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ORIGINAL ARTICLE

L-Glutamine and L-arginine protect against enterotoxigenic *Escherichia coli* infection via intestinal innate immunity in mice

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Abstract Dietary glutamine (Gln) or arginine (Arg) supplementation is beneficial for intestinal health; however, whether Gln or Arg may confer protection against Enterotoxigenic *Escherichia coli* (ETEC) infection is not known. To address this, we used an ETEC-infected murine model to investigate the protective effects of Gln and Arg. Experimentally, we pre-treated mice with designed diet of Gln or Arg supplementation prior to the oral ETEC infection and then assessed mouse mortality and intestinal bacterial burden. We also determined the markers of intestinal innate immunity in treated mice, including secretory IgA response (SIgA), mucins from goblet cells, as well as antimicrobial peptides from Paneth cells. ETEC colonized in mouse small intestine, including duodenum, jejunum, and ileum, and inhibited the mRNA expression of intestinal immune factors, such as polymeric immunoglobulin receptor (pIgR), cryptdin-related sequence 1C (CRS1C), and Reg3γ. We found that dietary Gln or Arg supplementation decreased bacterial colonization and promoted the activation of innate immunity (e.g., the mRNA expression of pIgR, CRS1C, and Reg3γ) in the intestine of ETEC-infected mice. Our results suggest that dietary arginine or glutamine supplementation may inhibit intestinal ETEC infection through intestinal innate immunity.

Keywords Enterotoxigenic *E. coli* · Glutamine · Arginine · Paneth cell · Innate immunity

Introduction

Enterotoxigenic *Escherichia coli* (ETEC) is a pathogenic bacterium and the leading cause of bacterial diarrhoeal disease. It causes diarrhea in travelers and children under the age

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of 5 in the developing world, which is thought to be responsible for the death of at least 300,000 every year (Rowe et al. 1970; Sears and Kaper 1996). ETEC is not only causing human infection, but also inducing diarrhea in piglets which causes a big economical loss in swine industry (Ren et al. 2015, 2016a). The mechanism for ETEC infection is dependent on its fimbrial adhesins, which interact with the brush border of enterocytes to initiate the colonization of ETEC and secretion of enterotoxins (Fleckenstein et al. 2010; Sears and Kaper 1996). ETEC infection also results in diarrhea and other intestinal functions, such as immune responses (Loos et al. 2012; Ren et al. 2014c, 2016a, 2017a; Wang and Hardwidge 2012; Wang et al. 2012), tight junction function (Kreisberg et al. 2011; Nakashima et al. 2013; Ngendahayo Mukiza and Dubreuil 2013; Nassour and Dubreuil 2014), and autophagy (Tang et al. 2014).

Mounting lines of evidence have shown that dietary supplements have beneficial effects in intestine against ETEC infection, such as *Clostridium butyricum* (Zhang et al. 2016), seaweed extracts (Heim et al. 2014), and chitosan (Xiao et al. 2014, 2016). Arginine (Arg) supplementation improves the reproductive performance of PCV2-infected pregnant mice, including decreased abortion rate of pregnant mice and mortality of neonates, and increased litter number, litter birth weight, as well as daily weight gain of neonates (Ren et al. 2012). Arg supplementation also increases the serum progesterone and nitric oxide (NO) levels, serum superoxide dismutase (SOD) activity, and total antioxidant capacity (T-AOC) in PCV2-infected pregnant mice (Ren et al. 2012). Glutamine (Gln) supplementation attenuates microscopic lesions in maternal tissues (e.g., liver, lung, and spleen) induced by PCV2, and alters the immune responses against PCV2 (Ren et al. 2013a, c). These findings suggest that dietary Arg or Gln supplementation may confer protective advantages against ETEC infection. In essence, dietary Arg or Gln supplementation alters intestinal microbiota and activated intestinal innate immunity, including activation of Toll-like receptors (TLR) signaling, expression of pro-inflammatory cytokines, and secretory immunoglobulin A (SIgA) (Ren et al. 2014a, b, 2016b, c, 2017b; Wu et al. 2016). Taken together, we hypothesized that dietary supplementation of Arg or Gln has beneficial effects against ETEC infection, and used an ETEC-infected murine model to dissect the antibacterial and immunomodulation effects of Arg and Gln.

Materials and methods

Bacterium

ETEC SEC 470 (serotype O4; oqxAB; F18; STa, STb, LT, SLT-IIe) isolate was obtained from a 39-day-old diarrheal piglet from Jingxi Province, China (Ren et al. 2014c). ETEC was cultured in LB medium.

Experimental design

Female Institute for Cancer Research (ICR) mice (6-week-old) were purchased from SLAC Laboratory Animal Central (Changsha, China). They were housed in sterile mouse colonies (temperature 20–30 °C; relative humidity 45–60%; lighting cycle 12 h/day). Mice had access to food and drinking water ad libitum. Glutamine and arginine were obtained from Ajinomoto Inc., Tokyo, Japan. Mice were randomly divided into eight groups: groups 1 mice ($n = 20$) were treated basal diet (Li and Neu 2009); group 2 mice ($n = 20$) were treated with basal diet with dietary 0.4% (w/w) arginine supplementation; group 3 mice ($n = 20$) were treated with basal diet with dietary 0.8% arginine supplementation; group 4 mice ($n = 20$) were treated with basal diet with dietary 1.5% arginine supplementation; group 5 mice ($n = 20$) were treated the basal diet; group 6 mice ($n = 21$) were treated with basal diet with dietary 0.5% glutamine supplementation; group 7 mice ($n = 21$) were treated with basal diet with dietary 1.0% glutamine supplementation; and group 8 mice ($n = 21$) were treated with basal diet with dietary 2.0% glutamine supplementation. The concentration of amino acids in the basal diet was reported previously (Ren et al. 2012, 2014a, b). After 1 week of pretreatment with their diets, mice in all groups except group 1 were orally infected with 5×10^8 *E. coli* SEC 470. ETEC infection in mice was conducted according to our previous method (Ren et al. 2014c). Mortality following infection was recorded within 24 h. All alive mice were sacrificed at 24 h post infection to collect samples. For the collection of the luminal contents of jejunum and ileum, whole jejunal or ileal luminal contents were collected by washing with phosphate buffered saline (PBS; pH 7.2–7.4). For duodenum, jejunum, and ileum collection, the intact duodenum, jejunum, and ileum samples (middle, about 3 cm) were collected after PBS (pH 7.2–7.4) washing. Samples were stored at −80 °C until processing.

Bacterial counting

To quantify the ETEC load in the duodenum, jejunum, and ileum, tissues (about 100 mg) were homogenized in saline, plated onto MacConkey Agar in serial dilutions, and incubated at 37 °C. Colony-forming units (CFU) were then counted after 16 h of incubation. To confirm that the bacteria were ETEC, the colonies were tested using PCR with primers (5′-CTGTATACGTGGCAG-3′) and (5′-ACTATGGTGAATGCTCAC-3′) obtained from ETEC *fedF* gene (GenBank accession no. Z26520). The number of bacteria in each mouse was calculated in terms of CFU/g.

Gene expression analysis

Total RNA was isolated from liquid nitrogen-frozen jejunum or ileum with TRIzol regent (Invitrogen, USA) and then treated with DNase I (Invitrogen, USA) in accordance with the manufacturer's instructions. Primers were reported in our previous studies (Ren et al. 2014a, b). β-Actin was used as the reference gene. One-step real-time PCR was performed according to our previous reports (Ren et al. 2013b, c). The relative expressions of the target genes were determined by real-time PCR performed using an ABI7900HT PCR system (Applied Biosystems, Forrest City, CA, USA). Real-time PCR was performed in triplicate for each cDNA sample, using SYBR Green I as a PCR core reagent in a final volume of 10 μL.

Levels of SIgA in the luminal contents of jejunum and ileum

Levels of secretory IgA (SIgA) present in the lumen of the jejunum and ileum were detected using ELISA Kits (Cusabio Biotech Co. Ltd., China) according to the manufacturer's instructions (Wu et al. 2016). Briefly, test samples were added at 100 μL per well in triplicate wells. Standard and negative controls were also added at 100 μL per well in triplicate wells. The plate was incubated at 37 °C for 2 h before adding the biotin antibody at 100 μL per well for 1 h of incubation at 37 °C. Then, HRP-avidin was added at 100 μL per well, followed by incubation at room temperature for 1 h. 90 μL of 3,3,5,5′-tetramethylbenzidine (TMB) substrate solution was added into each well, and incubated at room temperature for 15 min, followed by addition of stop solution at 50 μL per well. Absorbance at 450 nm [*A*(450)] was measured in a Microplate reader. Interpretation was made using calibration curve prepared according to the manufacturer's protocol.

Statistical analysis

Statistical analysis was performed using SPSS software, version 16 (Chicago, IL, USA). Mortality data between ETEC and arginine or between ETEC and glutamine group were analyzed using the Chi-square. Data about bacterial burden among ETEC, ETEC + 0.4% arginine, ETEC + 0.8% arginine, ETEC + 1.5% arginine, or among ETEC, ETEC + 0.5% glutamine, ETEC + 1.0% glutamine, ETEC + 2.0% glutamine, were analyzed by the one-way ANOVA method (Ren et al. 2014d). Data about gene expression of innate immune regulators or intestinal contents of SIgA between Control and ETEC, or between ETEC and ETEC + 0.4% arginine, or between ETEC and ETEC + 2.0% glutamine were analyzed by the Student's *t* test (Ren et al. 2014a). Differences with $P < 0.05$ were considered significant.

Results

Dietary arginine supplementation decreased mortality and bacterial burden

Six mice died within 24 h of exposure to ETEC. Dietary arginine supplementation excised survival advantages, with no dead mouse from mice treated with dietary 0.4% arginine supplementation, but one dead mouse from mice treated with dietary 0.8% arginine supplementation and two dead mice from mice treated with dietary 1.5% arginine supplementation (Table 1). Similar to the reduced mortality, dietary arginine supplementation reduced the bacterial burden in the duodenum, jejunum, and ileum (Fig. 1). In the duodenum, all dosages of dietary arginine supplementation significantly ($P < 0.05$) decreased the bacterial burden, compared to mice with basal diet (Fig. 1a). Mice with dietary 0.4% or 1.5% arginine supplementation had lower ($P < 0.05$) bacterial burden than mice with dietary 0.8% arginine supplementation (Fig. 1a). In the jejunum, mice with dietary 0.4 or 0.8% arginine supplementation had lower ($P < 0.05$) bacterial burden than mice with basal diet and mice with dietary 0.8% arginine supplementation had lower ($P < 0.05$) bacterial burden than mice with dietary 0.4 or 1.5% arginine supplementation (Fig. 1b). In the ileum, mice with dietary 0.4% arginine supplementation had lower ($P < 0.05$) bacterial burden than mice with basal diet or mice with dietary 0.8% arginine supplementation, while there was no difference in bacterial burden among mice with dietary 0.8% arginine supplementation, with dietary 1.5% arginine supplementation or with basal

Table 1 Mortality in different groups

Group	Total number	Dead number	Sig*
Control	20	0	Yes
ETEC	20	6	
ETEC + 0.4% arginine	20	0	Yes
ETEC + 0.8% arginine	20	1	No
ETEC + 1.5% arginine	20	2	No
ETEC + 0.5% glutamine	21	2	No
ETEC + 1.0% glutamine	21	2	No
ETEC + 2.0% glutamine	21	4	No

Mice (except from Control group) were infected with 5×10^8 *E. coli* SEC 470 for 24 h. The mortality of each group was collected

* Compared to ETEC group with the Chi-square analysis

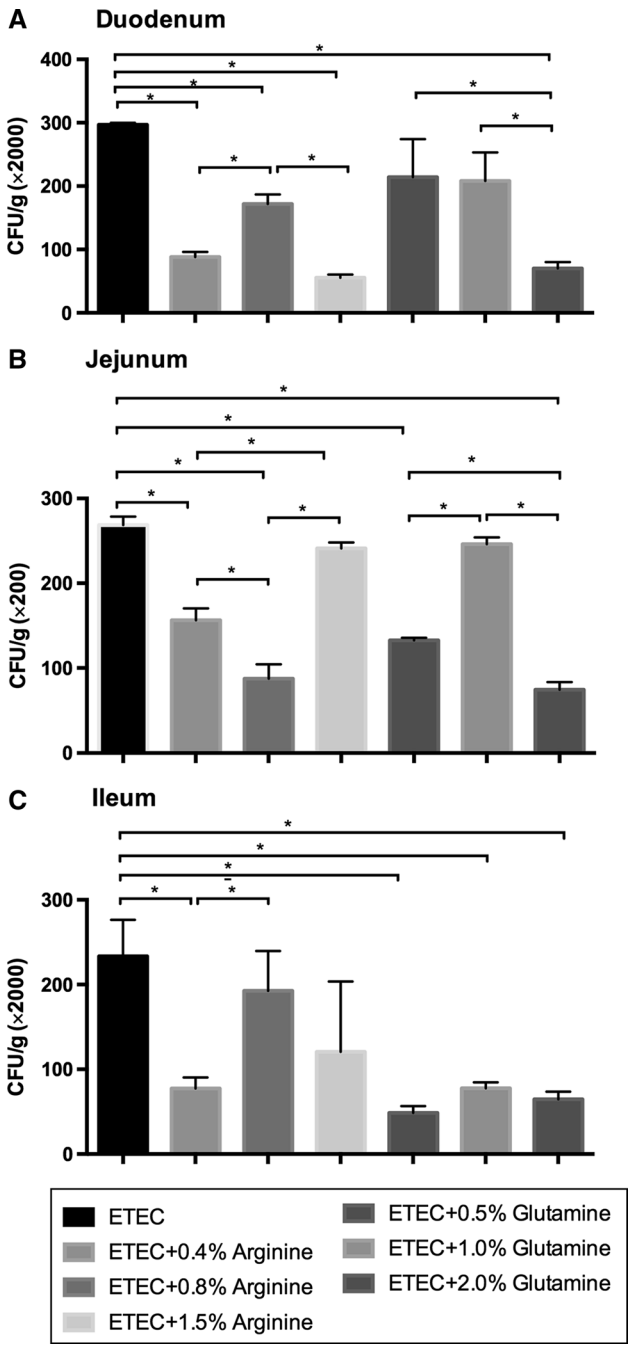

Fig. 1 Bacterial burden in mouse small intestine. **a** Bacterial burden in mice duodenum. **b** Bacterial burden in mice jejunum. **c** Bacterial burden in mice ileum. Mice were fed a basal diet (ETEC), or basal diet with dietary arginine supplementation with dosage of 0.4% (ETEC + 0.4% arginine), or 0.8% (ETEC + 0.8% arginine), or 1.5% (ETEC + 1.5% arginine), or basal diet with dietary glutamine supplementation with dosage of 0.5% (ETEC + 0.5% glutamine), or 1.0% (ETEC + 1.0% glutamine), or 2.0% (ETEC + 2.0% glutamine) for 1 week before enterotoxigenic *E. coli* (ETEC) infection. Twenty-four hours after ETEC infection, bacterial burden in the duodenum, jejunum, and ileum was assessed. Data are mean ± SEM, $n = 6$, $^*P < 0.05$. The finally ETEC burden in mice of duodenum and ileum should multiply 2000, while 200 for mouse jejunum

diet (Fig. 1c). In conclusion, dietary arginine supplementation decreases the mortality and bacterial burden in ETEC-infected mice, and dietary 0.4% arginine supplementation shows better effect.

Dietary glutamine supplementation decreased bacterial burden

Glutamine supplementation had little effect on mortality of ETEC-infected mice. The number of dead mice in mice treated with dietary 0.5, or 1.0, or 2.0% glutamine supplementation was 2, 2, and 4, respectively (Table 1). Like the arginine, dietary glutamine supplementation affected the bacterial burden in mouse small intestine (Fig. 1). In the duodenum, dietary 2.0% glutamine supplementation significantly ($P < 0.05$) decreased the bacterial burden, although dietary 0.5 or 1.0% glutamine supplementation had little effect on that (Fig. 1a). Mice with dietary 2.0% glutamine supplementation had lower ($P < 0.05$) bacterial burden than mice with dietary 0.5 or 1.0% glutamine supplementation (Fig. 1a). In the jejunum, dietary 0.5 or 2.0% glutamine supplementation significantly ($P < 0.05$) decreased the bacterial burden, although dietary 1.0% glutamine supplementation had little effect on that (Fig. 1b). Mice with dietary 2.0% glutamine supplementation had lower ($P < 0.05$) bacterial burden than mice with dietary 0.5 or 1.0% glutamine supplementation (Fig. 1b). In the ileum, all dosages of dietary glutamine supplementation significantly ($P < 0.05$) decreased the bacterial burden compared to the basal diet (Fig. 1c), while there was no difference in the bacterial burden among different dosages of glutamine supplementation (Fig. 1c). Overall, although dietary glutamine supplementation has little effect on the mortality of ETEC-infected mice, dietary 2.0% glutamine supplementation decreases the bacterial burden in mouse small intestine.

Dietary arginine or glutamine supplementation ameliorated intestinal innate immune response

As the greatest reduction in mortality and bacterial loads was seen with dietary with 0.4% arginine or 2.0% glutamine supplementation, further studies into intestinal immunity were conducted on samples collected from these groups. Of interest were SIgA, Paneth antimicrobials, and mucins produced from goblet cells.

To maintain homeostasis of the mucosa, SIgA is produced in the mucosal lining and secreted into the lumen to protect mucosal membranes against bacterial invasion. To determine the effect of ETEC in SIgA, mRNA expression of J-chain and the polymeric immunoglobulin receptor (pIgR), which is responsible for SIgA transportation through the cell, were detected. ETEC infection

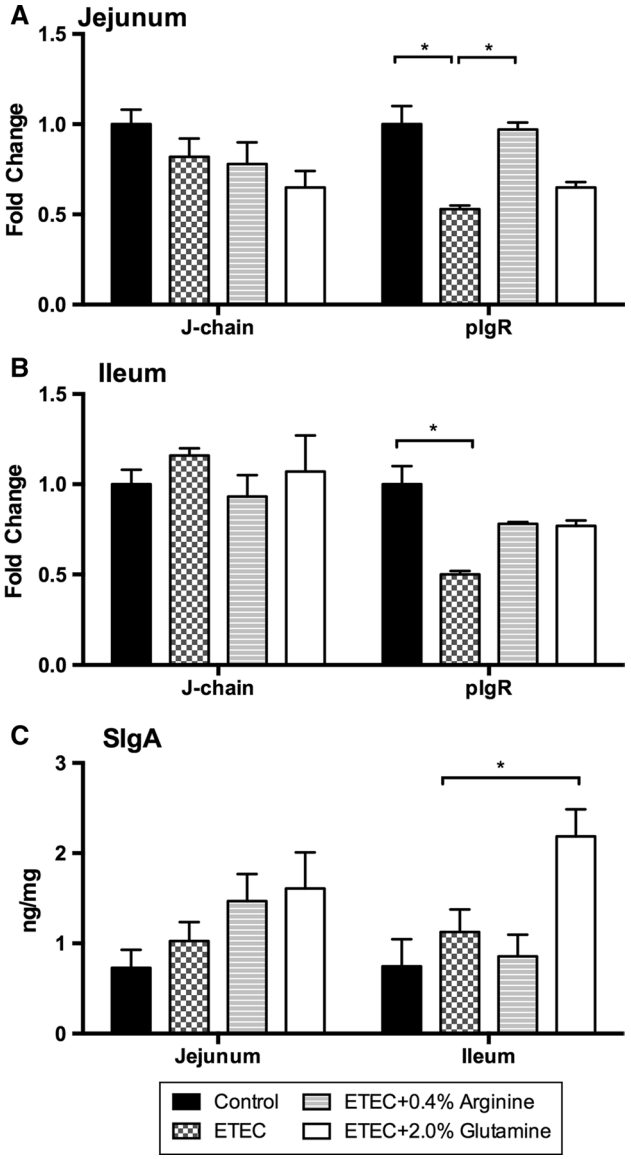

Fig. 2 Intestinal secretory IgA (SIgA) production. **a** mRNA expression of J-chain and polymeric immunoglobulin receptor (pIgR) in the jejunum. **b** mRNA expression of J-chain and pIgR in the ileum. **c** Levels of SIgA in the luminal contents of jejunum and ileum. Mice were fed a basal diet (ETEC), or basal diet with dietary 0.4% arginine supplementation (ETEC + 0.4% arginine), or basal diet with dietary 2.0% glutamine supplementation (ETEC + 2.0% glutamine) for 1 week before enterotoxigenic *E. coli* (ETEC) infection. Mice in control group were fed with basal diet and without ETEC infection. Data are mean ± SEM, $n = 6$, $^*P < 0.05$

had little effect on the mRNA expression of J-chain in mouse jejunum and ileum (Fig. 2a, b); however, it significantly ($P < 0.05$) inhibited the mRNA expression of pIgR in the jejunum and ileum (Fig. 2a, b). Dietary arginine or glutamine supplementation had little effect on the mRNA expression of J-chain in mouse jejunum and ileum (Fig. 2a, b). Dietary arginine supplementation promoted ($P < 0.05$) the mRNA expression of pIgR in the jejunum, but had little effect on it in the ileum (Fig. 2a, b). Dietary glutamine supplementation had little effect on the mRNA expression of pIgR in the jejunum and ileum (Fig. 2a, b). For SIgA contents in the luminal contents of the small intestine, ETEC infection had little effect on the concentration of SIgA in the luminal contents of jejunum and ileum (Fig. 2c). Dietary arginine supplementation had little effect on the concentration of SIgA in the luminal contents of jejunum and ileum (Fig. 2c). Although dietary glutamine supplementation had little effect on the concentration of SIgA in the luminal contents of jejunum, it significantly ($P < 0.05$) promoted the concentration of SIgA in the luminal contents of ileum (Fig. 2c).

Mucus is one of the various protective mechanisms employed by the gastrointestinal tract. Mucus contains mucin glycoproteins, the structure of which provides mechanical resistance against the adhesion and invasion of bacterial and viral pathogens (Vereecke et al. 2011). There are a number of different mucins. This study focused on the expression of Mucin 2, which is secreted, and Mucin 4, which is membrane-bound. ETEC infection had little effect on the mRNA expressions of Mucin 2 and 4 in mouse jejunum and ileum (Fig. 3a, b). Dietary arginine supplementation promoted ($P < 0.05$) the mRNA expression of Mucin 2 in the jejunum, but had little effect on it in the ileum (Fig. 3a, b). Dietary arginine supplementation had little effect on the mRNA expression of Mucin 4 in the jejunum and ileum (Fig. 3a, b). Dietary glutamine supplementation had little effect on the mRNA expression of Mucin 2 and 4 in mouse jejunum and ileum (Fig. 3a, b).

Antimicrobial peptides are secreted by Paneth cells in the small intestine to provide important mucosal defences against intestinal pathogens. Among the Paneth cell secretions in mice are α-defensins (cryptdin-1,-4, and-5), cryptdin-related sequence (CRS) peptides, C-type lectins (Reg3γ), lysozyme C, and RNase angiogenin 4 (Ang4) (Bevins and Salzman 2011). In the jejunum, ETEC infection inhibited ($P < 0.05$) the mRNA expression of lysozyme C, while had little effect on the mRNA expression of cryptdin-1,-4 and-5, CRS1C, CRS4C, Reg3γ and Ang4 (Fig. 4). Dietary arginine supplementation decreased ($P < 0.05$) the mRNA expression of cryptdin-4 and -5, CRS4C and Ang4, but promoted ($P < 0.05$) the mRNA expression of CRS1C (Fig. 4). Dietary glutamine supplementation decreased ($P < 0.05$) the mRNA expression of cryptdin-4, CRS4C, and Ang4, but had little effect on the mRNA expression of others (Fig. 4). In the ileum, ETEC infection inhibited ($P < 0.05$) the mRNA expression of CRS1C and Reg3γ, while had little effect on the mRNA expression of cryptdin-1,-4, and-5, CRS4C, lysozyme C, and Ang4 (Fig. 5). Dietary arginine supplementation decreased ($P < 0.05$) the mRNA expression of lysozyme C and CRS4C, but promoted ($P < 0.05$) the mRNA expression of CRS1C (Fig. 5).

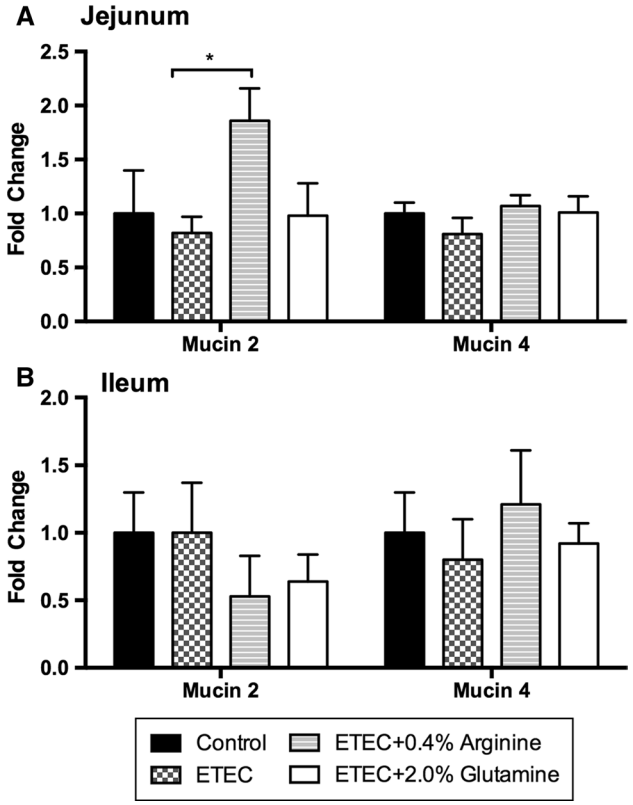

Fig. 3 Mucin2 and 4 expressions. **a** mRNA expression of Mucin2 and 4 in the jejunum. **b** mRNA expression of Mucin2 and 4 in the ileum. Mice were fed a basal diet (ETEC), or basal diet with dietary 0.4% arginine supplementation (ETEC + 0.4% arginine), or basal diet with dietary 2.0% glutamine supplementation (ETEC + 2.0% glutamine) for 1 week before enterotoxigenic *E. coli* (ETEC) infection. Mice in control group were fed with basal diet and without ETEC infection. Data are mean ± SEM, $n = 6$, $^*P < 0.05$

Dietary glutamine supplementation increased ($P < 0.05$) the mRNA expression of Reg3γ, but had little effect on the mRNA expression of others (Fig. 5).

Discussion

For a better understanding of the pathogenesis of ETEC infection in the intestine, an easy handling animal model is needed. By establishing an ETEC-infected mouse model with porcine ETEC isolate(Ren et al. 2014c), we found that ETEC can colonize in mouse small intestine, including the duodenum, jejunum, and ileum, and cause death. In addition, we found that ETEC infection inhibited the mouse intestinal innate immunity, such as mRNA expression of pIgR, lysozyme C, CRS1C, and Reg3γ. The result of pathogen recognition is the activation of a common set of signaling pathways, including nuclear factor (NF)-κB, activator protein-1 (AP-1), and mitogen-activated protein kinase (MAPK), to modulate the host's immune responses against the pathogen (Akira et al. 2006; Takeuchi and Akira 2010; Schroder and Tschopp 2010). However, the pathogen is usually equipped with countermeasures to inhibit the host's immune responses. For example, pathogenic *E. coli* inhibits the activation of NK-κB through its virulent proteins (Gao et al. 2009, 2013; Wan et al. 2011). Indeed, our previous study with proteomics and other molecular methods has found that ETEC infection inhibits the activation of NF-κB and MAPK pathways in the jejunum of piglets (Ren et al. 2016a). Similarly, we also found that ETEC infection inhibits the mRNA expression of TLRs, including TLR-2, 4, 5, 6, 7, 8, 9, and 10, as well as the mRNA expression of other indicators associated with intestinal immunity, including phospholipase A2, lysozyme, pIgR, and Mucin 2 in the jejunum of piglets (Ren et al. 2016a). These results suggest that ETEC colonizes to the small intestine through inactivating the normal immune responses in the small intestine.

Dietary arginine supplementation reduces the intestinal burden of ETEC and disease-associated mortality in mice. The dosage-dependent design of this study reveals that 0.4% arginine supplementation is optimal, which is similar with the previous conclusion that dietary 0.2–0.5% arginine supplementation shows the most significant immunostimulatory effects in mice injected with inactivated *Pasteruella multocida* (Ren et al. 2013d). Similarly, dietary glutamine supplementation confers benefits in mice infected with ETEC. Dietary glutamine supplementation is advantageous in improving reproductive performance in PCV2-infected pregnant mice (Ren et al. 2013c). The optimal dosage of glutamine supplementation to reduce the bacterial load in the small intestine is 2.0%, which is different from our earlier conclusion that the bacterial load of *P. multocida* and its associated virulence factors increase following a diet with glutamine supplementation (Ren et al. 2013b). The reason for this inconsistency has not been determined, but may be from variances in experimental methodology or differences in the infection model.

Arginine or glutamine may inhibit intestinal ETEC colonization through intestinal innate immunity. In this study, arginine or glutamine supplementation promotes mRNA expression of pIgR, Mucin 2, CRS1C, and Reg3γ, as well as the intestinal levels of SIgA. Similarly, previous study has shown that dietary arginine or glutamine supplementation affects intestinal microbiota and activation of intestinal innate immunity (Ren et al. 2014a, b; Wu et al. 2016). Glutamine supplementation increased both the abundance of SIgA in intestinal luminal contents and the number of IgA^+ plasma cells in the mouse ileum, may through the intestinal microbiota and subsequently T cell-dependent and T cell-independent pathways (Wu et al. 2016). Arginine or glutamine supplementation has shown various beneficial functions in intestine challenged with different stimulus (Tan et al. 2015; Wu et al. 2015; Leocadio et al. 2015; Wang et al. 2016; Li and Neu 2009), including epithelial DNA

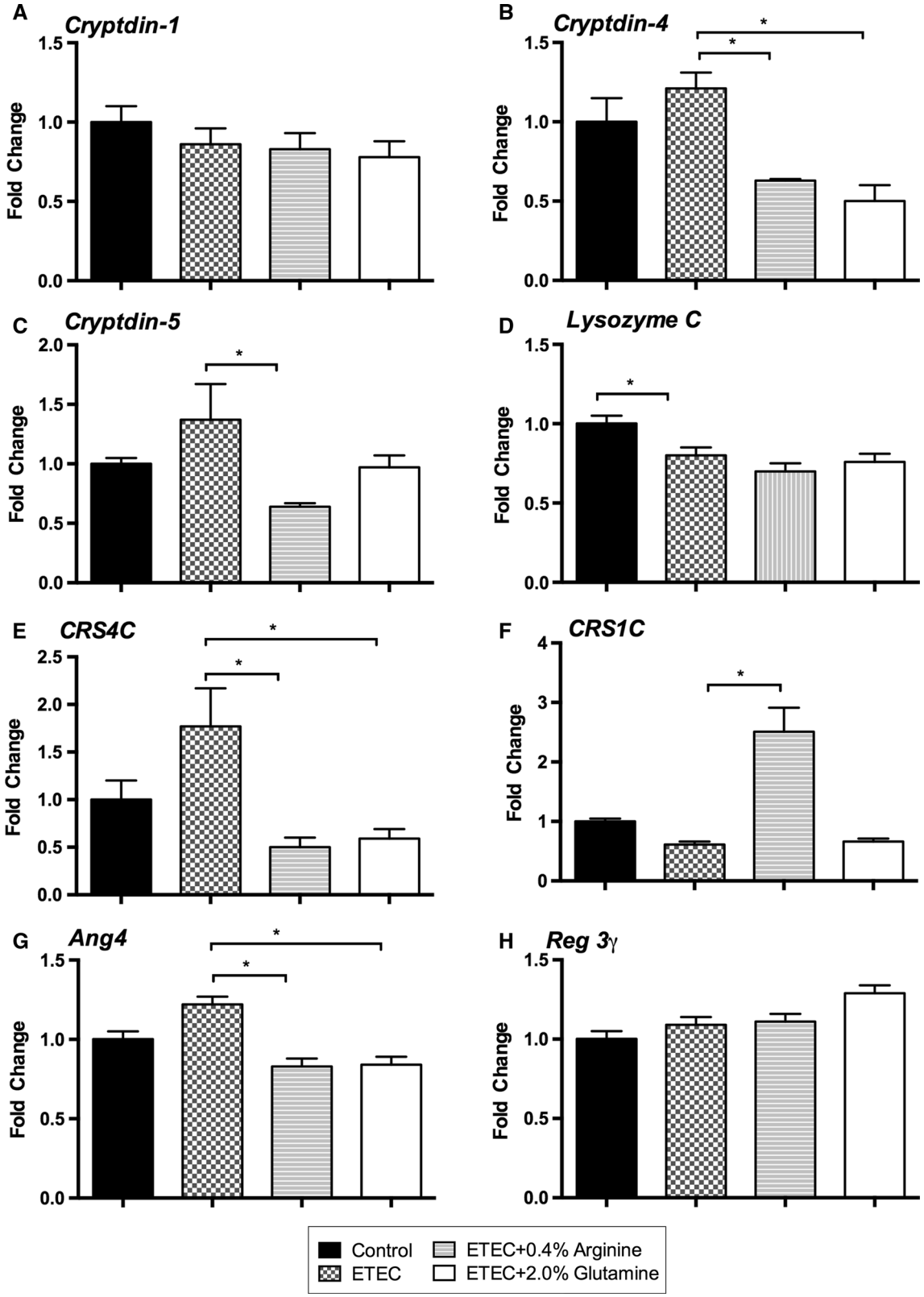

Fig. 4 mRNA expression of antimicrobial peptides secreted by Paneth cells in the jejunum. Mice were fed a basal diet (ETEC), or basal diet with dietary 0.4% arginine supplementation (ETEC + 0.4% arginine), or basal diet with dietary 2.0% glutamine supplementation (ETEC + 2.0% glutamine) for 1 week before enterotoxigenic *E. coli* (ETEC) infection. Mice in control group were fed with basal diet and without ETEC infection. Data are mean ± SEM, $n = 6$, $^*P < 0.05$

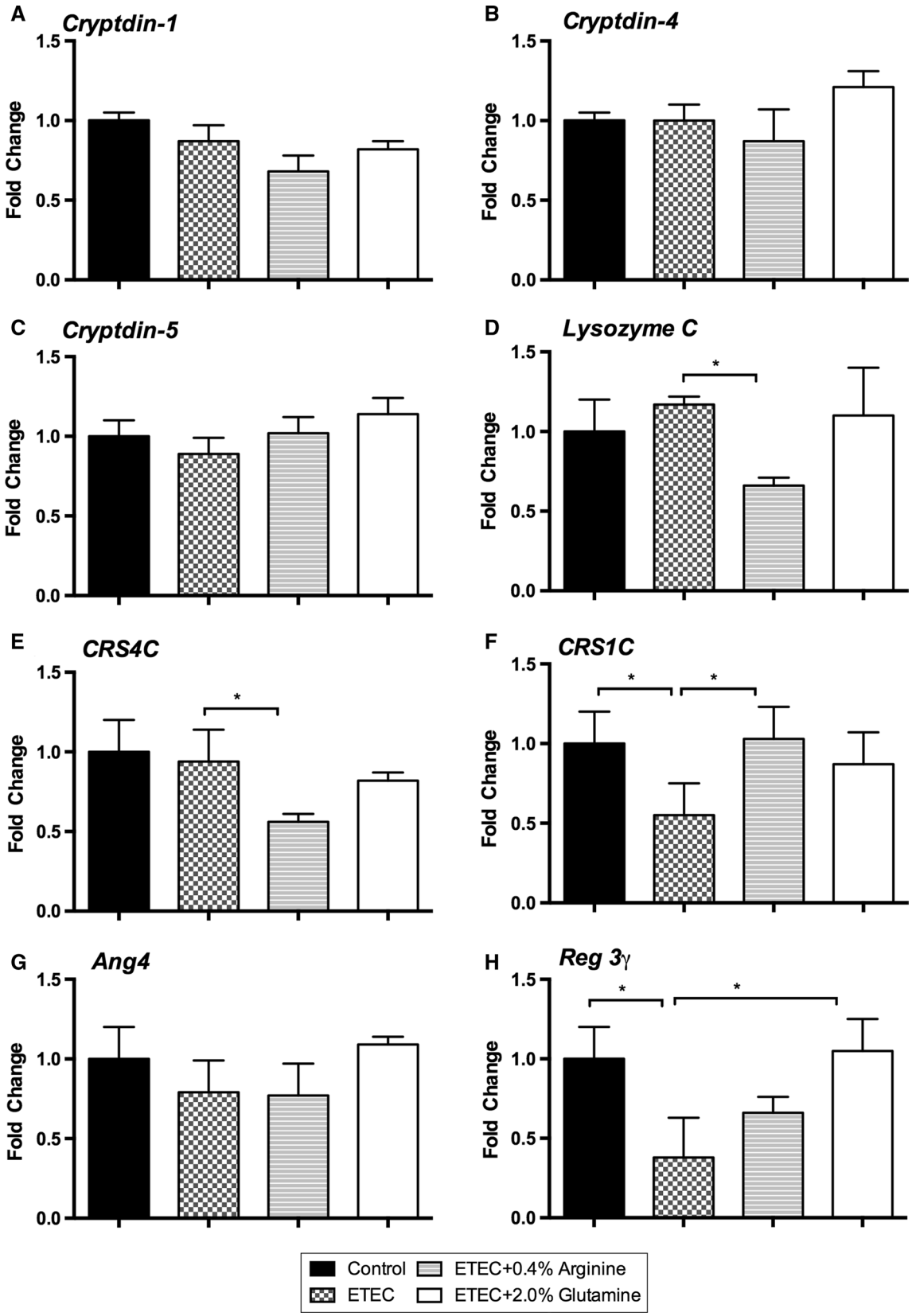

Fig. 5 mRNA expression of antimicrobial peptides secreted by Paneth cells in the ileum. Mice were fed a basal diet (ETEC), or basal diet with dietary 0.4% arginine supplementation (ETEC + 0.4% arginine), or basal diet with dietary 2.0% glutamine supplementation (ETEC + 2.0% glutamine) for 1 week before enterotoxigenic *E. coli* (ETEC) infection. Mice in control group were fed with basal diet and without ETEC infection. Data are mean ± SEM, $n = 6$, $^*P < 0.05$

synthesis and cell-cycle progression, mitochondrial bioenergetics, intestinal morphology and amino acid concentrations, mucosal recovery, intestinal permeability, and tight junction. However, whether arginine or glutamine supplementation inhibits ETEC intestinal colonization through these mechanisms is unknown. Interestingly, arginine or glutamine supplementation also inhibits the mRNA expression of cryptdins 4, lysozyme, CRS4C, and Ang4 in the jejunum or ileum of ETEC-infected mice. This is different from previous observation that arginine or glutamine supplementation promotes the mRNA expression of cryptdin 4, lysozyme, CRS4C, and Ang4 in the jejunum or ileum of healthy mice (Ren et al. 2014a, b). The underlying mechanism for this discrepancy remains to be explored; however, this indicates the complex relationship among intestinal nutrients, intestinal pathogens, and intestinal immunity.

In summary, ETEC colonizes in mouse small intestine, induces mouse mortality, and inhibits mouse intestinal innate immunity. Dietary arginine or glutamine supplementation regulates the outcome of ETEC infection, associating with its regulation in intestinal innate immunity.

Author contributions GL, WR, and YY conceived the experiment(s); GL, WR, JF, GG, JY, SC, and YP conducted the experiments; GL, WR, NAA, and VD analyzed the results; GL, WR, and CAH prepared the manuscript. All authors reviewed the manuscript.

Compliance with ethical standards

The protocol for this study was approved by the Committee on the Ethics of Animal Experiments of Institute of Subtropical Agriculture, Chinese Academy of Sciences (Permit Number: 201206-14), and it was conducted out in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of Institute of Subtropical Agriculture, Chinese Academy of Sciences.

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Conflict of interest The authors declare that they have no conflict of interest.

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