and eosin (H&E) before visualization under a microscope (Nikon, Eclipse Ci-L, Japan).

Inflammatory cytokine detection

The expressions of interleukin (IL)-6, IL-1β and TNF-α were detected by quantitative real-time polymerase chain reaction (PCR). According to the manufacturer's instructions, total RNA was extracted from the tissue of the uterus using MiniBEST Universal RNA Extraction Kit (Takara, Japan) and reverse transcribed into cDNA. The cDNA product was diluted with Fast qPCR Master Mix (High Rox, BBI, ABI) on a StepOne Plus fluorescent quantitative PCR instrument (ABI, Foster, CA, USA). Primers (Table 1) were designed using Primer Premier 5.0 software, and relative quantification of target gene expression was performed using the $2^{-\Delta\Delta Ct}$ method.

DNA extraction and library construction

Total genomic DNA from colorectal tissue was extracted using QIAamp 9 PowerFecal QIAcube HT kit (QIAGEN, 51531) according to the manufacturer's instructions. Concentration of DNA was verified using a NanoDrop (Thermo Fisher, 2000) and agarose gel electrophoresis.

Table 1. Primers for qPCR.

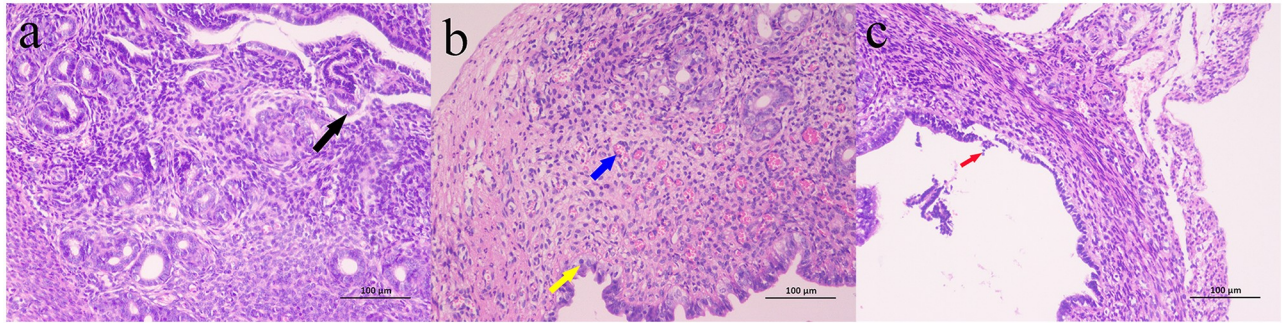| Name	Primer sequence	Product size(bp)
IL-6	Forward: TCTTGGGACTGATGCTGGTG	132
	Reverse: CATGTGTAATTAAGCCTCCGACT	
IL-1β	Forward: GTAATGAAAGACGGCACACCC	181
	Reverse: CAGGCTTGTGCTCTGCTTGTG	
TNF-α	Forward: TGTCTCAGCCTCTTCTCATTCC	152
	Reverse: TTTGTGAGTGTGAGGGTCTGG	
GAPDH	Forward: GGTTGTCTCCTGCGACTTCA	183
	Reverse: TGGTCCAGGGTTTCTTACTCC	

Fig 1. Effect of IOP on the histopathology of endometritis. (a). Control group. Local exfoliation of endometrial epithelial cells (black arrow). (b). LPS group. A small number of endometrial and glandular epithelial cells were swollen, the cytoplasm was loose and light stained (yellow arrow), and the lamina propria was heavily congested and dilated (blue arrow). (c). LPS+IOP Group. Local endometrial epithelium narrowed and a few endometrial epithelial cells shed (red arrow). (Hematoxylin and eosin staining, magnification 200 ×).

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The genomic DNA was used as template for PCR amplification with the barcoded primers and Tks Gflex DNAPolymerase (Takara, R060B). According to bacterial diversity analysis, V3-V4 variable regions of 16S rRNA genes was amplified with universal primers 343 F and 798 R (343F: 5′– `TACGGRAGGCAGCAG` –3′,798R: 5′–`AGGGTATCTAATCCT` –3′ [31]). The quality of amplifiers was confirmed by gel electrophoresis, purified by AMPure XP bead (Agencourt), followed by another round of PCR amplification. After purification of the AMPure XP bead, Qubit dsDNA analysis kit (Life Technologies, Q32854) was used to quantify the final amplitor. An equal number of purified amplicon were pooled for subsequent sequencing.

Bioinformatics analysis

Paired end—reads were preprocessed with Trimmomatic software [32], pruned and assembled with FLASH software after trimming [33]. The assembly parameters were as follows: minimum overlap 10 bp, maximum overlap 200 bp, and maximum error ratio 20%. Homologous sequences or those less than 200 bp were abandoned. In total, 75% of base readings above Q20 were retained. Then, the readings with the chimera were detected and removed. These steps were implemented using QIIME software (version 1.8.0) [34]. Vsearch software was used to generate operational taxonomic units (OTU) by removing primer sequences and clustering with a cut-off value of 97% similarity [35]. A representative reading for each OTU was selected using the QIIME package. All representative reads were annotate in line with the Silva database version 123 using the RDP classifier (confidence threshold was 70%) [36].

Statistical analysis

Statistical analyses were performed using GraphPad Prism 8 (GraphPad InStat Software, USA). Comparison between groups was performed using one-way ANOVA, and data were expressed as mean±SEM. $P < 0.05$ was considered statistically significant.

Results

IOP influences LPS-induced uterine inflammation

Compared with the control group, the lamina propria of the uterine tissues under LPS induction were swollen with a large number of capillary congestion and enhanced with eosinophils
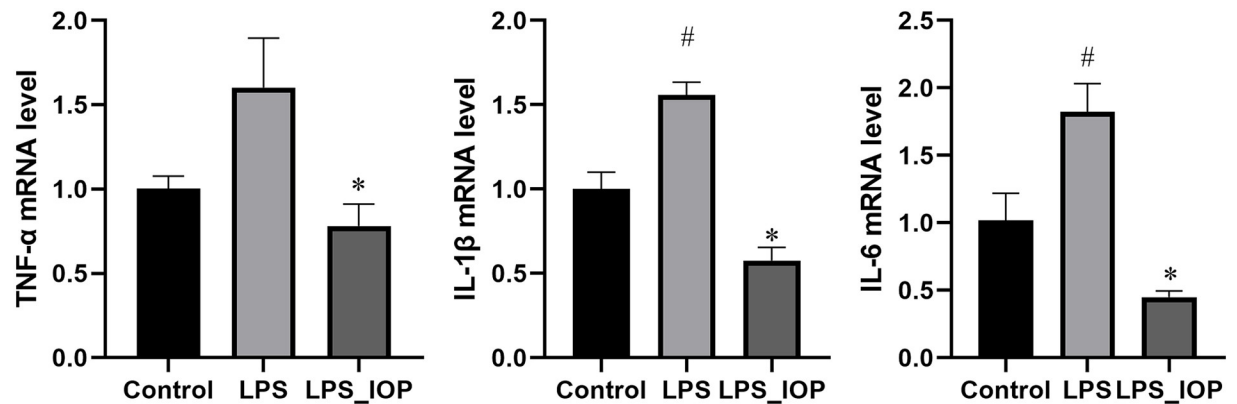
Fig 2. Effects of IOP on inflammatory cytokines TNF-α, IL-6, IL-1β. Mean ±SD was used for data processing. Three replicates were processed in each group. #$P < 0.05$ vs. Control group, *$P < 0.05$vs. LPS group.

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which improved after IOP treatment (Fig 1). Meanwhile, the levels of IL-6, IL-1β and TNF-α in uterine tissue were increased after LPS induction and decreased after IOP treatment (Fig 2).

The total structure of the gut microbiota

After quality control of the original data obtained from high-throughput sequencing, the Clean Tags were distributed between 85767 and 93865 The Clean Tags obtained through the removal of chimera relative to the valid Tag data were distributed between 78943 and 86592 and the final total OUT number was 6143 (S1 Table). The diversity of each group was analyzed by α diversity test; Shannon and Simpson indexes were measured by Wilcoxon Rank SUM test. The results (Fig 3a and 3b) showed that there was no significant difference in α diversity index between the groups. Simultaneously, we used β diversity analysis to compare the difference between the group samples. According to PCoA two-dimensional chart (Fig 3c), in mice with LPS-induced endometritis, a significant change in the microbial community was observed. However, there was not obviously altered in the microbiome after IOP treatment.
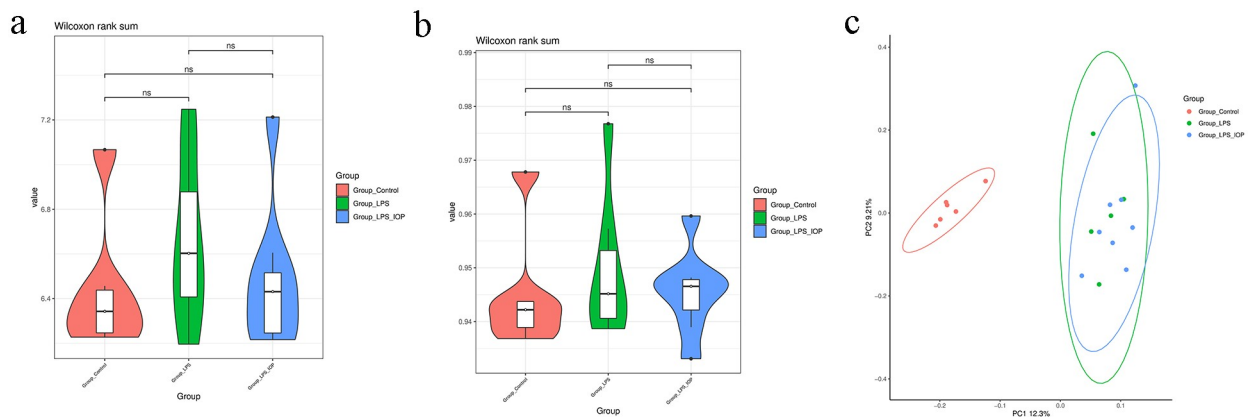

Fig 3. The total structure of the gut microbiota. (a). Shannon index of α diversity analysis. (b). Simpson index of α diversity analysis, ns means no difference. (c). Principal Co -ordinates analysis (*PCoA*) of β diversity.

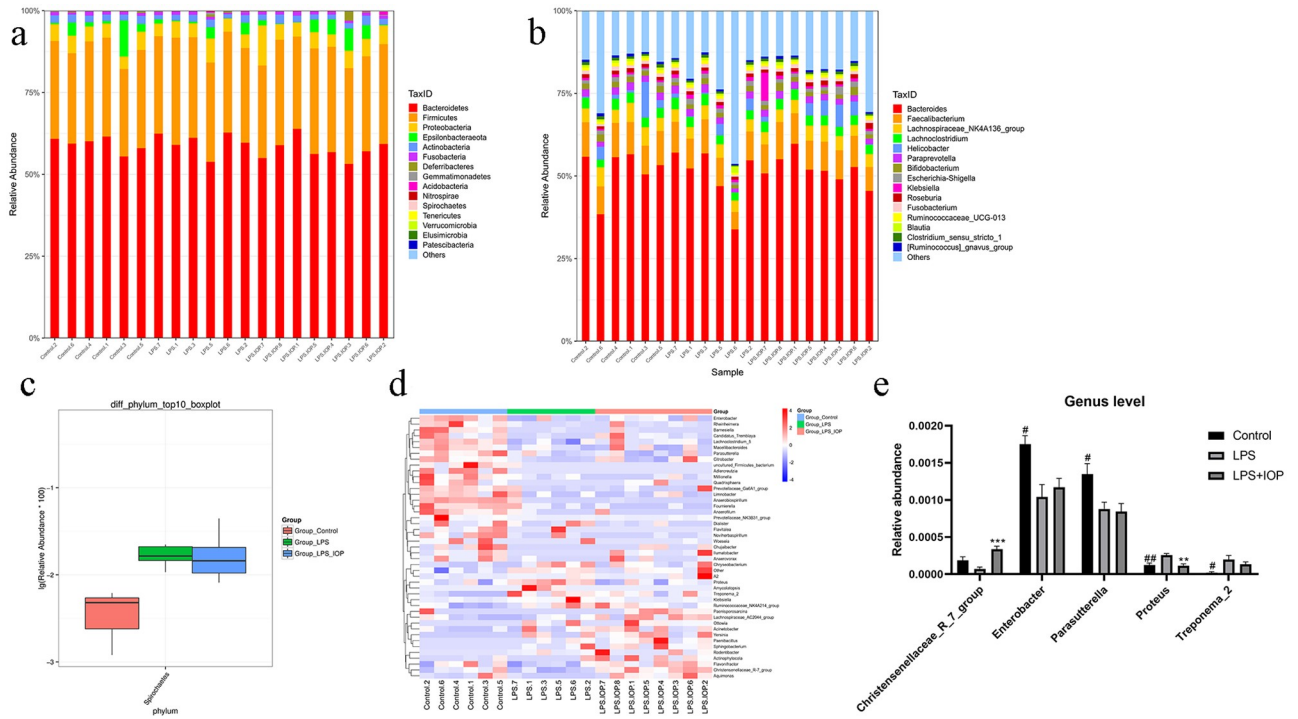https://doi.org/10.1371/journal.pone.0259570.g003

Fig 4. Changes in gut microbiota composition. (a). Species relative abundance of species at the phylum level. (b). Species relative abundance of species at the genus level. (c). Boxplot representing differences at the phylum level. (d). Heat map of differences at the genus level. Red indicates relatively high species abundance and blue indicates relatively low species abundance. (e). Significant species differences among the control group, LPS group and IOP interference group. #$P < 0.05$, ##$P < 0.01$,vs. Control group, **$P < 0.01$, ***$P < 0.001$vs. LPS group.

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Changes in gut microbiota composition

After obtaining the OTU annotation results where multiple OTUs corresponded to the same genus or species, the classification results were summarized to obtain the relative abundance of samples at each level (S2 Table). Fig 4a and 4b shows the relative abundance of TOP15 species in each group. The *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* are grouped at the level of Phylum, whereas *Bacteroides*, *Faecalibacterium*, *Lachnoclostridium*, *Helicobacter*, *Paraprevotella*, *Bifidobacterium* were grouped at the genus level. We also analyzed the different species of each group using a Kruskal Wallis algorithm (Fig 4c and 4d). At the phylum level, *Spirochaetes* was found to be differentially expressed, while species at the genus level included *Klebsiella*, *Lachnoclostridium_5*, *Enterobacter*, *Flavonifractor*, *Parasutterella*, *Treponema_2*, and *Christensenellaceae_R−7_group*. Fig 4e shows the difference microbiota of each group at the genus level, compared to the Control group, *Enterobacter* (P<0.05) and *Parasutterella* (P<0.05) were significantly decreased in LPS group, while *Proteus* (P<0.01) and *Treponema_2* (P<0.05) were increased, after IOP intervention, *Christensenellaceae_R−7_group* (P<0.001) was significantly increased, while *Proteus* (P<0.01) was decreased.

Discussion

Endometritis is a bacterial uterine disease that occurs in women and female animals such as cows and sows, affecting quality of life in women and also modern agricultural production [2, 37]. LPS causes inflammation by inducing the production of pro-inflammatory cytokines such as IL-6, IL-1β and TNF-α [38]. Our results showed that mouse uterine epithelial cells were

swollen and eosinophilic after LPS induction which was significantly improved by IOP treatment. At the same time, the levels of IL-6, IL-1β and TNF-α were increased after treatment with LPS and decreased by IOP. This indicates that IOP may ameliorate the symptoms of endometritis induced by LPS.

There is growing evidence that the diversity of gut microbes is linked to human health [39–41]. Previous studies have shown that the composition of the intestinal microflora has changed due to the induction of false evidence in endometrium and endometrial cancer [42], and the occurrence of endometritis is also related to intestinal microorganisms. To this end, we performed diversity analysis using 16S rRNA sequencing and found no clear effect of endometritis on the diversity of intestinal microflora. However, we did find differences between species of *Spirochaetes* at the phylum level, and in *Christensenellaceae_R.7_group*, *Parasutterella*, *Enterobacter*,*Treponema_2* and *Proteus* at the genus level in the composition of the gut microbiota.

Spirochaetes bacteria may be pathogenic [43], and we noted that the relative abundance of this species increased under the induction of LPS, while the abundance decreased after treatment with IOP, but not significantly. It indicated an increase in pathogenic bacteria in mice induced by LPS, whereas IOP intervention has little effect on bacteria in this phylum.

Christensenellaceae_R.7_group is a widespread human and animal microbe that been linked to conditions including obesity and inflammatory bowel disease [44], *Christensenellaceae_R.7_group* is not present in large amounts in obese people [39]; however, obese people have a higher risk of endometritis compared with more limited individuals [45]. LPS reduced the abundance of *Christensenellaceae_R.7_group*, while IOP significantly increased its abundance, even exceeding the normal level. This suggests that IOP may reduce LPS-induced endometritis in mice by increasing the abundance of *Christensenellaceae_R.7_group*.

Parasutterella has been shown to be an important microorganism that maintains gastrointestinal health in humans. Inflammatory bowel disease, obesity, diabetes, and fatty liver have been shown to be associated with the relative abundance of the species [46–49]. The relative abundance of *Parasutterella* was decreased after treatment with LPS and IOP. Although IOP intervention alleviates the symptoms of endometritis, it may not have an effect on *Parasutterella* and may even further reduce its relative abundance. In addition, *Parasutterella* has also been shown to be related to the homeostasis of bile acids [46] and the pathogenesis of cervicitis is related to the biosynthesis of primary bile acids. Not only that, cervicitis can cause a range of diseases including endometritis [50], which is consistent with the changes in relative abundance of *Parasutterella* observed in mice with endometritis.

In the previous uterine microbiota detection in patients with endometritis, fewer *Enterobacter* have been observed and it is speculated that the reduction in the number of *Enterobacter* may be related to the overgrowth of endometrial tissue [51], which is similar to the results observed in the intestine. In addition, as a pathogen [52], *Treponema_2* was increased after LPS induction, suggesting a role in inflammation. *Proteus* is a common symbiont of intestinal microbiome, which is widely considered to be pathogenic and also one of the pathogenic bacteria of endometritis [53]. The interference of IOP significantly reduced the abundance of *Proteus*, which also showed the function of IOP in alleviating endometritis. Interestingly enough, the effect of IOP on the gut microbiota is not obvious. The relationship between endometritis and the composition of intestinal flora and how the intestinal flora utilizes IOP under inflammation need further research. In summary, we further elucidated the role of IOP in endometritis, further analyzed the possible mechanism of action by analyzing the composition of intestinal microflora and identified the changes in the composition of microbial structure that may contribute to endometritis pathogenesis. For the specific role of changing microbiome in this is yet to be explained.

Supporting information

S1 Table. Raw data processing statistics.
(TXT)

S2 Table. Relative abundance of microbiota at phylum and genus levels.
(TXT)

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Validation: Binhong Hu, Yuqing Dong.

Writing – original draft: Binhong Hu, Yuqing Dong, Wenjing Zhou.

Writing – review & editing: Yichuan Ma, Rong Zhang, Songqing Liu.

References

1. Wallach E, Czernobilsky B. Endometritis and Infertility. Fertility and Sterility. 1978; 30(2):119–30. https://doi.org/10.1016/s0015-0282(16)43448-5 PMID: 354978

2. Kitaya K, Takeuchi T, Mizuta S, Matsubayashi H, Ishikawa T. Endometritis: new time, new concepts. Fertil Steril. 2018; 110(3):344–50. Epub 2018/07/02. https://doi.org/10.1016/j.fertnstert.2018.04.012 PMID: 29960704.

3. Andrews WW, Hauth JC, Cliver SP, Conner MG, Goldenberg RL, Goepfert AR. Association of asymptomatic bacterial vaginosis with endometrial microbial colonization and plasma cell endometritis in non-pregnant women. Am J Obstet Gynecol. 2006; 195(6):1611–6. Epub 2006/06/14. https://doi.org/10.1016/j.ajog.2006.04.010 PMID: 16769017.

4. Zhao H, Gong N. miR-20a regulates inflammatory in osteoarthritis by targeting the IkappaBbeta and regulates NK-kappaB signaling pathway activation. Biochem Biophys Res Commun. 2019; 518(4):632–7. Epub 2019/08/28. https://doi.org/10.1016/j.bbrc.2019.08.109 PMID: 31451219.

5. Zhou M, Yi Y, Hong L. Oridonin Ameliorates Lipopolysaccharide-Induced Endometritis in Mice via Inhibition of the TLR-4/NF-kappaBpathway. Inflammation. 2019; 42(1):81–90. Epub 2018/08/23. https://doi.org/10.1007/s10753-018-0874-8 PMID: 30132202.

6. Ju M, Liu B, He H, Gu Z, Liu Y, Su Y, et al. MicroRNA-27a alleviates LPS-induced acute lung injury in mice via inhibiting in fl ammation and apoptosis through modulating TLR4/MyD88/NF-kappaB pathway. Cell Cycle. 2018; 17(16):2001–18. Epub 2018/09/21. https://doi.org/10.1080/15384101.2018.1509635 PMID: 30231673.

7. Ursell LK, Haiser HJ, Van Treuren W, Garg N, Reddivari L, Vanamala J, et al. The intestinal metabolome: an intersection between microbiota and host. Gastroenterology. 2014; 146(6):1470–6. Epub 2014/03/19. https://doi.org/10.1053/j.gastro.2014.03.001 PMID: 24631493.

8. Marchesi JR, Adams DH, Fava F, Hermes GD, Hirschfield GM, Hold G, et al. The gut microbiota and host health: a new clinical frontier. Gut. 2016; 65(2):330–9. Epub 2015/09/05. https://doi.org/10.1136/gutjnl-2015-309990 PMID: 26338727.

9. Martin AM, Yabut JM, Choo JM, Page AJ, Sun EW, Jessup CF, et al. The gut microbiome regulates host glucose homeostasis via peripheral serotonin. Proc Natl Acad Sci U S A. 2019; 116(40):19802–4. Epub 2019/09/19. https://doi.org/10.1073/pnas.1909311116 PMID: 31527237.

10. Boer CG, Radjabzadeh D, Medina-Gomez C, Garmaeva S, Schiphof D, Arp P, et al. Intestinal microbiome composition and its relation to joint pain and inflammation. Nat Commun. 2019; 10(1):4881. Epub 2019/10/28. https://doi.org/10.1038/s41467-019-12873-4 PMID: 31653850.

11. Wei Y, Li Y, Yan L, Sun C, Miao Q, Wang Q, et al. Alterations of gut microbiome in autoimmune hepatitis. Gut. 2020; 69(3):569–77. Epub 2019/06/16. https://doi.org/10.1136/gutjnl-2018-317836 PMID: 31201284.

12. Liu R, Kang JD, Sartor RB, Sikaroodi M, Fagan A, Gavis EA, et al. Neuroinflammation in Murine Cirrhosis Is Dependent on the Gut Microbiome and Is Attenuated by Fecal Transplant. Hepatology. 2020; 71 (2):611–26. Epub 2019/06/21. https://doi.org/10.1002/hep.30827 PMID: 31220352.

13. Plottel CS, Blaser MJ. Microbiome and malignancy. Cell Host Microbe. 2011; 10(4):324–35. Epub 2011/10/25. https://doi.org/10.1016/j.chom.2011.10.003 PMID: 22018233.

14. King AE, Critchley HO. Oestrogen and progesterone regulation of inflammatory processes in the human endometrium. J Steroid Biochem Mol Biol. 2010; 120(2–3):116–26. Epub 2010/01/14. https://doi.org/10.1016/j.jsbmb.2010.01.003 PMID: 20067835.

15. Cameron EA, Sperandio V. Frenemies: Signaling and Nutritional Integration in Pathogen-Microbiota-Host Interactions. Cell Host Microbe. 2015; 18(3):275–84. Epub 2015/09/12. https://doi.org/10.1016/j.chom.2015.08.007 PMID: 26355214.

16. Liu L, Li M, Yu M, Shen M, Wang Q, Yu Y, et al. Natural polysaccharides exhibit anti-tumor activity by targeting gut microbiota. Int J Biol Macromol. 2019; 121:743–51. Epub 2018/10/21. https://doi.org/10.1016/j.ijbiomac.2018.10.083 PMID: 30342142.

17. Zhang X, Zhao S, Song X, Jia J, Zhang Z, Zhou H, et al. Inhibition effect of glycyrrhiza polysaccharide (GCP) on tumor growth through regulation of the gut microbiota composition. J Pharmacol Sci. 2018; 137(4):324–32. Epub 2018/08/29. https://doi.org/10.1016/j.jphs.2018.03.006 PMID: 30150145.

18. Liu G, Liang L, Yu G, Li Q. Pumpkin polysaccharide modifies the gut microbiota during alleviation of type 2 diabetes in rats. Int J Biol Macromol. 2018; 115:711–7. Epub 2018/04/28. https://doi.org/10.1016/j.ijbiomac.2018.04.127 PMID: 29702167.

19. Ma L, Chen H, Dong P, Lu X. Anti-inflammatory and anticancer activities of extracts and compounds from the mushroom Inonotus obliquus. Food Chem. 2013; 139(1–4):503–8. Epub 2013/04/09. https://doi.org/10.1016/j.foodchem.2013.01.030 PMID: 23561137.

20. Lu X, Chen H, Dong P, Fu L, Zhang X. Phytochemical characteristics and hypoglycaemic activity of fraction from mushroom Inonotus obliquus. J Sci Food Agric. 2010; 90(2):276–80. Epub 2010/04/01. https://doi.org/10.1002/jsfa.3809 PMID: 20355042.

21. Choi SY, Hur SJ, An CS, Jeon YH, Jeoung YJ, Bak JP, et al. Anti-inflammatory effects of Inonotus obliquus in colitis induced by dextran sodium sulfate. J Biomed Biotechnol. 2010; 2010:943516. Epub 2010/03/20. https://doi.org/10.1155/2010/943516 PMID: 20300439.

22. Chen YF, Zheng JJ, Qu C, Xiao Y, Li FF, Jin QX, et al. Inonotus obliquus polysaccharide ameliorates dextran sulphate sodium induced colitis involving modulation of Th1/Th2 and Th17/Treg balance. Artif Cells Nanomed Biotechnol. 2019; 47(1):757–66. Epub 2019/03/12. https://doi.org/10.1080/21691401.2019.1577877 PMID: 30856346.

23. Han Y, Nan S, Fan J, Chen Q, Zhang Y. Inonotus obliquus polysaccharides protect against Alzheimer's disease by regulating Nrf2 signaling and exerting antioxidative and antiapoptotic effects. Int J Biol Macromol. 2019; 131:769–78. Epub 2019/03/18. https://doi.org/10.1016/j.ijbiomac.2019.03.033 PMID: 30878614.

24. Tian J, Hu X, Liu D, Wu H, Qu L. Identification of Inonotus obliquus polysaccharide with broad-spectrum antiviral activity against multi-feline viruses. Int J Biol Macromol. 2017; 95:160–7. Epub 2016/11/21. https://doi.org/10.1016/j.ijbiomac.2016.11.054 PMID: 27865960.

25. Hu Y, Teng C, Yu S, Wang X, Liang J, Bai X, et al. Inonotus obliquus polysaccharide regulates gut microbiota of chronic pancreatitis in mice. AMB Express. 2017; 7(1):39. Epub 2017/02/16. https://doi.org/10.1186/s13568-017-0341-1 PMID: 28197985.

26. Tang Y, Xiao Y, Tang Z, Jin W, Wang Y, Chen H, et al. Extraction of polysaccharides from Amaranthus hybridus L. by hot water and analysis of their antioxidant activity. PeerJ. 2019; 7. https://doi.org/10.7717/peerj.7149 PMID: 31223543

27. Sarichai P, Buddhasiri S, Walters GE, Khantawa B, Kaewsakhorn T, Chantarasakha K, et al. Pathogenicity of clinical Salmonella enterica serovar Typhimurium isolates from Thailand in a mouse colitis model. Microbiology and Immunology. 2020; 64(10):679–93. https://doi.org/10.1111/1348-0421.12837 PMID: 32803887

28. Zhao G, Jiang K, Yang Y, Zhang T, Wu H, Shaukat A, et al. The Potential Therapeutic Role of miR-223 in Bovine Endometritis by Targeting the NLRP3 Inflammasome. Frontiers in immunology. 2018; 9:1916-. https://doi.org/10.3389/fimmu.2018.01916 PMID: 30186287.

29. Wu H, Zhao G, Jiang K, Li C, Qiu C, Deng G. Engeletin Alleviates Lipopolysaccharide-Induced Endometritis in Mice by Inhibiting TLR4-mediated NF-kappaB Activation. J Agric Food Chem. 2016; 64 (31):6171–8. Epub 2016/07/15. https://doi.org/10.1021/acs.jafc.6b02304 PMID: 27411287.

30. Wu H, Dai A, Chen X, Yang X, Li X, Huang C, et al. Leonurine ameliorates the inflammatory responses in lipopolysaccharide-induced endometritis. Int Immunopharmacol. 2018; 61:156–61. Epub 2018/06/08. https://doi.org/10.1016/j.intimp.2018.06.002 PMID: 29879659.

31. Nossa Carlos W, Oberdorf William E, Yang Liying et al. Design of 16S rRNA gene primers for454 pyrosequencing of the human foregut microbiome. [J]. World J. Gastroenterol., 2010, 16:4135–44. https://doi.org/10.3748/wjg.v16.i33.4135 PMID: 20806429

32. Bolger Anthony M, Lohse Marc, Usadel Bjoern. Trimmomatic: a flexible trimmer for Illumina sequence data. [J]. Bioinformatics, 2014, 30: 2114–20. https://doi.org/10.1093/bioinformatics/btu170 PMID: 24695404

33. Reyon Deepak, Tsai Shengdar Q, Khayter Cyd et al. FLASH assembly of TALENs for high-throughput genome editing. [J]. Nat. Biotechnol., 2012, 30: 460–5. https://doi.org/10.1038/nbt.2170 PMID: 22484455

34. Caporaso J Gregory, Kuczynski Justin, Stombaugh Jesse et al. QIIME allows analysis of high-throughput community sequencing data. [J]. Nat. Methods, 2010, 7: 335–6. https://doi.org/10.1038/nmeth.f.303 PMID: 20383131

35. Rognes Torbjørn, Flouri Tomáš, Nichols Ben et al. VSEARCH: a versatile open source tool for metagenomics. [J]. Peer J, 2016, 4: e2584. https://doi.org/10.7717/peerj.2584 PMID: 27781170

36. Wang Qiong, Garrity George M, Tiedje James M et al. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. [J]. Appl. Environ. Microbiol., 2007, 73: 5261–7. https://doi.org/10.1128/AEM.00062-07 PMID: 17586664

37. Brodzki P, Kostro K, Brodzki A, Lisiecka U. Determination of selected parameters for non-specific and specific immunity in cows with subclinical endometritis. Anim Reprod Sci. 2014; 148(3–4):109–14. Epub 2014/07/16. https://doi.org/10.1016/j.anireprosci.2014.06.021 PMID: 25022330.

38. Ahmad A, Wu J, Niu P, Zhao Y, Cheng Y, Chen W, et al. Impact of miR-223-3p and miR-2909 on inflammatory factors IL-6, IL-1ß, and TNF-α, and the TLR4/TLR2/NF-κB/STAT3 signaling pathway induced by lipopolysaccharide in human adipose stem cells. Plos One. 2019; 14(2). https://doi.org/10.1371/journal.pone.0212063 PMID: 30807577

39. AM Valdes JW, E Segal, TD Spector. Role of the gut microbiota in nutrition and health. 2018.

40. Sommer Felix R MC, Bang Corinna H M, Rehman Ateequr, Kaleta Christoph, Schmitt-Kopplin Phillippe D A, Weidinger Stephan, et al. Microbiomarkers in inflammatory bowel diseases: caveats come with caviar. 7. 2017. https://doi.org/10.1136/gutjnl-2016-313678 PMID: 28733278

41. Sommer F, Anderson JM, Bharti R, Raes J, Rosenstiel P. The resilience of the intestinal microbiota influences health and disease. Nat Rev Microbiol. 2017; 15(10):630–8. Epub 2017/06/20. https://doi.org/10.1038/nrmicro.2017.58 PMID: 28626231.

42. Borella F, Carosso AR, Cosma S, Preti M, Collemi G, Cassoni P, et al. Gut Microbiota and Gynecological Cancers: A Summary of Pathogenetic Mechanisms and Future Directions. ACS Infectious Diseases. 2021; 7(5):987–1009. https://doi.org/10.1021/acsinfecdis.0c00839 PMID: 33848139

43. Shanmuganandam S, Hu Y, Strive T, Schwessinger B, Hall RN. Uncovering the microbiome of invasive sympatric European brown hares and European rabbits in Australia. PeerJ. 2020; 8:e9564. Epub 2020/09/03. https://doi.org/10.7717/peerj.9564 PMID: 32874776.

44. Waters JL, Ley RE. The human gut bacteria Christensenellaceae are widespread, heritable, and associated with health. BMC Biol. 2019; 17(1):83. Epub 2019/10/30. https://doi.org/10.1186/s12915-019-0699-4 PMID: 31660948.

45. Kalkanbaeva CK, Shoonaeva ND, Asakeeva RS, Niyazova FR, Dzhakypova AK. Assessment of premorbid background in pregnant women with obesity of various degrees. Kazan medical journal. 2017; 98(6):913–7. https://doi.org/10.17750/kmj2017-913

46. Ju T, Kong JY, Stothard P, Willing BP. Defining the role of Parasutterella, a previously uncharacterized member of the core gut microbiota. ISME J. 2019; 13(6):1520–34. Epub 2019/02/12. https://doi.org/10.1038/s41396-019-0364-5 PMID: 30742017.

47. Morotomi M. The Family Sutterellaceae. In: Rosenberg E, DeLong EF, Lory S, Stackebrandt E, Thompson F, editors. The Prokaryotes: Alphaproteobacteria and Betaproteobacteria. Berlin, Heidelberg: Springer Berlin Heidelberg; 2014. p. 1005–12.

48. Shin NR, Whon TW, Bae JW. Proteobacteria: microbial signature of dysbiosis in gut microbiota. Trends Biotechnol. 2015; 33(9):496–503. Epub 2015/07/27. https://doi.org/10.1016/j.tibtech.2015.06.011 PMID: 26210164.

49. Blasco-Baque V, Coupe B, Fabre A, Handgraaf S, Gourdy P, Arnal JF, et al. Associations between hepatic miRNA expression, liver triacylglycerols and gut microbiota during metabolic adaptation to high-fat diet in mice. Diabetologia. 2017; 60(4):690–700. Epub 2017/01/21. https://doi.org/10.1007/s00125-017-4209-3 PMID: 28105518.

50. Zhang X, He M, Lei S, Wu B, Tan T, Ouyang H, et al. An integrative investigation of the therapeutic mechanism of Ainsliaea fragrans Champ. in cervicitis using liquid chromatography tandem mass spectrometry based on a rat plasma metabolomics strategy. J Pharm Biomed Anal. 2018; 156:221–31. Epub 2018/05/08. https://doi.org/10.1016/j.jpba.2018.04.048 PMID: 29729635.

51. Fang R-L, Chen L-X, Shu W-S, Yao S-Z, Wang S-W, Chen Y-Q. Barcoded sequencing reveals diverse intrauterine microbiomes in patients suffering with endometrial polyps. Am J Transl Res. 2016; 8 (3):1581–92. PMID: 27186283.

52. Choi DH, Park J, Choi JK, Lee KE, Lee WH, Yang J, et al. Association between the microbiomes of tonsil and saliva samples isolated from pediatric patients subjected to tonsillectomy for the treatment of tonsillar hyperplasia. Exp Mol Med. 2020; 52(9):1564–73. Epub 2020/09/04. https://doi.org/10.1038/s12276-020-00487-6 PMID: 32887934.

53. Spencer THI, Umeh PO, Irokanulo E, Baba MM, Spencer BB, Umar AI, et al. Bacterial isolates associated with pelvic inflammatory disease among female patients attending some hospitals in abuja, Nigeria. Afr J Infect Dis. 2014; 8(1):9–13. https://doi.org/10.4314/ajid.v8i1.3 PMID: 24653811.