Rhubarb Alleviates Acute Lung Injury by Modulating Gut Microbiota Dysbiosis in Mice

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Abstract
Intestinal microbiota disorders can aggravate pulmonary inflammation during acute lung injury (ALI). As a traditional Chinese herb, Rhubarb can regulated the gut microbiota. Therefore, this study was conducted to test the hypothesis that rhubarb alleviates gut microbiota dysbiosis and inflammation. Feces were collected from patients with ALI to detect the gut microbiota using 16S rDNA sequencing. Subsequently, a mouse model of ALI was established using lipopolysaccharide to investigate changes in the gut microbiota, the peripheral blood was attained for detecting the Th17/Treg cell ratio and the serum level of HDAC6 and HDAC9, and the effect of rhubarb treatment on the gut microbiota and Th17/Treg ratio were also evaluated. The results indicated that both the Firmicutes phylum decreased and the Bacteroidetes phylum increased were identified in patients with ALI, which induced the alternation of histone metabolites. The mice models also showed a similar imbalance in the Firmicutes/Bacteroidetes ratio at phylum of level. Rhubarb treatment alleviated the damaged lung tissue, accelerated Alistipes, Clostridium, and Lactobacillus proliferation at the level of genus, increased the level of HDAC6 in both the mice lung tissue and serum, and markedly reduced the Treg cells and increased the Th17 cells in the spleen tissue. The study suggested that both patients and mouse models with ALI presented gut microbiota dysbiosis, and lead to a Th17/Treg cell imbalance in ALI mouse. Rhubarb promoted Alistipes, Clostridium, and Lactobacillus proliferation, increased the HDAC6 concentration, restored the Th17/Treg cell balance, and protected against ALI.

Introduction
The lungs are the most vulnerable target organ and are usually damaged early during sepsis and pulmonary injuries. Acute lung injury (ALI) and its more severe clinical manifestation, acute respiratory distress syndrome (ARDS), are common responses to various infectious and noninfectious etiologies, including severe sepsis, pneumonia, severe acute pancreatitis, and transfusion-related acute lung injury, and can lead to uncontrollable inflammation with a cascade effect [1–3]. Neutrophil infiltration may be key effector cells to initiate inflammation in the pathogenesis of ALI/ARDS [4, 5]. Progressive hypoxemia and respiratory distress syndrome are common clinical manifestations in patients with ALI. Despite the development of various therapeutic techniques, the mortality rate from ARDS can reach 70–90% [6]. In 2005, the incidence of ALI increased to 306 per 100,000 person-years for people aged 75–84 years in the USA [7].

The human body contains numerous microflora, equaling ~10 times the number of human cells. A “healthy gut microbiota” comprises a diverse range of intestinal microorganisms, which depend on host and environmental interactions [8]. A healthy microbiota protects against dysbiosis-related diseases, such as allergic sensitization, eczema, and asthma [9]. In patients with ALI/ARDS, the intestinal microbiota are thought to be disordered and aggravate inflammation in the lungs [10, 11]. Kapur et al. indicated that gut microbiota increased plasma macrophage inflammatory protein-2 levels as well as pulmonary neutrophil accumulation during ALI [12]. Increasing evidence indicates that a Th17/Treg cell imbalance is related to the development of several disorders, and patients with ALI exhibit increased Th17 cells [13]. Another research from Kapur et al. also found Treg cell maybe critical effectors that protect against ALI, Treg cell depletion lead to antibody-mediated acute lung injury in vivo [14]. A healthy gut microbiota and its metabolites contribute to regulating

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the Th17/Treg cell balance via epigenetic mechanisms. Luo et al. showed that regulating the intestinal microbiota dysbiosis increased the short-chain fatty acid (SCFA) levels and restored the Th17/Treg cell balance [15]. Therefore, alterations in specific gut microorganisms and the effect of metabolites on the Th17/Treg cell imbalance and inflammation during LPS-induced ALI should be explored.

Rhubarb, a traditional herb with various pharmacological activities, playing anti-inflammatory effects, reduces intestinal permeability and bacterial translocation, and modulates gut microbiota dysbiosis [16]. In the gut, rhubarb supplementation improved intestinal ecosystem disorders and induced antimicrobial peptide expression [17]. Rhubarb also attenuated intestinal microbiota dysbiosis, relieved intestinal mucosal barrier damage, and inhibited intestinal inflammatory responses during acute pancreatitis [18]. Therefore, rhubarb regulated the gut microbiota and relieved the inflammation. Emodin, extracted from rhubarb, has shown anti-inflammatory properties for treating pancreatitis, atherosclerosis, asthma, and ALI [19, 20]. Xiao et al. indicated that emodin relieved pulmonary edema and MCP-1 and E-selectin secretions and inhibited LPS-induced pulmonary damage [19]. Furthermore, emodin was shown to alter the gut microbiota structure, reduce the number of harmful bacteria, increase the number of beneficial bacteria, and ameliorate chronic kidney disease [21]. However, few published studies have explored the effect of rhubarb on the gut microbiota and inflammation during ALI development.

We hypothesized that rhubarb can alleviate intestinal microflora disorders and inflammation during ALI. To test this hypothesis, we evaluated gut microbiota dysbiosis in patients with ALI. We also used a mouse model of ALI induced by intratracheal administration of LPS to investigate changes in the intestinal microbiota and the Th17/Treg cell ratio. We also explored the effect of rhubarb on the gut microbiota and inflammation.

Materials and Methods

Subjects and Protocol

Consecutive patients were considered eligible if they met the Berlin definition of ALI/ARDS [22]: (1) acute onset; (2) oxygenation index (pressure partial pressure of oxygen (PaO2)/fraction of inspiration oxygen (FiO2) <200 mmHg to ≤300 mmHg (1 mmHg = 0.133 kPa); (3) chest imaging showing patchy shadows in both lungs; and (4) pulmonary arterial entrapment pressure ≤18 mmHg or no clinical evidence of increasing left atrial pressure.

Exclusion Criteria

Exclusion criteria were as follows: (1) less than 18 years old; (2) rapid progression to ARDS; (3) acute left heart failure or cardiogenic pulmonary edema; (4) patients with related intestinal diseases, such as ulcerative colitis and irritable bowel syndrome; (5) patients who had taken probiotics, antibiotics or immunosuppressants within the previous 2 weeks; and (6) tumors, diabetes, liver or kidney dysfunction, connective tissue disease, or other inflammatory diseases that may affect the Th17/Treg cell ratio and intestinal flora balance. Finally, eleven patients who met the inclusion criteria were recruited into the study. Twenty-five age- and sex-matched healthy volunteers with no history of chronic disease served as controls. All subjects provided written informed consent. The Ethics Committee of Zhejiang Hospital approved the study (Grant NO: 2019-28 (K)), which was conducted according to the 1975 Declaration of Helsinki (as revised in 1983).

Samples Collection and Analysis

Feces were collected from each subject in the morning and stored at −70 °C until used. Tubes with heparin or ethylenediamine tetraacetate acid (EDTA) were used to collect the peripheral blood, and serum was obtained after centrifugation and stored at −70 °C for subsequent analyses. Blood gas analysis and white blood cell counts, neutrophils and C-reactive protein (CRP) were immediately tested in the clinical chemistry laboratory of Zhejiang Hospital.

ALI Induction and Intervention

C57BL/6 mice (Shanghai Slake Experimental Animal Co., Ltd., Shanghai, China) were obtained and housed at 20–26 °C and 40–70% humidity. The ALI model was established via intratracheal instillation of LPS; the control group was instilled with the same amount of normal saline. Because trichostatin A (TSA) can inhibit the histone deacetylation (HDAC), and valproic acid (VPA), one of SCFA, can regulate the size and function of Treg cells, which regarded as the positive control in the paper. The 36 mice were randomly divided into the ALI group (n = 6), control (n = 6), low-dose rhubarb (50 mg/kg) (n = 6), high-dose rhubarb (150 mg/kg) (n = 6), TSA (1 mg/kg) (n = 6), and VPA (200 mg/kg) groups (n = 6). The treatments were administered once daily for 5 days; the ALI and control groups received the same amount of normal saline via gavage. All experimental protocols were also approved by the Ethics Committee of Zhejiang Hospital. All methods were performed in accordance with the relevant guidelines and regulations.
Sample Collection

The mice were sacrificed by cervical dislocation. As mentioned above, peripheral blood were collected and serum were extracted for analysis. Feces samples were obtained from the cecum and stored at −70 °C for detecting the gut microbiota. Lung tissue and spleen tissue were obtained for subsequent analyses.

Lung W/D Ratios and Histopathology

Pulmonary edema can be reflect by lung wet/dry (W/D). Firstly, the left of lung was removed and weighted to determine the wet weight, then, play at oven at 80 °C for 48 h and attain the dried weight. The lung W/D ratio was calculated according to the formula of wet weight/dry weight. Lung tissues were fixed with 4% paraformaldehyde for 24 h, dehydrated, routinely processed, and embedded in paraffin. The tissues were sectioned at 5-μm thick and stained with hematoxylin and eosin (HE) to analyze the pathological damage to the lung tissues.

Enzyme-Linked Immunosorbent Assay (ELISA)

The lung tissue was ground and diluted 5 times with phosphate-buffered saline, then the lung tissue and peripheral blood were centrifuged at 3000 rpm for 15 min and stored at −70 °C for detection. HDAC9-ELISA kits (CSB-EL010245MO, Wuhan, China) and HDAC6-ELISA kits (CSB-EL010242MO, CUSABIO, Wuhan, China) were used per the manufacturer’s instructions to quantify the concentration of HDAC9 and HDAC6 in the serum and lung tissue.

Flow Cytometry Assay

As mentioned before, the spleen tissue also was ground and supernatant was obtained after centrifuged. Lymphocytes extracted from the spleen were stained with FITC-conjugated anti-CD4 (ebioscience, 11-0042-82, USA), and incubate at 4 °C for 30 min, centrifuged 500×g for 5 min, and discarded the supernatant. The lymphocytes were resuspended with 1 mL of freshly prepared cell fixation, and incubated at 4 °C for 30 min. After centrifuged 1000×g for 5 min, and the cells were resuspended with 100ul PBS and added PE-conjugated anti-FOXP3 (ebioscience, 12-5773-82). The number of CD4+ FOXP3+ Treg cells was detected via flow cytometry assays. In addition, lymphocytes isolated from spleen were inoculated into 1 mL RPMI1640 containing 10% FBS, and stimulated with PMA (1 μL) and ionomycin (1 μL) for 3.5 h. After staining the cells with FITC-conjugated anti-CD4 (ebioscience, 11-0042-82) and PE-IL-17A (ebioscience, 12-7177-81), the number of CD4+ IL-17+ Th17 cells was also detected via flow cytometry assays. Flow cytometry analysis was performed on a FACSCalibur flow cytometer (BD Biosciences, San Jose, CA, USA) equipped with CellQuest software (BD Biosciences).

Fecal Bacterial DNA Extraction and 16S rDNA Sequencing

The QIAamp DNA Stool Mini kit (Qiagen, Hilden, Germany) was used to extract fecal bacterial DNA per the manufacturer’s protocols. The V3–V4 hypervariable regions of the qualified bacterial 16S rDNA were amplified via PCR using the GeneAmp PCR System 9700 (ABI Co., USA). The primers (upstream primer: 5’-CTACGGGNGGCWGCAG-3’; downstream primer: 5’-GACTACHVGGGTWTCTAAAT-3’) were synthesized by Sangon Biotech (Shanghai, China). PCR was performed in 5-μL volumes containing 0.1 units of Taq polymerase (Qiagen, Hilden, Germany), 10 ng of whole-genome-amplified genomic DNA, 2.5 pmol of each PCR primer, and 2.5 pmol of dNTP. The processing cycle was as follows: predenaturation at 95 °C for 5 min, denaturation at 95 °C for 30 s, renaturation at 58 °C for 15 s, and extension at 72 °C for 1 min. The entire process was repeated for 40 cycles, followed by a final extension step at 72 °C for 10 min. The QIAquick PCR purification kit (Qiagen, Hilden, Germany) was used to recover and purify the PCR products, which were added into the MiSeq Reagent Kit v3 (llumina, San Diego, CA, USA) and sequenced by Illumina MiSeq PE300 (Illumina, San Diego, CA, USA).

Bioinformatics Analysis of the Gut Microbiota

After sequence alignment analysis using USEARCH software, all sequences were clustered into operational taxonomic units (OTUs) according to 97% similarity. Metabolic pathways were used to explore the relationship between the intestinal microflora and metabolism using PICRUSt analysis. The α-diversity indices were used to evaluate the gut microbial community diversity and abundance. Principal coordinate analysis based on Bray–Curtis distance and UniFrac analysis was performed to compare the global microbiota composition in each group. The relative species composition abundances were demonstrated at the phylum, class, order, family, genus, and species levels among groups. Differential taxonomic features among groups were obtained using linear discriminant analysis (LDA) effect size (LEfSe) to identify taxonomic features that differed among groups. The top 15 species were used to construct the Spearman correlation using the Corrplot package of R 24 software.
Statistical Analysis

Statistical analyses were performed in R24 and GraphPad Prism 5.0 (GraphPad Software Inc., La Jolla, CA, USA). Wilcoxon rank sum test was used to analyze the difference of diversity index among groups at different levels. Metastats analysis was used to compare the species with significant differences among groups at each taxonomic level. Spearman correlation was also performed among species. Data are presented as the mean ± SD. Differences within groups were analyzed using t-tests, variance analysis was used for continuous variables, and chi-square tests were used for categorical variables. Significance was defined as \( P < 0.05 \).

Results

Participants’ Basic Clinical Characteristics

Table 1 shows the participants’ basic clinical characteristics. 11 patients with ALI and 25 healthy subjects were included in the study. The mean age of the patients with ALI was 65.5 ± 14.85 years and control group was 60.42 ± 11.35 years. The partial pressure of oxygen (PaO2), partial pressure of carbon dioxide (PaCO2), PaO2/FiO2, and CRP of the ALI patients differed significantly from those of the control group (all \( P < 0.05 \)).

Intestinal Flora Sequencing in the ALI Patients

More than 40,000 sequences were obtained via Illumina MiSeq PE300 sequencing with an integrity ≥ 86.13% (Suppl Fig. 1). After sequence alignment analysis using Usearch software to remove low-quality sequences, 692 OTUs were obtained with 97% similarity. The species distribution differences were analyzed in the gut microbiotas between the ALI patients and healthy controls. The phylum of Firmicutes and Bacteroidetes were the most abundant, with a proportion of 90%. At phylum level, the percentages of Firmicutes and Bacteroidetes were 49.39% and 38.07% in the ALI patients and 64.57% and 26.34% in the controls, respectively, which suggested Firmicutes was significantly reduced and Bacteroidetes was markedly elevated in patients with ALI (Fig. 1A, B). The results suggested that gut microbiota disorder was identified in ALI patients.

Associations Between Signaling Pathways and the Intestinal Microbiota in ALI Patients

A Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) analysis is applied to predict different functional pathways of microorganisms between ALI patients and healthy subjects. Three-layer analyses were performed to evaluate the alternation of signaling pathway. The first layer demonstrated that gut microbiota dysbiosis lead to the alternation of metabolism in ALI patients (Fig. 2A). Further analysis revealed amino acid metabolites were disorders (Fig. 2B). The third-layer analysis indicated that histone metabolites were altered in ALI patients (Fig. 2C). Thus, changes in the intestinal flora affected histone metabolism in patients with ALI.

Animal Model of ALI

LPS-Induced ALI Model

To verify the clinical results, a mouse model of ALI was induced by instilling varying LPS concentrations. Pathological damage to the lungs was evaluated via HE staining. HE staining indicated that increasing LPS concentrations aggravated the degree of lung injury. The ALI model induced with 3 mg/kg of LPS was used in subsequent experiments. Rhubarb treatment markedly attenuated the lung tissue damage (Fig. 3A). Lung W/D weight ratios also certified that rhubarb treatment relieved pulmonary edema, compared with ALI model (Fig. 3B).

Effect of Rhubarb on the Gut Microbiota

A Venn diagram was used to analyze the similarities and differences in OTUs among groups. Twenty-one OTUs were common among the six groups, and each group had its own unique OTUs (Fig. 4A). According to the sample number and species OTUs, the species accumulation boxplot and observed species were used to evaluate the richness and diversity of species, the curve had reached a plateau (Suppl Fig. 2A, B), which indicated that the sample in our study was relatively large enough to reflect the species abundance. β-Diversity analysis showed that species diversity had differed markedly among groups (Suppl Fig. 2C, D).
The top 20 species with the highest abundance were selected to analyze the distribution differences at the level of phylum, class, order, family, and genus, taxon assignments (Fig. 4B) was used to demonstrate the relative abundances in the microbiota. The gut microbiome consisted largely of Firmicutes, Bacteroidetes, Proteobacteria, and Tenericutes at the level of phylum, of which, Firmicutes and Bacteroidetes accounted for 86% of the total bacteria. At the class levels, Bacteroidia and Clostridia were prevalent, accounting for most of the microbiota, and at the order levels, Bacteroidales and Lactobacillales were the most abundant genera. Interestingly, the alternation of Firmicutes/Bacteroidetes ratio in the animal models was consistent with the results of the clinical study. In addition, the low-concentration rhubarb treatment repressed Anaeroplasmatales and did not significantly affect the Lactobacillales or Clostridiales abundances at the order level. Increasing the rhubarb concentration effectively promoted Lactobacillus proliferation at the genus level, which suggested that treatment with high-concentration rhubarb inhibited Bacteroides growth by promoting Clostridium and Lactobacillus elevation in mice with ALI.

**Effect of Rhubarb on HDAC and Th17/Treg Ratios**

Peripheral blood and lung tissue were used to detect the effect of rhubarb on HDAC6 and HDAC9. High-concentration rhubarb significantly increased the HDAC6 in both the lung tissue and serum (Fig. 5A) but did not affect the HDAC9 concentration (Fig. 5B), indicating that the elevated HDAC6 occurred after the rhubarb treatment. The Th17/Treg cell ratio was calculated to further evaluate the effect of rhubarb on HDAC6 acetylation function. Peripheral blood and spleen lymphocytes were collected to calculate the Treg
cells via flow cytometry. The number of Treg cells decreased in the peripheral blood, and increased in spleen tissue from control or ALI animal model (Suppl Fig. 3). These results indicated that numerous Treg cells were produced in the spleen to replenish the Treg cells consumed in the peripheral blood during ALI. Because of the lymphocyte shortage in the peripheral blood, the spleen was used to detect Treg cells in the next experiment. The number of Th17 and Treg cells was calculated via flow cytometry after treatment. The Th17 cells were significantly increased (*P < 0.05), and Treg cells were significantly reduced after rhubarb treatment (**P < 0.01) (Fig. 5C, D, Suppl Fig. 4). Thus, rhubarb restored Th17/Treg cell ratios by increasing HDAC6 levels and exerting anti-inflammatory effects.

### Discussion

The present research demonstrated that LPS-induced ALI led to a disproportionate Firmicutes/Bacteroidetes ratio with increased Bacteroidetes and decreased Firmicutes in the gut microbiota. Rhubarb treatment in mice alleviated gut microbiota dysbiosis, promoted *Alistipes*, *Clostridium*, and *Lactobacillus* proliferation at the genus level, and also increased HDAC functioning, induced Th17 cells to differentiate and mature, and exerted anti-inflammatory functions.

The gut microbiota is not typically altered in healthy individuals but can be affected by various disorders. Human microbiome plan have explored the differences in structure and abundance of the intestinal microflora under healthy and disease conditions to identify the role of the gut microbiota during disease development. Dysbiosis in the gut microflora also occurs in many disorders [9]. ALI animal model was induced by LPS, accompanied by neutrophil infiltration certified by HE staining, and lung W/D weight ratios elevated, which had accordance with the previous researches [5, 23]. In addition, ALI activated the systemic inflammatory response and secretion of inflammatory mediators, leading to intestinal mucosal barrier damage and intestinal bacterial translocation, which affected the intestinal flora diversity and abundance. As showed in Venn diagram, gut microbiota was altered during ALI or after intervention. We investigated the microflora characteristics in fecal matter from humans and mouse using 16S rDNA sequencing and found significant differences in the species and their distributions between ALI patients and controls. The results showed higher Bacteroidetes abundances in the ALI patients than in the control group. Interestingly, compared with the control group mice, the model group mice had an increased abundance of Bacteroidetes and decreased Firmicutes richness, which had accordance with the clinical research. The phylum of Firmicutes/Bacteroidetes ratio plays a key regulatory role in ALI, this regulation was positively correlated among similar phyla and negatively correlated among different phyla. These data were consistent with those of previous studies on intestinal microbiota alterations in animal models of ALI. Li et al. found decreased Firmicutes in LPS-induced ALI models [10]. Sze et al. demonstrated that instilling LPS in the lungs led to acute changes in the cecal bacterial microbiota [11]. Therefore, dysbiosis of the gut microbiota with the imbalance of Firmicutes/Bacteroidetes may induce inflammation during ALI.

As we known, SCFAs, gut microbiota-derived bacterial products, regulate the size and function of Treg cells, affect the Th17/Treg cell ratio, and maintain the balance between pro- and anti-inflammatory factors [24, 25]. Several species strains, such as *Alistipes*, *Clostridium*, *Bifidobacterium*, *Butyrivibrio*, and *Lactobacillus*, are important sources of SCFAs. Rhubarb treatment effectively repaired the intestinal mucosal barrier and increased the abundances of *Bifidobacterium* and *Lactobacillus*. Neyrinck et al. found that rhubarb extract restored the intestinal microbial ecosystem during alcohol-induced hepatic injury [26]. An extracts from rhubarb ameliorated gut microbiota dysbiosis, with an increase in probiotic *Lactobacillus* and other SCFA-producing species. Consistent with previous reports, the present study demonstrated that rhubarb supplementation contributed to Firmicutes proliferation and increased *Alistipes*, *Clostridium*, and *Lactobacillus* at the genus level, and attenuated the gut microbiota dysbiosis, and play anti-inflammation effect. Both *Alistipes* and *Lactobacillus* are SCFA-producing bacterium that is thought to be related to the health status of individuals, to promote the Treg cell and have an anti-inflammatory effect, Liu et al. study also revealed that the abundances of *Alistipes* decreased during inflammatory status. *Clostridium*, as a probiotic strains [27]. In addition, Li et al. also indicated that supplement *Clostridium* increased the percentage and total number of Tregs and attenuated the inflammation induced by allergen [28].

As Kapur et al. reported that Treg cell act as anti-inflammation effect, play protect against ALI [14]. TSA can inhibit HDAC and induce histone H3 acetylation of the FOXP3 gene promoter in Treg cells, which restores the Th17/Treg cell balance [29]. A published study revealed that inhibiting HDAC6 blocked NF-κB activation by inhibiting IκB phosphorylation after an LPS challenge and alleviated LPS-induced acute lung inflammation [30]. Conversely, several
papers have drawn opposite conclusions. Menden et al. indicated that HDAC6 inhibition augmented LPS-induced acute lung inflammation [31]. The imbalance in peripheral circulating Th17 and Treg cell frequencies gradually increased from mild to severe in ARDS patients, and a positively correlated with disease severity. Zhang et al. reported that the Th17/Treg cell ratio in bronchiolar lavage fluid was higher after ALI induced by smoke inhalation [32]. The results of Zhang’s study were inconsistent with our results, we found that the HDAC6 concentration was elevated in serum, and the Th17/Treg ratio was decreased in the spleen in LPS-induced ALI. Rhubarb treatment increased the HDAC6 levels in the lung tissue and serum, thus reducing the Treg cells, increasing the Th17 cells, and restoring the Th17/Treg cell imbalance induced by LPS in the spleen. In addition, Luo et al. study reported that Rhubarb Peony Decoction decreases expressing of IL17A and contributing to the restoration of Th17/Treg balance in colon [33]. Another research from Sharma et al. demonstrated that emodin can inhibit splenocyte proliferation and the formation/releasing of Th17 cell cytokines in vitro [34]. Different from previous studies, which detect the numbers of Th17/Treg in colon or cell culture, several points accounted for the difference, spleen tissue was used to detect the Treg/Th17 percentage due to

Fig. 3 ALI model was established by LPS, lung injury aggravated was formed after LPS intervention. After rhubarb or other intervention, the damage of lung tissue was remarkable improved (A). Lung W/D weight ratios also certified that pulmonary edema relieved after rhubarb or other intervention, compared with control (B).
shortage of Treg cells in peripheral blood. In order to inhibit the inflammation during ALI, Treg cells were consumed in the peripheral blood, then reproduced in the spleen to replenish the consumed cells, which induced the Treg cells decreased in spleen tissue. Rhubarb treatment attenuated the intestinal microbiota disorder and elevated the HDAC6 levels, which induced Th17 cell production and restored the splenic Th17/Treg cell balance, which providing evidence of mechanisms of rhubarb immunomodulation.

A limitation of this study was that only 11 ALI patients were included, which may have increased the chance of bias. Second, we did not provide probiotics against the gut microbiota dysbiosis. Third, no SCFAs were detected in the present study. Future research should further explore the function of metabolic products in the gut microbiota and the effect of probiotics on inflammation in LPS-induced ALI.
HDAC6 and HDAC9 was analysis by ELISA (A, B). Th17 and Treg cell numbers were determined via flow cytometry from spleen tissue (C, D). After high-dose rhubarb treatment, the level of HDAC6 was significantly higher than other groups from lung tissue (*P < 0.05). Compared with control group, the level of HDAC6 in all groups was higher in the peripheral blood serum (*P < 0.05) (A). Whether peripheral blood or lung tissue, no change was identified in HDAC9 activity (B). Th17 cells were flag as CD4+IL-17+and Treg were CD4+CD25+Foxp3+. Th17 cells were significantly increased (C) (*P < 0.05), and Treg cells were significantly reduced after rhubarb treatments (D) (**P < 0.01)

Conclusion

The current study showed that ALI with intestinal microbiota dysbiosis presented reduced Firmicutes and elevated Bacteroidetes in both human patients and ALI-induced mice, leading to a Th17/Treg cell imbalance and aggravated inflammation. Rhubarb played an anti-inflammatory role by contributing to Alistipes, Clostridium, and Lactobacillus proliferation at the level of genus.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1007/s00284-022-02811-x.

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Author Contributions

TYT drafted the manuscript, WF and YW collected the samples and analyze data, TZ contributed to perform detection and prepare tables, LZJ conceived and planned the study design. All authors read and approved the final manuscript.

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Data Availability

The raw materials were uploaded to the NCBI BioProject (NO: SRP357547).

Declarations

Conflict of interest

The authors declare that they have no competing interests.

Ethical Approval

The study was conducted according to the World Medical Association Declaration of Helsinki in 1975, as revised in 1983, and was approved by the Ethic Committee of Zhejiang Hospital (Grant NO: 2019-28 (K)).

Consent to Participate

All subjects provided their informed written consent.

Consent for Publication

Not applicable.

References

1. Gotts Jeffrey E, Matthey Michael A (2014) Treating ARDS: new hope for a tough problem. Lancet Respir Med 2(2):84–85. https://doi.org/10.1016/S2213-2600(13)70285-6

2. Semple JW, McVey MJ, Kim M, Rebetz J, Kuebler WM, Kapur R (2018) Targeting transfusion-related acute lung injury: the journey from basic science to novel therapies. Crit Care Med 46:e452–e458. https://doi.org/10.1097/CCM.0000000000002989

3. Semple JW, Rebetz J, Kapur R (2019) Transfusion-associated circulatory overload and transfusion-related acute lung injury. Blood 133:1840–1853. https://doi.org/10.1182/blood-2018-10-860809

4. Rebetz J, Semple JW, Kapur R (2018) The pathogenic involvement of neutrophils in acute respiratory distress syndrome and transfusion-related acute lung injury. Transfus Med Hemother 45:290–298. https://doi.org/10.1159/000492950

5. Kapur R, Kasetty G, Rebetz J, Egesten A, Semple JW (2019) Osteopontin mediates murine transfusion-related acute lung injury via stimulation of pulmonary neutrophil accumulation. Blood 134:74–84. https://doi.org/10.1182/blood.2019000972

6. Calfee CS, Eisner MD, Ware LB, Thompson BT, Parsons PE, Wheeler AP, Korpak A, Matthyah MA, Acute Respiratory Distress Syndrome Network, National Heart, Lung and Blood Institute (2007) Trauma-associated lung injury differs clinically and biologically from acute lung injury due to other clinical disorders. Crit Care Med 35:2243–2250. https://doi.org/10.1097/01.CCM.0000280434.33451.87

7. Rubenfeld GD, Caldwell E, Peabody E, Weaver J, Martin DP, Neff M, Stern EJ, Hudson LD (2005) Incidence and outcomes of acute lung injury. N Engl J Med 353:1685–1693. https://doi.org/10.1056/NEJMoa050333

8. Sommer F, Anderson JM, Bharti R, Rosenstiel P (2017) The resilience of the intestinal microbiota influences health and disease. Nat Rev Microbiol 15:630–638. https://doi.org/10.1038/nrmicro.2017.58

9. Zimmermann P, Messina N, Mohn WW et al (2019) Association between the intestinal microbiota and allergic sensitization, eczema, and asthma: A systematic review. J Allergy Clin Immunol 143:467–485

10. Li Y, Liu XY, Ma MM, Qi ZJ, Zhang QX, Li Z, Cao GH, Li J, Zhu WW, Wang XZ (2014) Changes in intestinal microbiota in rats with acute respiratory distress syndrome. World J Gastroenterol 20:5849–5858. https://doi.org/10.3748/wjg.v20.i19.5849

11. Sze MA, Tsuruta M, Yang SW, Oh Y, Man SF, Hogg JC, Sin DD (2014) Changes in the bacterial microbiota in gut, blood, and lungs following acute LPS instillation into mice lungs. PLoS ONE 9:e111228. https://doi.org/10.1371/journal.pone.0111228

12. Kapur R, Kim M, Rebetz J, Hallström B, Björkman T, Takabe-French A, Kim N, Liu J, Shanmugabhavanthan S, Milošević S, McVey MJ, Speck ER, Semple JW (2018) Gastrointestinal microbiota contributes to the development of murine transfusion-related acute lung injury. Blood Adv 2(13):1651–1663. https://doi.org/10.1182/bloodadvances.2018018903

13. Yu ZX, Ji MS, Yan J, Cai Y, Liu J, Yang HF, Li Y, Jin ZC, Zheng JX (2015) The ratio of Th17/Treg cells in early acute respiratory distress syndrome. Crit Care 19:82. https://doi.org/10.1186/s13054-015-0811-2

14. Kapur R, Kim M, Aslam R, McVey MJ, Tabuchi A, Luo A, Liu J, Li Y, Shanmugabhavanthan S, Speck ER, Zufferey A, Yousef G, Zhang H, Rondina MT, Weyrich AS, Porcelijn L, Kuebler WM, Slutsky AS, Semple JW (2017) T regulatory cells and dendritic cells protect against transfusion-related acute lung injury via IL-10. Blood 129:2557–2569. https://doi.org/10.1182/blood-2016-12-758185
15. Luo A, Leach ST, Barres R, Hesson LB, Grimm MC, Simar D (2017) The microbiota and epigenetic regulation of T helper 17 regulatory T cells: in search of a balanced immune system. Front Immunol 8:417. https://doi.org/10.3389/fimmu.2017.00417

16. Huang Z, Xu Y, Wang Q, Gao X (2019) Metabolism and mutual biotransformations of anthraquinones and anthrones in rhubarb by human intestinal flora using UPLC-Q-TOF/MS. J Chromatogr B Anal Technol Biomed Life Sci 1104:59–66. https://doi.org/10.1016/j.jchromb.2018.10.008

17. Wang Z, Elekwachi C, Jiao J, Wang M, Tang S, Zhou C, Tan Z, Forster RJ (2017) Changes in metabolically active bacterial community during rumen development, and their alteration by rhubarb root powder revealed by 16S rRNA amplicon sequencing. Front Microbiol 8:159. https://doi.org/10.3389/fmicb.2017.00159

18. Yao P, Cui M, Li Y, Deng Y, Wu H (2015) Effects of rhubarb on intestinal flora and toll-like receptors of intestinal mucosa in rats with severe acute pancreatitis. Pancreas 44:799–804. https://doi.org/10.1097/MPA.0000000000000339

19. Song YD, Li XZ, Wu YY, Shen Y, Liu FF, Gao PP, Sun L, Qian F (2018) Emodin alleviates alternatively activated macrophage and asthmatic airway inflammation in a muring asthma model. Acta Pharmacol Sin 39:1317–1325. https://doi.org/10.1038/aps.2017.147

20. Xiao M, Zhu T, Zhang W, Wang T, Shen YC, Wan QF, Wen FQ (2014) Emodin ameliorates LPS-induced acute lung injury, involving the inactivation of NF-κB in mice. Int J Mol Sci 15:19355–19368. https://doi.org/10.3390/ijms151119355

21. Zeng YQ, Dai Z, Lu F, Lu Z, Liu X, Chen C, Qu P, Li D, Hua Z, Qu Y, Zou C (2016) Emodin via colonic irrigation modulates gut microbiota and reduces uremic toxins in rats with chronic kidney disease. Oncotarget 7:17468–17478. https://doi.org/10.3390/ijms171119355

22. ARDS Definition Task Force, Ranieri VM, Rubenfeld GD, Thompson BT, Ferguson ND, Caldwell E, Fan E, Camporota L, Slutsky AS (2012) Acute respiratory distress syndrome: the Berlin definition. JAMA 307:2526–2533

23. Kapur R, Rebetz J, van der Velden S, Semple JW (2021) Pancreatic involvement in murine antibody-mediated transfusion-related acute lung injury? Transfusion 61:987–989. https://doi.org/10.1111/trf.16240

24. Smith PM, Howitt MR, Panikov N, Michaud M, Gallini CA, Bohlooly-y M, Glickman JN, Garrett WS (2013) The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. Science 341:569–573

25. Arpaia N, Campbell C, Fan X, Dikiy S, van der Veken J, deRoos P, Liu H, Cross JR, Pfeffer K, Coffey PJ, Rudensky AY (2013) Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. Nature 504:451–455. https://doi.org/10.1038/nature12726

26. Neyrinck AM, Etxeberria U, Taminiau B, Daube G, Van Hul M, Everard A, Cani PD, Bindels LB, Delzenne NM (2017) Rhubarb extract prevents hepatic inflammation induced by acute alcohol intake, an effect related to the modulation of the gut microbiota. Mol Nutr Food Res. https://doi.org/10.1002/mnfr.201500899

27. Jialing L, Yangyang G, Jing Z, Xiaoyi T, Ping W, Liwei S, Simin C (2020) Changes in serum inflammatory cytokine levels and intestinal flora in a self-healing dextran sodium sulfate-induced ulcerative colitis murine model. Life Sci 263:118587. https://doi.org/10.1016/j.lfs.2020.118587

28. Li YN, Huang F, Liu L, Qiao HM, Li Y, Cheng HJ (2012) Effect of oral feeding with Clostridium leptum on regulatory T-cell responses and allergic airway inflammation in mice. Ann Allergy Asthma Immunol 109:201–207. https://doi.org/10.1016/j.anai.2012.06.017

29. Tao R, de Zoeten EF, Ozkaynak E, Chen C, Wang L, Porrett PM, Li B, Turka LA, Olson EN, Greene MI, Wells AD, Hancock WW (2007) Deacetylase inhibition promotes the generation and function of regulatory T cells. Nat Med 13:1299–1307. https://doi.org/10.1038/nm1652

30. Liu L, Zhou X, Shetty S, Hou G, Wang Q, Fu J (2019) HDAC6 inhibition blocks inflammatory signaling and caspase-1 activation in LPS-induced acute lung injury. Toxicol Appl Pharmacol 370:178–183. https://doi.org/10.1016/j.taap.2019.03.017

31. Menden H, Xia S, Mabry SM, Noel-MacDonnell J, Rajasingh J, Ye SQ, Sampath V (2017) Histone deacetylase 6 regulates endothelial MyD88-dependent canonical TLR signaling, lung inflammation, and alveolar remodeling in the developing lung. Am J Physiol Lung Cell Mol Physiol 317:L332–L346. https://doi.org/10.1152/ajplung.00247.2018

32. Zhang F, Li MY, Lan YG, Wang CB (2016) Imbalance of Th17/Tregs in rats with smoke inhalation-induced acute lung injury. Sci Rep 6:21348. https://doi.org/10.1038/srep21348

33. Luo S, Wen R, Wang Q, Zhao Z, Nong F, Fu Y, Huang S, Chen J, Zhou L, Luo X (2019) Rhubarb peony decoction ameliorates ulcerative colitis in mice by regulating gut microbiota to restoring Th17/Treg balance. J Ethnopharmacol 231:39–49. https://doi.org/10.1016/j.ejep.2018.08.033

34. Sharma R, Tiku AB (2016) Emodin inhibits splenocyte proliferation and inflammation by modulating cytokine responses in a mouse model system. J Immunotoxicol 13:20–26. https://doi.org/10.3109/1547691X.2014.995243

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