International Journal of Peptide Research and Therapeutics (2021) 27:1605–1613
https://doi.org/10.1007/s10989-021-10186-z

Protective Effects of Pidotimod Against *Salmonella* Infections

Yuanhao Zhou[1] · Xiaolin Ye[2] · Baikui Wang[1] · Jiafu Ying[1] · Zihan Zeng[1] · Li Tang[1] · Qi Wang[1] · Peng Zou[1] · Xiaoli Zhan[1] · Luoqin Fu[1,3,4]

Accepted: 22 February 2021 / Published online: 8 April 2021

Abstract

Pidotimod has been shown to exhibit immunomodulatory activities and exert protective effects against bacterial infections. This study aimed at evaluating the protective effects of pidotimod against *Salmonella* infections in mice. C57BL/6 mice were assigned into 6 groups: Control group; Low-dose pidotimod group (PL, 12.5 μg/gram of body weight); High-dose pidotimod group (PH, 200 μg/gram of body weight); ST infection group (ST); Low-dose pidotimod and ST infection group (PL + ST); and High-dose pidotimod and ST infection group (PH + ST). Mice in each group were gavaged 200 μL ddH_2O with or without pidotimod per day before *Salmonella enterica* Typhimurium (ST) challenge. It was shown that high-dose pidotimod pretreatment alleviated weight loss, inhibited ST associated spleen and liver damage, suppressed serum TNF-α levels and elevated IL-10 levels. Moreover, it elevated $CD4^+/CD8^+$ T cell ratio in the blood. In conclusion, oral pidotimod can effectively enhance clinical responses to *Salmonella* infections.

Keywords Pidotimod · Antimicrobial properties · *Salmonella* · Immunity

Introduction

Pidotimod ((R)-3-[(S)-(5-oxo-2-pyrrolidinyl) carbonyl]-thiazolidine-4-carboxylic acid) is a synthetic dipeptide molecule with biological and immunological activities on both innate and adaptive immune systems (Riboldi et al. 2009). Among its functions, it enhances and stimulates immune cell functions, including neutrophil phagocytosis (Auteri et al. 1992; Ferrario et al. 2015); it induces phenotypic and functional maturation of mucosal dendritic cells; it plays a crucial role in the cross-talk between innate and adaptive immunity (Puggioni et al. 2019); and, it stimulates protective effects against bacterial and viral infections without any therapeutic interactions (Coppi et al. 1994; Manzardo et al. 1994). Regarding its antibacterial mechanisms, pidotimod can upregulate the expression of TLR2 (Toll Like Receptors), with a significant increase in NF-κB protein expression and NF-κB nuclear translocation (Carta et al. 2013). Moreover, pidotimod promotes the mRNA expression levels of antimicrobial peptides and augments the expression of genes involved in inflammatory responses (Esposito et al. 2015). All in all, pidotimod had a certain antibacterial ability.

Salmonella, a member of the *Enterobacteriaceae* family (Bopp 1999), causes enteric diseases in humans and animals (Dougan et al. 2011). Globally, *Salmonella* are highly

✉ Xiaoli Zhan
zhanxiaoli1988@163.com

✉ Luoqin Fu
fuluoqin@gmail.com

[1] Key Laboratory of Molecular Animal Nutrition of the Ministry of Education, National Engineering Laboratory of Biological Feed Safety and Pollution Prevention and Control, Key Laboratory of Animal Feed and Nutrition of Zhejiang Province, Institute of Animal Nutrition and Feed Sciences, College of Animal Sciences, Zhejiang University, Hangzhou 310058, Zhejiang Province, China

[2] University Hospital and Medical Faculty of the Heinrich-Heine University Düsseldorf, 40204 Düsseldorf, Germany

[3] Department of General Surgery, Chun'an First People's Hospital (Zhejiang Provincial People's Hospital Chun'an Branch), Hangzhou 311700, Zhejiang Province, China

[4] State Key Laboratory of Radiation Medicine and Protection, School of Radiation Medicine and Protection & School for Radiological and Interdisciplinary Sciences (RAD-X), Collaborative Innovation Center of Radiation Medicine of Jiangsu Higher Education Institutions, Soochow University, Suzhou 21513, Jiangsu Province, China

associated with foodborne diseases and impair the quality of livestock products in animal husbandry (Anderson and Kendall 2017; Zhen et al. 2018). *Salmonella enterica* Typhimurium (ST), a frequent cause of acute and self-limiting foodborne diarrhea, is an ideal study model that has been used to reveal key principles of enteropathogenic infections (Wotzka et al. 2017). This study aimed at evaluating the efficacy of pidotimod against *Salmonella* infections.

Materials and Methods

Study Animals and Ethical Statements

Healthy male C57BL/6 mice aged 5–6 weeks were purchased from Slac Animal Inc. (Shanghai, China) and housed in the environment without pathogenic microorganisms. Mice were maintained at 20–25 ℃ with a 12:12, light/dark cycle and given adequate standard feed and drinking water. The experimental procedures involving mice were approved by the Institutional Animal Care and Use Committee of Zhejiang University.

Bacterial Strains and Culture Conditions

Salmonella Typhimurium CMCC 50115 (ST), which is an attenuated strain (Wei et al. 2019), was obtained from the Institute of Preventive Veterinary Medicine, Zhejiang University (China). It was inoculated into Luria–Bertani (LB) medium and incubated at 37 ℃ with shaking at 180 rpm/min overnight. The ST liquid culture was centrifuged at 5000 rpm for 5 min and concentrated to 2×10^9 CFU/mL in sterile PBS.

Experimental Design

Healthy male C57BL/6 mice were randomly assigned into 6 groups ($n = 10$ per group): Control, Low-dose pidotimod group (PL), High-dose pidotimod group (PH), ST infection group (ST), Low-dose pidotimod and ST infection group (PL + ST), as well as High-dose pidotimod and ST infection group (PH + ST).

The experiment was performed in two phases. The first phase involved prevention for 14 days. Mice in the control and ST groups were gavaged 200 μL ddH_2O per day. Mice in the PL and PL + ST groups were gavaged 200 μL ddH_2O containing pidotimod (12.5 μg per gram body weight) on the basis of normal drinking water per day, while PH and PH + ST groups administered with 200 μg/body weight of pidotimod. Weight changes were measured after every two days. The second phase involved ST infection which was immediately after the end of the prevention stage. Mice in the ST, PL + ST and PH + ST groups were inoculated with 4×10^8 CFU of ST in 200 μL PBS by gavage, while the other groups were administered with 200 μL of PBS. After two days of infection, mice were weighed and the samples collected after euthanized.

Preparation of Tissue Samples

The spleen, liver and colon were collected and cleaned with normal saline to remove blood and mesangial membrane. The spleen and liver were weighed to calculate the splenic and liver indices [splenic/liver index = spleen/liver weight (mg) divided by body weight (g)]. The procedure of making pathological sections used in this study was performed as previously described (Güçlü et al. 2011) with some modifications. Briefly, spleen, liver and colon sections were fixed in 4% paraformaldehyde. Hematoxylin–eosin (H&E) staining of the sections was performed through dehydration, paraffin embedding, slicing, dewaxing, staining and dehydration sealing. Histopathological sections were observed using an optical microscope.

Analysis of Bacterial Load in the Spleen

The spleen was placed in a sterile environment, rinsed with pre-cooled sterile PBS, weighted and homogenized. The homogenate was plated on *Salmonella*–Shigella agar after serial dilutions and incubated. Colonies were counted after 24 h.

Determination of GOT and GPT in the Liver and Serum

A tissue homogenate of the liver was prepared. Subsequently, glutamic pyruvic transaminase (GPT) and glutamic oxaloacetic transaminase (GOT) activities were determined in the tissue homogenate and serum according to the manufacturer's protocols (Nanjing Jiancheng Bioengineering Institute, China).

Serum Preparation and Cytokine Determination

Blood was collected from the femoral artery of mice and kept still at 4 ℃ overnight. Serum was separated by centrifugation at 3000 g for 20 min at 4 ℃. Cytokine (TNF-α and IL-10) concentrations in the serum were determined using ELISA kits (eBioscience, USA) according to the manufacturer's instructions.

Flow Cytometry Analysis of Blood Lymphocytes

After two days of *Salmonella* infection, mononuclear lymphocytes from mice in the ST-infected groups were extracted as previously described (Trobonjaca et al. 2002). The

lymphocyte suspension was blocked using 5% goat serum for 15 min and co-incubated with anti-mouse CD3 (FITC) (eBioscience, USA) and anti-mouse CD4 (PE) (eBioscience, USA) or anti-mouse CD3 (FITC) and anti-mouse CD8 (PE) (eBioscience, USA) at 4 ℃ for 30 min, respectively. Stained cells were analyzed by FACS using an LSR II Analyzer (BD). Blood $CD3^+CD4^+$ to $CD3^+CD8^+$ ($CD4^+$/ $CD8^+$) ratios were then calculated.

Statistical Analysis

Data were analyzed using the IBM SPSS Statistics 22 and represented as mean ± SD. Comparison of means between two groups was done using the Student's *t* test, while comparisons of means for multiple groups were performed by one-way ANOVA. $P < 0.05$ was considered significant while $P < 0.01$ was considered extremely significant. The OriginPro 9.0 software was used to draw graphics.

Results

Pidotimod Alleviated ST Infection in Mice

Pidotimod administration (in both PH and PL groups) did not exert a significant effect on body weight of mice before ST challenge ($P > 0.05$) (Fig. 1a). Regarding the protective effect of pidotimod against ST infection, compared to mice in the ST group, weight loss in the PH + ST group was significant ($P < 0.05$) (Fig. 1b). Moreover, mice in the PL + ST group did not achieve a good effect on infection relief ($P > 0.05$). These results reveal that high-dose pidotimod can effectively alleviate ST infection associated weight loss.

There was no significant difference in splenic index between mice in pidotimod treated groups (both of the PH and PL groups) and the control group ($P > 0.05$) (Fig. 1c). After two days of infection, splenic index of mice in the ST group was significantly higher than that of the control group ($P < 0.05$) while the splenic index of mice in the PH + ST group was significantly lower than that of the ST group ($P < 0.05$) (Fig. 1c). Pidotimod did not exert any significant effects on the splenic index of normal mice. However, high-dose pidotimod relieved ST infection associated increase in splenic index. Furthermore, compared to the control group, high-dose pidotimod was shown to significantly reduce the liver index ($P < 0.05$) (Fig. 1d). The liver index of mice in the ST group was found to be significantly elevated ($P < 0.05$) while liver indices of mice in the PL + ST and PH + ST groups were significantly lower than that of the ST group ($P < 0.05$) (Fig. 1d). These results imply that pidotimod could effectively relieve the elevated liver index caused by ST infection.

Regarding *Salmonella* burden in the spleen, although pidotimod suppressed the quantities of *Salmonella* in mice spleen, the differences among groups were not significant ($P > 0.05$) (Fig. 1e).

Pidotimod Alleviated ST Infection Associated Tissue Structure Damage

Spleen structures of mice in the control group were clear with complete shapes (Fig. 2a). Moreover, the shape of the lymphoid follicles, which occurred in large numbers, was clear. Splenic morphologies in the PL and PH groups were similar to that of the control mice. After 2 days of infection, splenic structures of mice in the ST group were found to have been severely damaged, lymphoid follicles had shrunk, while the number of lymphoid follicles had also reduced, when compared to the control group. Splenic structures of mice in the PL + ST group was similar to that of the ST group, but without lymphoid follicles. However, the splenic structure of mice in the PH + ST group was better than that of the ST group, the texture was relatively clear while the lymphoid follicles were intact. These results imply that high-dose pidotimod could significantly alleviate ST infection associated splenic damage and exert a preventive effect.

There was no significant difference in liver structures between mice in the PL and PH groups compared to the control mice (Fig. 2b). After 2 days of infection, acute inflammatory cell infiltration and focal necrosis were observed in the hepatocytes of mice in the ST group. In addition, ST induced acute inflammatory cell infiltration was found to be significantly alleviated in the pidotimod groups (PH + ST and PL + ST groups). These results show that pidotimod can significantly alleviate ST infection induced liver inflammation.

Colon morphologies of mice in the control, PL and PH groups were complete with clear mucosal and gland structures. Moreover, there was no inflammatory cell infiltration (Fig. 2c). After 2 days of infection, inflammatory cell infiltration was elevated in the colonic mucosa and submucosa of mice in the ST group, while the structure of the gland was not clear. Colonic mucosa was clear while inflammatory cell infiltration was inhibited among the pidotimod groups (PH + ST and the PL + ST groups), compared to the ST group. These findings imply that pidotimod alleviates ST infection associated colon tissue lesions.

Pidotimod Alleviated ST Associated Increase in GOT and GPT Activities in Serum

Compared to mice in the control group, GOT activities in the liver of mice in the pidotimod groups (PH and PL groups) were significantly decreased ($P < 0.05$) (Fig. 3a). At the same time, pidotimod administration

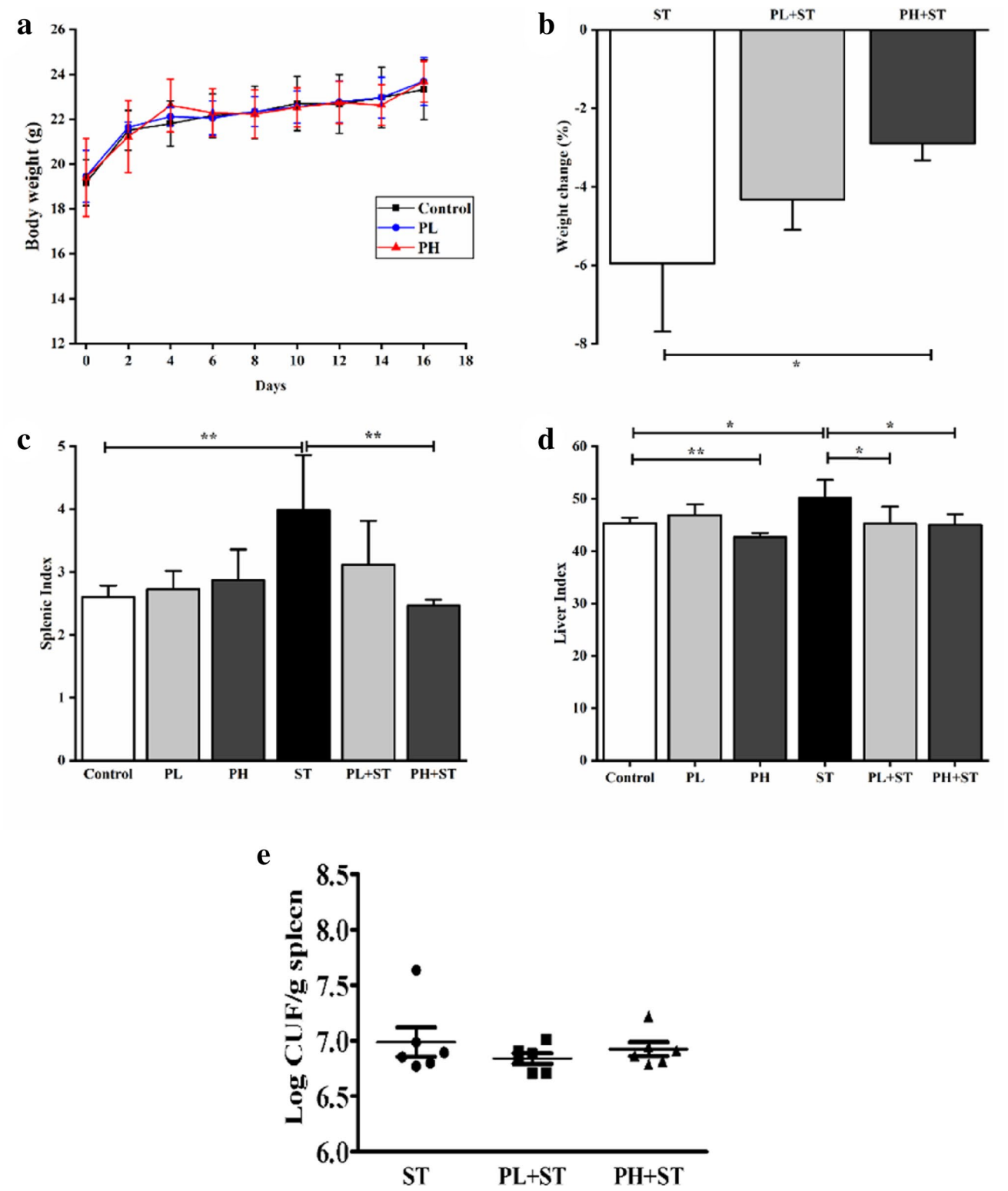

Fig. 1 Weight change, splenic index, liver index and ST translocation in spleen of mice. **a** Effects of pidotimod on body weights of normal mice. **b** The proportion of weight change in ST-infected mice. Data is presented as the means ± SD for n = 10; $*P < 0.05$. **c** Effects of pidotimod on splenic indices of mice. **d** Effects of pidotimod on liver indices of mice. **e** Effects of pidotimod on ST translocation of spleen in mice. Data is presented as the means ± SD for n = 6; $*P < 0.05$, $**P < 0.01$.

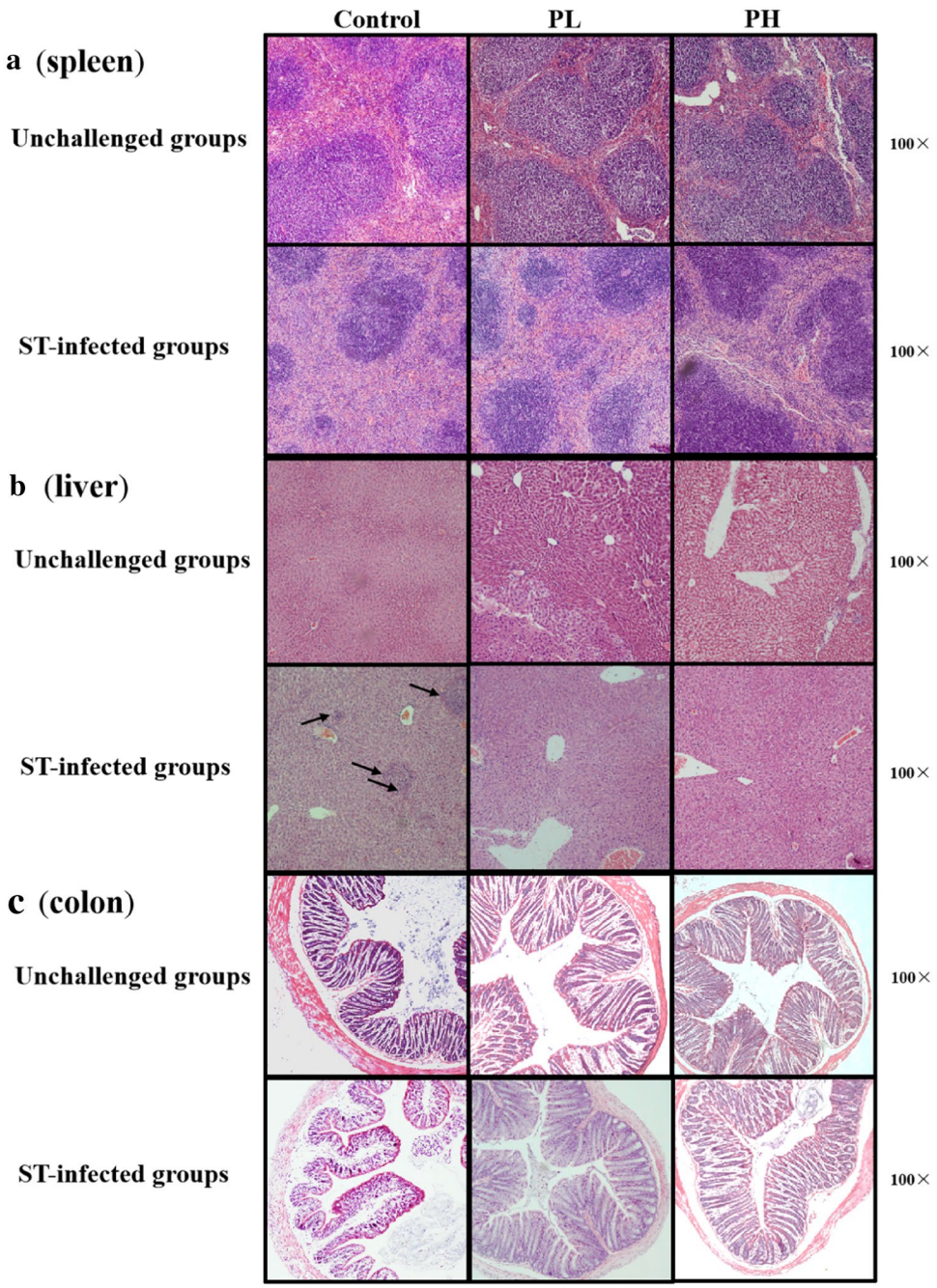

Fig. 2 Histological morphology was determined by H&E staining. **a** Histological morphology of spleen. **b** Histological morphology of liver. Black arrows → focal necrosis. **c** Histological morphology of colon

significantly reduced the activities of GOT and GPT in the serum ($P < 0.05$) (Fig. 3c and d). After two days of infection, compared to mice in the ST group, pretreatment with high-dose pidotimod was shown to significantly inhibit the elevated GOT and GPT activities in the serum ($P < 0.05$) (Fig. 3c, d). Therefore, to a certain extent, ST infection-induced liver damage can be alleviated by pidotimod.

Pidotimod Inhibited the Secretion of TNF-α and Promoted IL-10 Secretion in ST-Infected Mice

Serum TNF-α levels of normal mice were too low to be detected (Fig. 4a). After two days of infection, TNF-α levels in serum of mice in the ST group was shown to have sharply increased. However, TNF-α levels of mice in the PL + ST group were significantly low than those of the ST

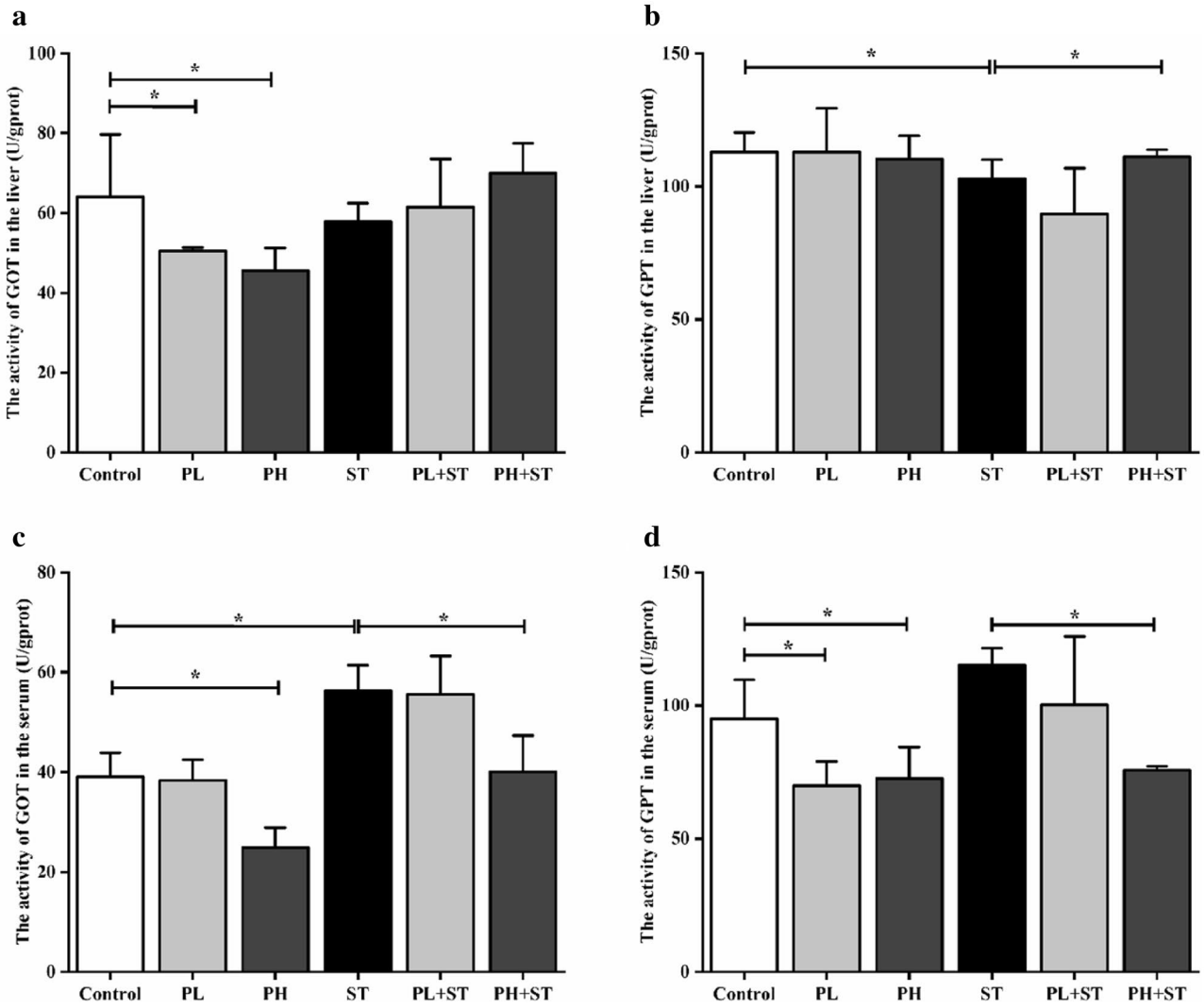

Fig. 3 GOT and GPT activities in the liver and serum. **a** GOT activity in the liver. **b** GPT activity in the liver. **c** GOT activity in the serum. **d** GPT activity in the serum. Data is presented as the means ± SD for n = 4; *$P < 0.05$

group ($P < 0.05$). Higher IL-10 levels were recorded in normal mice (significantly higher in the PH group, $P < 0.05$) (Fig. 4b). After two days of infection, IL-10 levels of mice in the PH + ST group were significantly elevated when compared to the ST group ($P < 0.05$). These results indicate that higher doses of pidotimod enhances the secretion of IL-10 in normal mice and ST-infected mice, and inhibits the secretion of TNF-α in ST-infected mice.

Pidotimod Elevated the $CD4^+/CD8^+$ Ratio in the Blood of ST-Infected Mice

Flow cytometry revealed that, after two days of infection, the $CD4^+/CD8^+$ cell ratios in the blood of pidotimod administered groups (PH + ST and the PL + ST groups) were significantly elevated compared to those of the ST group ($P < 0.05$) (Fig. 5).

Discussion

This study revealed the effects of pidotimod in normal and ST-infected mice. Other studies have documented that active dosages of pidotimod against bacterial infections in mice range from 0.01 to 100 μg/g (Coppi et al. 1994). As for our study, we used two experimental concentrations (12.5 μg/g and 200 μg/g). We found that although pidotimod did not exert a significant effect on body weights in normal mice, pretreatment with a high-dose of pidotimod alleviated ST infection induced weight loss. These results are consistent with those of Coppi et al. who concluded that pretreatment

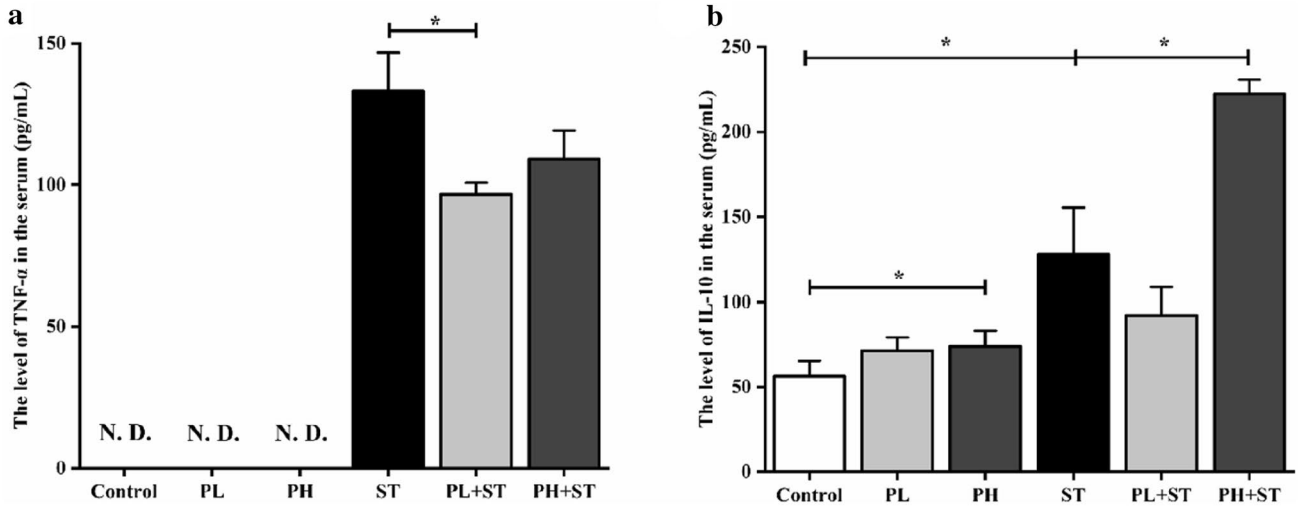

Fig. 4 Cytokine levels in serum were quantified by ELISA. **a** TNF-α levels in serum. *N. D.* not detected. **b** IL-10 levels in serum. Data is presented as the means ± SD (n = 3; $*P < 0.05$)

with pidotimod has protective effects against bacterial infections in mice (Coppi et al. 1994). It is worth mentioning that the spleen plays an important role in resistance to infection (Morris and Bullock 1919), and the splenic index reflects immune functions of the body during infections (Hadidi et al. 2008). We found that high-dose pidotimod relieved the increase in splenic index caused by pathogenic microbe infection and the acute inflammatory cell infiltration. However, in our study, pidotimod could not significantly reduce *Salmonella* burden in the spleen, which was an interesting phenomenon. In addition, *Salmonella* can migrate to the liver when the intestinal barrier is destroyed (Mathur et al. 2012). It also attacks the liver, impairing its functions (Esposito et al. 2018). We found that pidotimod could also relieve ST infection associated increase in liver indices of mice. Moreover, pidotimod alleviated ST infection associated liver inflammation and colon tissue lesions. These results suggest that it could alleviate the tissue damage caused by *Salmonella* infections, in tandem with the findings of Zhao et al. who reported that pidotimod could significantly decrease the number of medium-sized inflammatory foci of neutrophil infiltrates in the liver of mice after parasitic infections (Zhao et al. 2013).

Infection with pathogenic microbes, such as *Salmonella*, often leads to a significant inflammatory response (Dube et al. 2001). In this study, pidotimod suppressed the serum level of pro-inflammatory cytokine, TNF-α, and elevated the level of anti-inflammatory cytokine, IL-10. It has been reported that pidotimod can modify the immune responses that are triggered by TLR ligands and attenuate TLR-induced inflammation (Fogli et al. 2014). In addition, other studies have shown that pidotimod can be used in the treatment of inflammation-associated diseases (Caruso and Fiorentini 2016). We postulate that one of the ways in which pidotimod resists ST infection is by relieving inflammation. GOT and GPT can be used as important liver injury indicators (Huang et al. 2006). We found that high doses of pidotimod relieved elevated serum GOT and GPT levels after ST infection, implying that pidotimod may be effective in alleviating *Salmonella*-induced liver damage.

$CD4^+$ T cells play important roles in immune protection (Zhu and Paul, 2008). $CD8^+$ T cells also respond to viral infections and participate in defenses against bacterial and protozoal infections (Wong and Pamer, 2003). The $CD4^+$/$CD8^+$ T cell ratio is closely associated with the immune ability of the body, and is increasingly becoming a valuable indicator of immune activation (Bruno et al. 2017). A high ratio indicates that the immune function is relatively strong (Amadori et al. 1995), while a decreased $CD4^+/CD8^+$ ratio is associated with impaired cellular immunity (Wang et al. 2017). Pidotimod stimulated cellular immune responses by promoting lymphocyte proliferation (Fogli et al. 2014). In this experiment, pidotimod could significantly increase the $CD4^+/CD8^+$ T cell ratio in the blood of ST-infected mice, implying that pidotimod enhances immune cell functions against ST infection. This finding is consistent with that of other studies that showed that pidotimod improved the level of T-lymphocyte subtypes in recurrent respiratory tract infections (Niu et al. 2019).

Conclusions

A high dose of pidotimod (200 μg pidotimod per gram of body weight) exhibits protective effects against ST infection in mice. These findings highlight the significance of

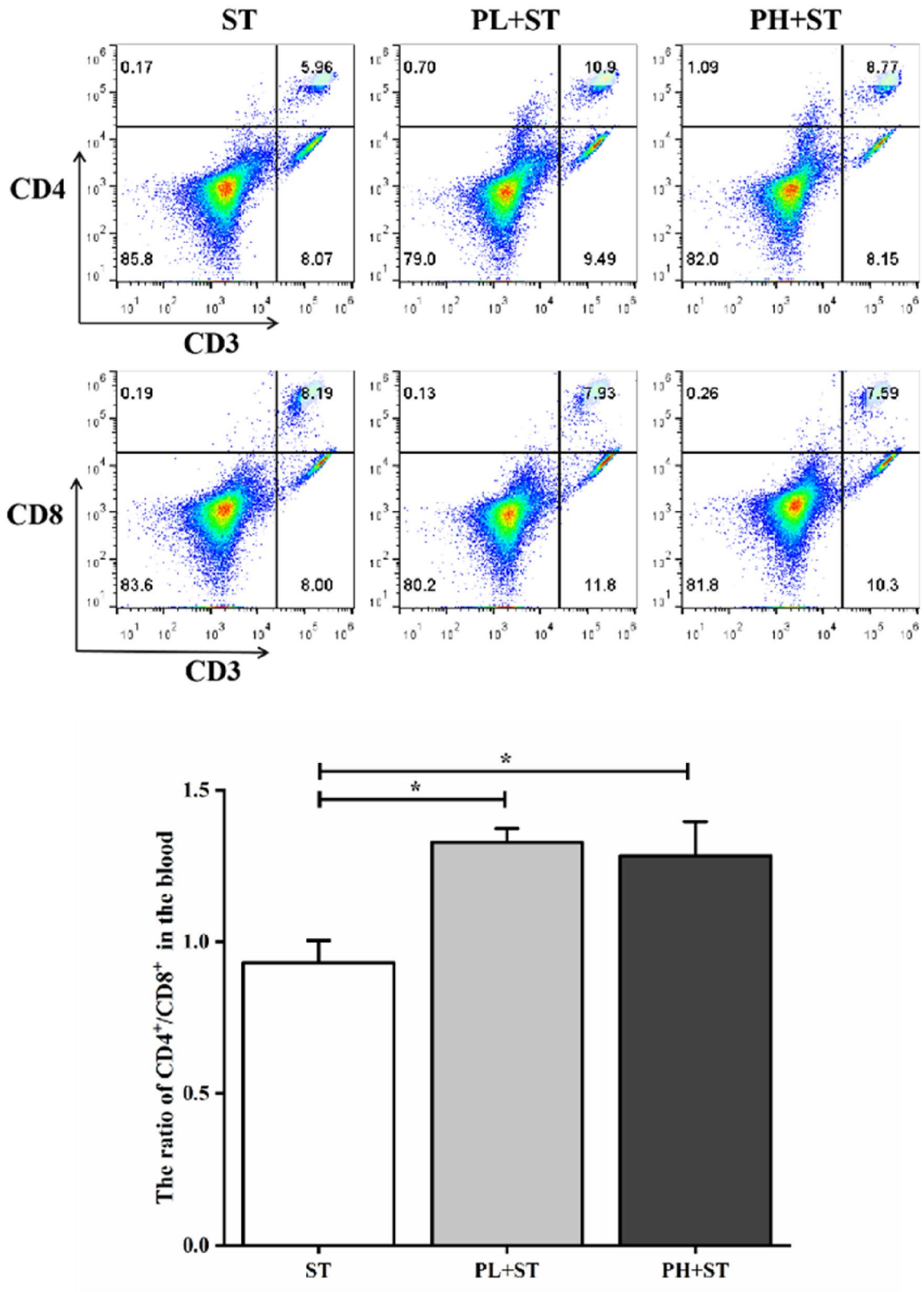

Fig. 5 $CD4^+/CD8^+$ cell ratios in blood. Data is presented as the means ± SD (n = 3; $*P < 0.05$)

pidotimod as a positive immunomodulator in defense against ST challenge and provides a theoretical basis for the application of pidotimod in animal husbandry to resist bacterial infections.

Author Contributions XZ and LF conceptualized the experiments; YZ and LF performed the experiments; YZ wrote the original draft; YZ and LF analyzed the data; XY and the rest of the authors revised the manuscript. All authors have read and agreed to the published version of the manuscript.

Declarations

Conflict of interest The authors declare no conflict of interest.

References

Amadori A, Zamarchi R, De Silvestro G, Forza G, Cavatton G, Danieli GA, Clementi M, Chieco-Bianchi L (1995) Genetic control of the CD4/CD8 T-cell ratio in humans. Nat Med 1:1279–1283

Anderson CJ, Kendall MM (2017) *Salmonella enterica* Serovar Typhimurium strategies for host adaptation. Front Microbiol 8:1983

Auteri A, Pasqui AL, Bruni F, Saletti M, Di Renzo M, Bova G (1992) Effect of pidotimod, a new immunostimulating agent, on some aspects of immune response vitro study. Pharmacol Res 26(Suppl 2):196–197

Bopp CA (1999) *Escherichia*, *Shigella*, and *Salmonella*. Manual of clinical microbiology. SM Press, Washington

Bruno G, Saracino A, Monno L, Angarano G (2017) The revival of an "old" marker: CD4/CD8 ratio. AIDS Rev 19:81–88

Carta S, Silvestri M, Rossi GA (2013) Modulation of airway epithelial cell functions by Pidotimod: NF-kB cytoplasmatic expression and its nuclear translocation are associated with an increased TLR-2 expression. Ital J Pediatr 39:29

Caruso A, Fiorentini S (2016) Pidotimod for use in the treatment of inflammation-associated diseases. Google Patents

Coppi G, Falcone A, Manzardo S (1994) Protective effects of pidotimod against experimental bacterial infections in mice. Arzneimittelforschung 44:1417–1421

Dougan G, John V, Palmer S, Mastroeni P (2011) Immunity to salmonellosis. Immunol Rev 240:196–210

Dube PH, Revell PA, Chaplin DD, Lorenz RG, Miller VL (2001) A role for IL-1 alpha in inducing pathologic inflammation during bacterial infection. Proc Natl Acad Sci USA 98:10880–10885

Esposito S, Garziano M, Rainone V, Trabattoni D, Biasin M, Senatore L, Marchisio P, Rossi M, Principi N, Clerici M (2015) Immunomodulatory activity of pidotimod administered with standard antibiotic therapy in children hospitalized for community-acquired pneumonia. J Transl Med 13:1–10

Esposito E, Campolo M, Casili G, Lanza M, Franco D, Filippone A, Peritore AF, Cuzzocrea S (2018) Protective effects of xyloglucan in association with the polysaccharide gelose in an experimental model of gastroenteritis and urinary tract infections. Int J Mol Sci 19:1844

Ferrario BE, Garuti S, Braido F, Canonica GW (2015) Pidotimod: the state of art. Clin Mol Allergy 13:8

Fogli M, Caccuri F, Iaria ML, Giagulli C, Corbellini S, Campilongo F, Caruso A, Fiorentini S (2014) The immunomodulatory molecule pidotimod induces the expression of the NOD-like receptor NLRP12 and attenuates TLR-induced inflammation. J Biol Regul Homeost Agents 28:753–766

Güçlü BK, Kara K, Çakır L, Çetin E, Kanbur M (2011) Carnitine supplementation modulates high dietary copper-induced oxidative toxicity and reduced performance in laying hens. Biol Trace Elem Res 144:725–735

Hadidi S, Glenney GW, Welch TJ, Silverstein JT, Wiens GD (2008) Spleen size predicts resistance of rainbow trout to *Flavobacterium psychrophilum* challenge. J Immunol 180:4156–4165

Huang X, Choi Y, Im H, Yarimaga O, Yoon E, Kim H (2006) Aspartate aminotransferase (AST/GOT) and alanine aminotransferase (ALT/GPT) detection techniques. Sensors-Basel 6:756–782

Manzardo S, Falcone A, Pinzetta A, Ieva G, Coppi G (1994) General pharmacology of pidotimod and testing for drug interactions. Arzneimittelforschung 44:1441–1447

Mathur R, Oh H, Zhang D, Park SG, Seo J, Koblansky A, Hayden MS, Ghosh S (2012) A mouse model of *Salmonella typhi* infection. Cell 151:590–602

Morris DH, Bullock FD (1919) The importance of the spleen in resistance to infection. Ann Surg 70:513–521

Niu H, Wang R, Jia Y, Cai Y (2019) Pidotimod, an immunostimulant in pediatric recurrent respiratory tract infections: a meta-analysis of randomized controlled trials. Int Immunopharmacol 67:35–45

Puggioni F, Alves-Correia M, Mohamed MF, Stomeo N, Mager R, Marinoni M, Racca F, Paoletti G, Varricchi G, Giorgis V, Melioli G, Canonica GW, Heffler E (2019) Immunostimulants in respiratory diseases: focus on Pidotimod. Multidiscip Respir Med 14:31

Riboldi P, Gerosa M, Meroni PL (2009) Pidotimod: a reappraisal. Int J Immunopathol Pharmacol 22:255–262

Trobonjaca Z, Kroger A, Stober D, Leithauser F, Moller P, Hauser H, Schirmbeck R, Reimann J (2002) Activating immunity in the liver. II. IFN-beta attenuates NK cell-dependent liver injury triggered by liver NKT cell activation. J Immunol 168:3763–3770

Wang K, Shen T, Siegal GP, Wei S (2017) The CD4/CD8 ratio of tumor-infiltrating lymphocytes at the tumor-host interface has prognostic value in triple-negative breast cancer. Hum Pathol 69:110–117

Wei S, Huang J, Liu Z, Wang M, Zhang B, Lian Z, Guo Y, Han H (2019) Differential immune responses of C57BL/6 mice to infection by *Salmonella enterica* serovar Typhimurium strain SL1344, CVCC541 and CMCC50115. Virulence 10:248–259

Wong P, Pamer EG (2003) CD8 T cell responses to infectious pathogens. Annu Rev Immunol 21:29–70

Wotzka SY, Nguyen BD, Hardt W (2017) Salmonella typhimurium diarrhea reveals basic principles of enteropathogen infection and disease-promoted DNA exchange. Cell Host Microbe 21:443–454

Zhao Y, Huang B, Huang S, Zheng H, Li YQ, Lun ZR, Shen J, Wang Y, Kasper LH, Lu F (2013) Evaluation of the adjuvant effect of pidotimod on the immune protection induced by UV-attenuated *Toxoplasma gondii* in mouse models. Parasitol Res 112:3151–3160

Zhen W, Shao Y, Gong X, Wu Y, Geng Y, Wang Z, Guo Y (2018) Effect of dietary *Bacillus coagulans* supplementation on growth performance and immune responses of broiler chickens challenged by *Salmonella enteritidis*. Poultry Sci 97:2654–2666

Zhu J, Paul WE (2008) CD4 T cells: fates, functions, and faults. Blood 112:1557–1569