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ORIGINAL ARTICLE

Dietary L-glutamine supplementation increases *Pasteurella multocida* burden and the expression of its major virulence factors in mice

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Abstract This study was conducted to determine the effects of graded doses of L-glutamine supplementation on the replication and distribution of *Pasteurella multocida,* and the expression of its major virulence factors in mouse model. Mice were randomly assigned to the basal diet supplemented with 0, 0.5, 1.0 or 2.0 % glutamine. *Pasteurella multocida* burden was detected in the heart, liver, spleen, lung and kidney after 12 h of *P. multocida* infection. The expression of major virulence factors, toll-like receptors (TLRs), proinflammatory cytokines (interleukin-1 beta, interleukin-6, and tumor necrosis factor alpha) and anti-oxidative factors (GPX1 and CuZnSOD) was analyzed in the lung and spleen. Dietary 0.5 % glutamine supplementation has little significant effect on these parameters, compared to those with basal diet. However, results showed that a high dose of glutamine supplementation increased the *P. multocida* burden ($P < 0.001$) and the expression of its major virulence factors ($P < 0.05$) as compared to those with a lower dose of supplementation. In the lung, high dose of glutamine supplementation inhibited the proinflammatory responses ($P < 0.05$) and TLRs signaling ($P < 0.05$). In the spleen, the effect of glutamine supplementation on different components in TLR signaling depends on glutamine concentration, and high dose of glutamine supplementation activated the proinflammatory response. In conclusion, glutamine supplementation increased *P. multocida* burden and the expression of its major virulence factors, while affecting the functions of the lung and spleen.

Keywords Glutamine · *Pasteurella multocida* · Virulence factor · TLR signaling

Abbreviations

IL-1 β	Interleukin-1 beta
IL-6	Interleukin-6
TNF-α	Tumor necrosis factor alpha

Introduction

L-Glutamine (Gln), is an amino acid crucial for optimal immune responses (Li et al. 2007; Ren et al. 2011; Wu and Flynn 1995), fetal growth and survival (Wu et al. 2010; Ren et al. 2011, 2012a), metabolic regulation (Bonetto et al. 2011; Cooksey and McClain 2011), intracellular redox status and anti-oxidative reactions (Wu 2009; Xi et al. 2011), and alimentary tract health (Brasse-Lagnel et al. 2010). However, the function of Gln is not limited to

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these aspects and plays a role in anti-infection. For example, dietary Gln supplementation reduces inflammation and increases the abundance of mRNAs for anti-inflammatory cytokines in mice after *Helicobacter pylori* infection at 6 weeks post-infection (Hagen et al. 2009). The results of other studies have shown that Gln supplementation reduces the expression of the genes for mucosal cytokines (interleukin-1 beta, IL-1β; interleukin-6, IL-6; and interleukin-10, IL-10) and increases the abundance of tight-junction proteins (claudin-1 and occludin) in *Escherichia coli*-infected weanling piglets (Ewaschuk et al. 2011). Also, we have reported that dietary Gln supplementation is beneficial for ameliorating reproductive failure in porcine circovirus-infected mice (Ren et al. 2011) and for enhancing immune responses in porcine circovirus type 2-infected mice (Ren et al. 2013a).

Collectively, these studies suggest that Gln does exercise beneficial functions in infectious models. However, before recommending its use in clinical practice, it is important to elucidate several critical points. For example, it is critical to identify the suitable time, dose and means of supplementation to optimize its functions under conditions of infection. Most importantly, we lack much-needed experimental data about the effect of Gln supplementation on the replication and distribution of bacteria or the expression of their virulence factors in vivo. Also, regulatory functions of Gln in acute infectious diseases need further investigation. Because concentrations of glutamine in the plasma are reduced in response to infection (Boutry et al. 2012; Zhou et al. 2012), it is important to determine the effects of Gln supplementation on the replication and distribution of a pathogen and the expression of its virulence factors in animals. This goal was achieved in the present study using a mouse model with *Pasteurella multocida* infection.

Materials and Methods

Preparation of the bacterium

The *P. multocida* serotype A (CQ2) strain used in this study was isolated from the lung of clinically infected cattle, which was dead with pneumonia (Ren et al. 2013a, b, c). The bacterium was identified as the *P. multocida* serotype using the 16s rRNA sequence and multiplex capsular PCR typing system (Townsend et al. 2001). The bacterial isolate was cultured in Martin's broth agar medium containing 5 % horse serum. The pathogenicity of the isolate was tested in healthy mice by intraperitoneal inoculation of the 2 days' culture, and all the mice succumbed to infection between 36 and 48 h post-infection. Bacteria re-isolated from these infected mice were used to infect mice.

Experimental design

120 Female KM mice (body weight 18–22 g) were obtained from the Laboratory Animal Center of Third Military Medical University, Chongqing, China. The mice were housed in a pathogen-free mouse colony (temperature, 20–30 °C; relative humidity, 45–60 %; lighting cycle, 12 h/day) and had free access to a standard rodent diet and drinking water (Ren et al. 2012b). Animals were randomly divided into four groups ($n = 30$ per group): (1) mice were fed the basal diet supplemented with 0.5 % Gln (Ajinomoto Inc., Tokyo, Japan) (0.5 % Gln + basal diet); (2) mice were fed the basal diet supplemented with 1.0 % Gln (1.0 % Gln + basal diet); (3) mice were fed the basal diet supplemented with 2.0 % Gln (2.0 % Gln + basal diet); and (4) mice were fed the basal diet (the control group). At day 7 after initiation of Gln supplementation, mice were challenged by an intraperitoneal injection of *P. multocida* serotype A (CQ2) at the dose of 2.2×10^5 CFU (LD50). All mice were killed to collect the heart, liver, spleen, lung and kidney at 12 h post-infection for bacterial counting and further molecular analysis. This study was performed according to the guidelines of the Laboratory Animal Ethical Commission of the Southwest University.

Counting of bacteria

The number of viable bacteria in the heart, liver, spleen, lung and kidney was measured by homogenizing tissues in saline, plating serial dilutions on Martin broth agar and counting CFU after 16 h of growth at 37 °C.

Real-time quantitative (RT-PCR)

Total RNA was isolated from the liquid nitrogen-frozen spleen and lung using TRIZOL regents (Invitrogen, USA) and then treated with DNase I (Invitrogen, USA) according to the manufacturer's instructions (Geng et al. 2011). Primers (Table 1) were designed with Primer 5.0 according to the mouse gene sequence. β-actin was used as an internal control to normalize target gene transcript levels. For determining expression of virulence factors, the 16S rRNA was used as the reference gene. Real-time PCR was performed according to our previous study (Ren et al. 2011). Briefly, 1 μl cDNA template was added to a total volume of 10 μl containing 5 μl SYBR Green mix, 0.2 μl Rox, 3 μl dd-H_2O and 0.4 μl each of forward and reverse primers. We used the following protocol: (i) pre-denaturation for 10 s at 95 °C; (ii) an amplification and quantification program consisting of repeated 40 cycles (5 s at 95 °C and 20 s at 60 °C); (iii) a melting curve program (60–99 °C with a heating rate of 0.1 °C/s and fluorescence measurement). The relative levels of genes were expressed

Table 1 Primers used in this study

Primer name	Accession number	Primer seq (5′–3′)	Product size
TLR1	NM_030682.1	F: GTCAAAGCTTGGAAAGAATCTGAAG	76
		R: AATGAAGGAATTCCACGTTGTTTC	
TLR2	NM_011905.3	F: GAATTGCATCACCGGTCAGAA	64
		R: CCTCTGAGATTTGACGCTTTGTC	
TLR4	NM_021297.2	F: TTCAGAACTTCAGTGGCTGGATT	64
		R: CCATGCCTTGTCTTCAATTGTTT	
TLR5	NM_016928.2	F: CAGTCCTGGAGCCTGTGTTGT	56
		R: ACCCGGCAAGCATTGTTCT	
TLR6	NM_011604.3	F: TGAATGATGAAAACTGTCAAAGGTTAA	78
		R: GGGTCACATTCAATAAGGTTGGA	
TLR7	NM_133211.3	F: TGCCACCTAATTTACTAGAGCTCTATCTTTAT	101
		R: TAGGTCAAGAACTTGCAACTCATTG	
TLR8	NM_133212.2	F: GAAGCATTTCGAGCATCTCC	188
		R: GAAGACGATTTCGCCAAGAG	
TLR9	NM_031178.2	F:CTCCATCTCCCAACATGGTTCT	76
		R: GCCAGCACTGCAGCCTGTA	
MYD88	NM_010851.2	F: GCATGGTGGTGGTTGTTTCTG	108
		R: GAATCAGTCGCTTCTGTTGG	
IL-1 beta	NM_008361.3	F: ATGAAAGACGGCACACCCAC	175
		R: GCTTGTGCTCTGCTTGTGAG	
IL 6	NM_031168.1	F: TGCAAGAGACTTCCATCCAGT	71
		R: GTGAAGTAGGGAAGGCCG	
TNF-α	NM_013693.2	F: AGGCACTCCCCCAAAAGAT	192
		R: TGAGGGTCTGGGCCATAGAA	
ompA	AET15767.1	F: AAACCATCAAGTAGCGGGTAG	146
		R: AATTTCGCAGTAGAGTTAGGCTATG	
ompH	AAK02472.1	F: TCGGTGATGATGTTGGTGTGTCTGACTA	199
		R: TATAAACCCGCTACAACGAAACCACGAC	
pfhB2	AAK02143.1	F: AACGCAGAGATTAGGAGTACGACAC	138
		R: ACCATAAATACGCCCAGCATTAGT	
PlpE	AAK03601.1	F: AGAGGGGCTTGAAAGGAGGATTGTTGACTA	191
		R: GCTGGAAATCGTGCTGACCGTGTAG	
HasR	AAK03706.1	F: AGGGTATCCAGAAGATGCGATTG	136
		R: AGATTGAAGATAACGTAGCCCGATG	
pm0979	AAK03063.1	F: ACGAAACGCACACCATCTTGATAAG	95
		R: GGTAAAGCAGATAGCGCAACAGTAACAAT	
pm0442	AAK02526.1	F: TGGAAGAAGTGAAAGCCGCTACTG	135
		R: CCATCTTTGTCGCCGTAGCAG	
16S rRNA	E05329.1	F: ACGCTGGCGGCAGGCTTAAC	101
		R: ATTCCCAAGCATTACTCACCCGTCC	
Beta-actin	NM_007393.3	F: GTCCACCTTCCAGCAGATGT	117
		R: GAAAGGGTGTAAAACGCAGC	
GPx1	NM_008160.6	F: GAAGAACTTGGGCCATTTGG	76
		R: TCTCGCCTGGCTCCTGTTT	
CuZnSOD	NM_011434.1	F: TGGTGGTCCATGAGAAACAA	105
		R: GTTTACTGCGCAATCCCAAT	

as a ratio of the target gene to the control gene using the formula $2^{-(\Delta\Delta Ct)}$, where $\Delta\Delta Ct = (Ct$ for a target gene $-$ Ct for the β-actin gene) in a treatment group $-$ (Ct for a target gene $-$ Ct for the β-actin gene) in the control (Fu et al. 2010).

Statistical analysis

Data, expressed as means ± the standard error of the mean (SEM), were statistically analyzed by the one-way analysis of variance using the SPSS 16.0 software (Chicago, IL, USA). Differences among treatment means were determined using the Student–Newman–Keuls multiple comparison test. Log transformation of variables was performed when variance of data was not homogenous among treatment groups, as assessed using the Levene's test (Wei et al. 2012). P values ≤ 0.05 were taken to indicate statistical significance.

Results

High doses of Gln supplementation increases bacterial burden in tissues

Based on the bacterial counting analysis, *P. multocida* were detected in the heart, liver, spleen, lung and kidney after 12 h of infection. In the heart, dietary supplementation with 1.0 or 2.0 % Gln increased ($P < 0.001$) bacterial loads as compared to the control and the 0.5 % Gln group. Meanwhile, the bacterial burden was much higher ($P < 0.001$) in the 2.0 % Gln group than in the 1.0 % Gln group, but no significant difference ($P > 0.05$) was detected between the control and the 0.5 % Gln group (Fig. 1a). Similar results were observed in the spleen (Fig. 1a). In the lung, dietary supplementation with 1.0 or 2.0 % Gln increased ($P < 0.001$) bacterial loads when compared with the control and the 0.5 % Gln group, whereas no significant difference was found between the control and the 0.5 % Gln group, or between the 2.0 % Gln and the 1.0 % Gln groups (Fig. 1a). In the kidney, the bacterial number in the 1.0 % Gln and 2.0 % Gln groups was higher ($P < 0.001$) than that in the control and the 0.5 % Gln group, and was also higher ($P < 0.001$) in the 0.5 % Gln group than in the control (Fig. 1a). Dietary supplementation with 1.0 or 2.0 % Gln increased ($P < 0.001$), but dietary supplementation with 0.5 % Gln decreased ($P < 0.001$), the numbers of bacteria in the liver as compared to the control group (Fig. 1a). The bacterial burden was much higher ($P < 0.001$) in the 2.0 % Gln group than in the 1.0 % Gln group in the liver (Fig. 1a). Collectively, dietary supplementation with 2 % Gln enhanced ($P < 0.001$) bacterial replication in vivo as compared to the 1 % Gln, 0.5 % Gln and the control groups. Additionally, the bacterial number was much higher ($P < 0.001$) in the 1 % Gln group than in the 0.5 % Gln and the control groups (Fig. 1b). No difference in bacterial numbers was found between the 0.5 % Gln and the control groups (Fig. 1b).

Glutamine supplementation affects the expression of virulence factors in vivo

Next, we determined the effect of dietary Gln supplementation on the expression of major virulence factors in *P. multocida,* including ompA, ompH, pm0442, pm0979, PfhB2, plpE and hasR (Wilkie et al. 2012). We analyzed the lung and spleen, because the lung is the main target of *P. multocida* and the spleen plays an important role in immune responses to infection. In the lung, the most intriguing finding was that dietary supplementation with 2.0 % Gln enhanced ($P < 0.05$) the expression of virulence factors, including pm0979, plpE, ompA, pm0442 and hasR when compared with the control and the 0.5 % Gln group (Fig. 2a). In the spleen, mRNA levels for virulence factors (plpE and pm0442) in the 0.5 % Gln group were higher ($P < 0.05$) than those in the other groups (Fig. 2b). Similar to results for the lung, splenic mRNA levels for hasR were higher ($P < 0.05$) in the 1.0 % Gln and 2.0 % Gln groups than in the control and the 0.5 % Gln group (Fig. 2b). Collectively, a high dose of Gln supplementation stimulated expression of virulence factors in the lung and spleen.

Glutamine supplementation affects TLR expression in vivo

TLRs are a class of proteins that play an important role in the activation of the innate immune system following infection (Tan et al. 2010). Thus, analysis of TLR expression could help explain why a high dose of Gln supplementation increased bacterial burden and expression of virulence factors in vivo. In the lung, no significant difference in the expression of most TLRs was found between the 0.5 % Gln group and the control, except for TLR 7 and TLR 8. However, dietary supplementation with 2.0 % Gln reduced ($P < 0.05$) the expression of TLRs, including TLR1, TLR2, TLR4, TLR5, TLR6, TLR7, TLR8 and TLR9 when compared with a lower dose of Gln supplementation (Fig. 3a). However, results for the spleen varied among the types of TLR (Fig. 3a). Nonetheless, a high dose of Gln supplementation attenuated TLR expression in the lung.

Glutamine supplementation affects inflammation

Inflammation is a cascade of immune responses triggered by cytokines, such as IL-1β, IL-6 and tumor necrosis factor

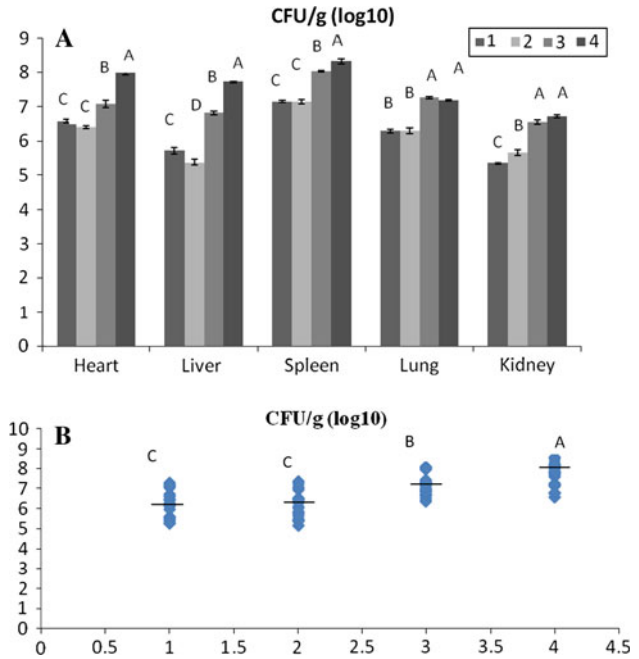

Fig. 1 The bacterial burden in different tissues (**a**) or in the whole body (**b**) of mice at 12 h after *Pasteurella multocida* infection. Mice were fed the basal diet supplemented with 0 % (*1*), 0.5 % (*2*), 1.0 % (*3*) or 2.0 % (*4*) Gln before being challenged with *Pasteurella multocida.* Data are mean ± SEM, $n = 6$, *A–B* mean values sharing *different superscripts* within each variable differ ($P < 0.001$)

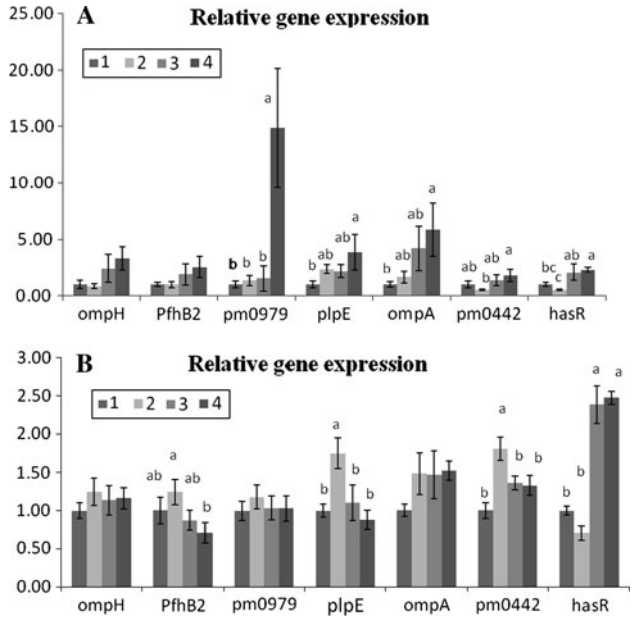

Fig. 2 Expression of major virulence factors of *Pasteurella multocida* in the lung (**a**) and spleen (**b**) of mice at 12 h after *Pasteurella multocida* infection. Mice were fed the basal diet supplemented with 0 % (*1*), 0.5 % (*2*), 1.0 % (*3*) or 2.0 % (*4*) Gln before being challenged with *Pasteurella multocida.* Data are mean ± SEM, $n = 6$, *a–b* mean values sharing *different superscripts* within each variable differ ($P < 0.05$)

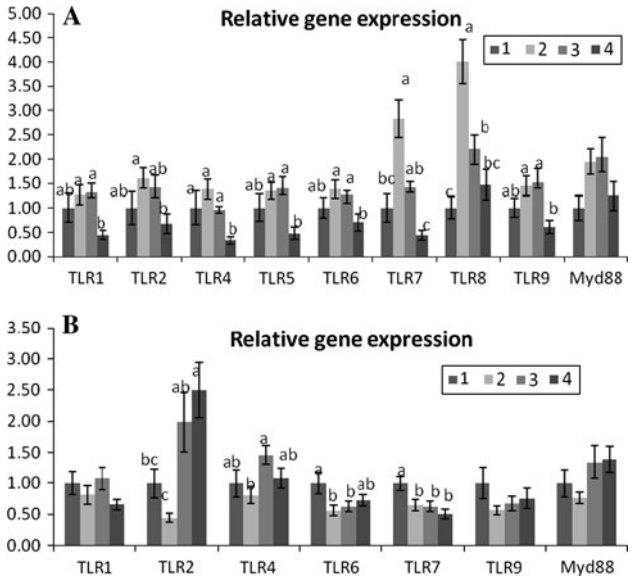

Fig. 3 Expression of toll-like receptors (TLRs) in the lung (**a**) and spleen (**b**) of mice at 12 h after *Pasteurella multocida* infection. Mice were fed the basal diet supplemented with 0 % (*1*), 0.5 % (*2*), 1.0 % (*3*) or 2.0 % (*4*) Gln before being challenged with *Pasteurella multocida.* Data are mean ± SEM, $n = 6$, *a–b* mean values sharing *different superscripts* within each variable differ ($P < 0.05$)

alpha (TNF-α), and immune cells in response to physical injury, noxious stimuli or microbes and their toxins. In the lung, dietary supplementation with 0.5 % Gln enhanced the expression of proinflammatory cytokines, including IL-1β and TNF-α. Dietary supplementation with 2.0 % Gln decreased ($P < 0.05$) the expression of IL-1β, IL-6 and TNF-α in the lung, compared to the control, 0.5 % Gln and 1 % Gln groups (Fig. 4a). Contrary results were obtained in the spleen (Fig. 4b). In contrast, expression of GPX1 or CuZnSOD in the lung and spleen did not differ among the treatment groups (Fig. 4a, b).

Discussion

L-Glutamine is the most abundant free amino acid in the plasma and skeletal muscle of humans and many other animals (Wu 2013a; Yao et al. 2012). Although food ingredients for animals contain a high content of Gln (Hou et al. 2012; Li et al. 2011a), only ~30 % of dietary Gln enters the portal circulation in mammals due to extensive catabolism by the small intestine (Bensaci et al. 2012; Tan et al. 2012; Wu 2010a, b). While Gln is synthesized from L-glutamate (Hou et al. 2011; Lei et al. 2012; Yao et al. 2012), Gln cannot be fully replaced by L-glutamate in animal nutrition, particularly for the mucosal integrity and function of the small intestine and lymphoid organs (Reza et al. 2013a, b; Wu et al. 1996, 2011a, b). Increasing evidence shows that Gln coordinates many immunological

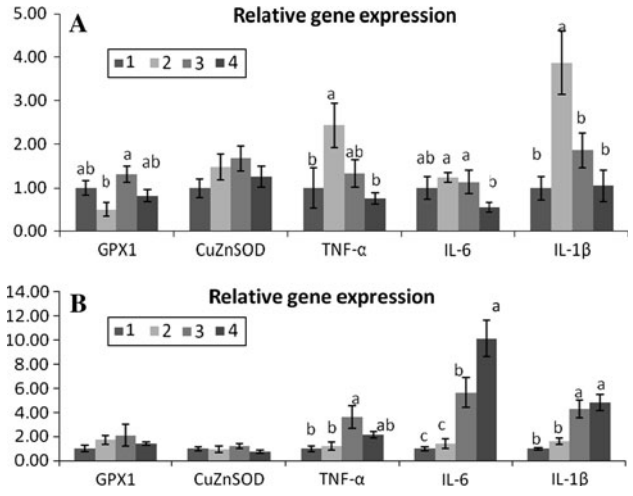

Fig. 4 Abundance of mRNAs for proinflammatory cytokines (IL-1β, IL-6 and TNF-α) and anti-oxidative factors (GPX1 and CuZnSOD) in the lung (**a**) and spleen (**b**) of mice at 12 h after *Pasteurella multocida* infection. Mice were fed the basal diet supplemented with 0 % (*1*), 0.5 % (*2*), 1.0 % (*3*), or 2.0 % (*4*) Gln before being challenged with *Pasteurella multocida.* Data are mean ± SEM, $n = 6$, *a–b* mean values sharing *different superscripts* within each variable differ ($P < 0.05$)

and physiological responses to infection (Li et al. 2007). For example, the results of one study indicate that alanyl-glutamine increases the survival rate of mice infected with *Clostridium difficile* by reducing intestinal mucosal injury (Rodrigues et al. 2013). Thus, Gln is now used as an immune-enhancing nutrient in clinical nutrition and metabolic care (Wu et al. 2013).

Although no differences in most of the measured variables were detected between the control and the 0.5 % Gln group, dietary supplementation with a higher dose of Gln increased the bacterial burden in mice infected with *P. multocida* in vivo. This result can partially explain our previous observation that a high content of Gln in the diet caused a high rate of mortality in mice after *P. multocida* infection (data not shown). The possible explanation is that a high dose of supplemental Gln leads to metabolic disorders (e.g., elevated levels of ammonia) (Holecek et al. 2011; Li et al. 2011b) and impaired blood flow (Wu et al. 2011a; Wu and Meininger 2009) in the host, favoring bacterial replication or inhibiting the host defense system. Additional data from future studies are needed to test this hypothesis. Indeed, beneficial or adverse effects of any nutrient on animals (including humans) critically depend on its content and its ratio to other substances in the diet. For example, pretreatment with a high dose of *Lactobacillus rhamnosus*, a probiotic, may negate its preventative effects in *E. coli*-challenged piglets, possibly by disturbing the established microbial ecosystem and interfering with mucosal immune responses against potential enteric pathogens (Li et al. 2012). Gln is extensively utilized by microorganisms in the lumen of the small intestine (Dai et al. 2010, 2011). This amino acid also regulates the utilization of other amino acids (including arginine) by bacteria (Dai et al. 2012a, b, 2013), therefore modulating the activity of the intestinal immune system. However, it remains to be determined whether dietary Gln supplementation affects the microbial ecosystem and mucosal immunity in the intestine of *P. multocida*-infected mice. Interestingly, we recently observed that a high dose of Gln (2.0 %) supplementation could activate the intestinal innate immune response in mice infected with *Citrobacter rodentium* or enterotoxigenic *E. coli* (Ren, unpublished data). Therefore, it appears that a beneficial role for glutamine supplementation also depends on infectious models. In support of this view, Gln supplementation exerted a protective role in porcine circovirus-infected mice or piglets (Ren et al. 2011, 2012a, 2013a).

Another novel and important finding of this study is that dietary supplementation with a high dose of Gln increased expression of virulence factors of *P. multocida* in the lung. A possible reason is that an excessive amount of Gln inhibits the activation of TLRs in cells of the immune system. TLR4 binds to lipopolysaccharide and plays an important role in the activation of the innate immune system following infection by Gram-negative bacteria (Miller et al. 2012). TLR2 can form a heterodimer with either TLR1 or TLR6, and TLR9, which is a receptor for CpG DNA in bacterial genomes, to recognize lipoteichoic acid (LTA), lipoproteins, and peptidoglycans in bacteria (Medzhitov 2007). However, parenteral nutrition with a daily Gln supplementation (0.35 g/kg) does not affect the expression of TLR2 and 4 in peripheral blood monocytes in trauma patients (Perez-Barcena et al. 2010). Thus, the biological functions of Gln on TLRs depend on its intake from the diet, as dietary supplementation with 0.5 % Gln did not influence expression of TLR 2 and 4 in the lung or spleen of mice. Similarly, Gln supplementation had little effect on mRNA levels for proteins of the MyD88-dependent pathway, but we did not quantify the actual abundance of proteins in the mouse lung. Future studies are warranted to determine the effects of Gln supplementation on the TRIF-dependent pathway, another arm of the cellular TLR signaling. Meanwhile, it remains unknown whether Gln supplementation affects the expression of Nod-like receptors, the cytoplasmic counterparts of TLRs. As high doses of Gln supplementation inhibited TLR expression, they reduced the expression of proinflammatory cytokines, including IL-1β, IL-6 and TNF-α. This result is of nutritional significance because inflammatory responses are intended to disarm or destroy invading microorganisms, remove irritants and set the stage for tissue repair or wound healing. Indeed, results of previous studies support the notion that Gln enhances immunity and improves

anti-inflammatory responses through diverse pathways, including attenuated expression of nuclear factor kappa B, signal transducer and activator of transcription and mitogen-activated protein kinases, and activation of peroxisome proliferator-activated receptor-γ and heat shock factor-1 (Ban et al. 2011; Kretzmann et al. 2008; Li et al. 2007).

Another salient result of this study is that a high dose of Gln supplementation increased the bacterial burden in the spleen, but had mixed effects on expression of major virulence factors of *P. multocida* and TLRs. Also, unlike the results for the lung, a high dose of glutamine promoted the proinflammatory response in the spleen. The reasons for this discrepancy remains unknown and may be related to different microenvironments between the lung and spleen at molecular, cellular and tissue levels, as well as different pathways for glutamine metabolism between these two organs. Further studies are required to elucidate mechanisms responsible for the regulatory role of Gln in the expression of virulence factors. Nonetheless, increased expression of virulence factors in response to Gln supplementation is expected to promote antibody production in vivo, which would make Gln a useful adjuvant in vaccine immunization. Indeed, we previously reported that arginine or proline supplementation increased antibody levels in the serum of mice immunized with the inactivated *P. multocida* vaccine (Ren et al. 2013b, c).

Other intriguing findings of this study are that dietary supplementation with Gln stimulated expression of IL-1β and TNF-α in the lung (low dose) and spleen (high dose). There is a report that IL-1β and TNF restrict the growth of intracellular *M. tuberculosis* through a caspase-dependent mechanism (Jayaraman et al. 2013). Thus, we surmise that Gln supplementation may enhance the clearance of pathogens in mice infected with *P. multocida*. Thus, in addition to its regulatory roles in metabolism and physiology (Chiu et al. 2012; Xi et al. 2011, 2012), Gln is truly a functional amino acid in nutrition and immunity of mammals (Wu 2013b).

In conclusion, the results of this study indicate that dietary supplementation with 1 and 2 % Gln increases the *P. multocida* burden and the expression of major virulence factors in a mouse model. The Gln supplementation inhibits the cellular TLR signaling and proinflammatory responses in the lung, but has mixed effects on the spleen. Caution should be taken when Gln is supplemented to the diets of immunologically challenged animals so as to optimize immune responses in specific target tissues and organs. To our knowledge, this is the first study to determine the effects of dietary Gln supplementation on bacterial distribution and the expression of its major virulence factors in an acute infectious mouse model. These findings have important implication for enhancing the efficiency of animal production and for improving preventive medicine in humans.

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Conflict of interest The authors declare no conflict of interest.

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