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Effects of dietary glutamine supplementation on lung injury induced by lipopolysaccharide administration

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Hou YC, Pai MH, Chiu WC, Hu YM, Yeh SL. Effects of dietary glutamine supplementation on lung injury induced by lipopolysaccharide administration. *Am J Physiol Lung Cell Mol Physiol* 296: L288–L295, 2009. First published December 5, 2008; doi:10.1152/ajplung.90479.2008.—Acute lung injury (ALI) is a critical syndrome associated with respiratory dysfunction, and neutrophils are considered to be central to the pathogenesis of ALI. This study investigated the effects of glutamine (Gln) on neutrophil recruitment in a model of lipopolysaccharide (LPS)-induced ALI. C57BL/6 mice were fed a standard diet either with casein as the nitrogen source or with 25% of total nitrogen replaced by Gln. After 10 days, intratracheal instillation of LPS was used to induce ALI. Mice were killed at 0, 6, 12, and 24 h after LPS administration (n = 10/group). Bronchoalveolar lavage fluid and lung tissues were collected for further analysis. The results showed that, compared with the control group, lipid peroxide levels in the lungs were higher at 12 and 24 h after LPS administration in the Gln group. CXC chemokines as well as tumor necrosis factor-α were significantly elevated and reached peaks at 6 h in the Gln group, which was earlier than in the control group. Histopathological findings showed that the thickening of alveolar septal space was extensive in the Gln group 24 h and 2 wk after LPS. Also, greater amounts of collagen had accumulated in lung tissue in the Gln group. This study indicates that dietary Gln administration resulted in higher inflammatory cytokine production, with more neutrophils recruited at the early stage of ALI. These results were consistent with the histopathological findings that Gln supplementation causes more severe interstitial inflammation and fibrosis in a model of ALI induced by LPS.

acute lung injury; neutrophil recruitment

ACUTE LUNG INJURY (ALI) is a clinical syndrome associated with respiratory dysfunction, and acute respiratory distress syndrome (ARDS) is the most severe form of ALI, which is partially responsible for the high mortality in critically ill patients (30). ALI results from direct or indirect insults leading to pulmonary inflammation that is characterized by leukocyte recruitment and injuries to the endothelium and epithelium that increase the permeability of the alveolar capillaries, leading to hypoxemia (29). Neutrophils are the most abundant leukocytes in the blood and are crucial to the innate immune response. Their activation leads to the release of multiple cytotoxic products including reactive oxygen species, proteolytic enzymes, and proinflammatory mediators that may enhance the inflammatory response and consequently result in lung injury (6). Neutrophils are considered to be central to the pathogenesis of most forms of ALI (19). The accumulation of activated neutrophils in the lungs is an early step in the pulmonary inflammatory process that leads to ALI (1).

Glutamine (Gln) is the most abundant free amino acid in the body. Gln serves as an important energy source for many rapidly proliferating cells, especially enterocytes and immune cells, and is important for optimal leukocyte activation and proliferation (5, 35). Several studies have demonstrated that Gln has immunomodulating properties (14, 33). Enteral Gln-containing formulas are commonly used in the nutrition regimens of critically ill patients. There are some studies suggesting that Gln supplementation improves outcome in clinical and experimental models of ALI (7, 21, 27, 28). Previous animal studies showed that Gln attenuates intravenous lipopolysaccharide (LPS)-induced lung injury (21, 34). Also, studies performed by Singleton et al. (27, 28) demonstrated that Gln prevents the activation of nuclear factor (NF)-κB and enhances pulmonary heat shock protein expression, thus attenuating lung injury following gut-derived sepsis. However, an in vitro study found that Gln increases the expression of NADPH oxidase, catalyzes superoxide anion production, and increases oxidative stress in neutrophils (22). A study by Pithon-Curi et al. (23) also found that Gln administration delays the apoptosis of neutrophils. Because accumulation of active neutrophils and excessive superoxide anion in the lungs is the initial step of ALI, we speculated that Gln supplementation may prolong the infiltration time of recruited neutrophils and produce more reactive oxidants in the lung, thus exaggerating injury to the lungs. This hypothesis may differ from the favorable Gln effects mentioned above (21, 27, 28, 34). However, the animal models with lung injury used previously were indirectly induced by gut-derived peritonitis or intravenous injection of endotoxin that was very different from the model of ALI used in this study. At present, the impact of Gln administration on direct ALI remains unclear. Therefore, we designed a model of direct ALI to investigate the effects of Gln administration on neutrophil recruitment and proinflammatory cytokine production. ALI was induced by intratracheal administration of the gram-negative bacterial endotoxin LPS. This is a well-established animal model of direct lung injury used to imitate bacteria-infected pneumonia (3). Direct instillation of LPS into the lungs via the trachea was thought to have an advantage over inhalation, because the actual dose delivered to the lungs of each animal can be ensured (8).

MATERIALS AND METHODS

Experimental animals. Six-week-old C57BL/6 male mice were used in this study. Mice were maintained in a temperature- and humidity-controlled room with a 12:12-h light-dark cycle and were fed ad libitum before the studies. The study protocol was approved by

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the Animal Care Committee of Taipei Medical University (approval no. LAC-94-0094). The care of the laboratory animals was in full compliance with the *Guide for the Care and Use of Laboratory Animals* (National Research Council, 1996) as reviewed by the Animal Care Committee.

Study protocols. After 1 wk of acclimation, mice were randomly assigned to a control group ($n = 55$) or a Gln group ($n = 55$). Control mice were fed a common semipurified diet, while the Gln group received a diet in which part of the casein was replaced by Gln, which provided 25% of the total amino acid nitrogen. This amount of Gln has been proven to have an immunomodulatory effect in rodents (16, 32). The two diets were isonitrogenous and similar in energy and nutrient distributions. The calorie content of the diets was 4.18/g (Table 1). After the mice were fed the diets for 10 days, ALI was induced by the intratracheal instillation of LPS from *Escherichia coli* O111:B6 (Sigma, St Louis, MO). Mice were weighed and anesthetized with intraperitoneal pentobarbital (80 mg/kg body wt). A gavage needle was inserted into the trachea via the mouth, and 20 μg of LPS suspended in 50 μl of sterilized phosphate-buffered saline (PBS) was slowly instilled into the lung (17). This dose of LPS was chosen because in a preliminary study we found that neutrophils were recruited into the alveolar space within 24 h after intratracheal instillation with this dose. All mice became conscious after anesthesia for 1 h and recovered their ambulatory ability gradually. After ALI induction, the experimental diets were given for the remaining hours or days. Ten mice were killed in each group at 0, 6, 12, and 24 h after a single dose of LPS, respectively, and 15 mice in both groups were allowed to live for up to 2 wk. Survival of the mice was recorded every day for 14 days. At the end of the study, mice were anesthetized with ether and killed by cardiac puncture. The trachea was exposed by a midline incision and intubated directly with a polyethylene 20-gauge catheter. Two 1-ml aliquots of PBS were instilled and sequentially removed by gentle hand suction with a 1-ml syringe for the collection of bronchoalveolar lavage fluid (BALF). The recovered volume of BALF was 1.5–1.7 ml for each animal. A part of the lung tissue was harvested for the histopathology analysis, and the remaining tissues were stored at −80°C for further assays.

Cell counts in BALF. BALF was centrifuged at 1,500 *g* for 10 min. The supernatants were retained for the cytokine assays, and the cell pellets were suspended in 100 μl of PBS. Cells were double-stained with phycoerythrin (PE)-labeled anti-mouse Ly6G (Clone 1A8, BD Pharmingen) and fluorescein isothiocyanate (FITC)-labeled anti-mouse CD45 (Clone 30-F11, BD Pharmingen) antibodies. CD45 is the marker of white blood cells (WBC), and mouse Ly6G is predominantly expressed on neutrophils in peripheral organs. Isotype-matched antibodies were used as the control. Fluorescence data were collected by flow cytometry (Coulter, Miami, FL). Cells were gated on the basis of forward scatter and side scatter profiles and analyzed for the expressions of CD45 and Ly6G. Cells were counted at a constant flow rate. WBC or neutrophils per minute indicate the absolute cell numbers counted in a given time. Neutrophil counts per minute divided by WBC per minute represent the percentage of neutrophils in total WBC.

Table 1. *Composition of semipurified diets*

	Control	Glutamine
Soybean oil	100	100
Casein	200	150
Glutamine	0	41.7
Salt mixture	35	35
Vitamin	10	10
Methyl cellulose	31	31
Choline chloride	1	1
Methionine	3	3
Corn starch	620	628.3

Concentrations are expressed in g/kg. Salt mixture contained the following (mg/g): 500 calcium phosphate dibasic, 74 sodium chloride, 52 potassium sulfate, 220 potassium citrate monohydrate, 24 magnesium oxide, 3.5 manganese carbonate, 6 ferric citrate, 1.6 zinc carbonate, 0.3 cupric carbonate, 0.01 potassium iodate, 0.01 sodium selenite, and 0.55 chromium potassium sulfate. Vitamin mix contained the following (mg/g): 0.6 thiamine hydrochloride, 0.6 riboflavin, 0.7 pyridoxine hydrochloride, 3 nicotinic acid, 1.6 calcium pantothenate, 20 DL-α-tocopherol acetate, 0.25 cholecalciferol, and 0.005 menaquinone.

Determination of tumor necrosis factor-α, keratinocyte-derived chemokine, and macrophage inflammatory protein-2 in BALF. Tumor necrosis factor (TNF)-α, keratinocyte-derived chemokine (KC), and macrophage inflammatory protein (MIP)-2 levels in the BALF were measured with enzyme-linked immunosorbent assay (ELISA) kits (R & D Systems, Minneapolis, MN). Antibodies specific for mouse TNF-α, KC, and MIP-2 were coated onto the wells of the microtiter strips provided. Procedures followed the manufacturer's instructions.

Western blotting for NF-κB in lungs. Frozen lung tissues were homogenized in ice-cold buffer (10 mM Tris·HCl pH 7.5, 1% Triton X-100) containing protease inhibitor cocktail (Complete, Roche Diagnostics), and protein concentration was determined with a Bradford Protein Assay Reagent kit (Bio-Rad, Richmond, CA). Forty micrograms of protein was loaded and separated on 12.5% SDS-PAGE and transferred onto a polyvinylidene difluoride (PVDF) membrane in a wet transfer apparatus. Membranes were blocked with 5% nonfat milk in PBS containing 0.1% Tween 20 (PBS-T) for 1 h and then incubated with rabbit anti-NF-κB p65 antibodies (1:1,000; Millipore, Billerica, MA) or mouse anti-actin antibodies (1:2,000; Millipore) for 2.5 h. After membranes were washed four times (5 min each) in PBS-T, goat anti-rabbit IgG or anti-mouse IgG-horseradish peroxidase conjugates (Millipore) were applied at 1:10,000 dilutions for 1 h. Membranes were washed three times with PBS-T over 30 min, and blots were developed with enhanced chemiluminescence reagents (PerkinElmer Life Sciences, Waltham, MA) and exposed to X-ray films. Relative intensity was measured to quantify protein level. All blots were normalized against actin to adjust the protein loaded.

Concentration of thiobarbituric acid-reactive substances in the lungs. The evaluation of lipid peroxides was carried out by the detection of thiobarbituric acid-reactive substances (TBARS). The production of TBARS (assumed to be mainly malondialdehyde and its precursors) in lung homogenates was determined by a previously described method (9). A 20% lung homogenate was prepared at 4°C in PBS. Trichloroacetic acid (10%) was added to lung homogenate and centrifuged to precipitate the protein; 0.8 ml of 0.22% H_2SO_4, 0.1 ml of 10% phosphotungstic acid, and 0.2 ml of 0.67% 2-thiobarbituric acid were added to 20 μl of supernatant and heated for 60 min in boiling water. The pink product was extracted with 1-butanol and measured with a fluorescence spectrophotometer at 515-nm excitation and 555-nm emission. The TBARS were expressed as nanomolar, and 1,1,3,3-tetramethoxypropane was used as the standard.

Histopathology. At 0 h, 24 h, and 2 wks after a single dose of LPS, lung tissues were harvested and fixed with 4% paraformaldehyde. After being steeped in a 30% sucrose solution overnight, the tissue was embedded in a frozen tissue matrix (Leica Instruments). Lungs were then sectioned into 5-μm-thick sections, mounted on glass slides, and stained. Hematoxylin and eosin (H & E) staining was used to determine the morphology of the lungs. Digital images at ×40 magnification per section were captured. Five fields per section were analyzed for thickening of the septal space, which was presented as areas of the interalveolar septum. Images were assessed with Image-Pro Plus software (Media Cybernetics, Silver Spring, MD). Staining intensities for nucleus, air space, and total area were determined by calculating the pixel values within each image, and the areas of the interalveolar septum were calculated by the following formula: total area − air space − nucleus areas (μm^2). Masson's trichrome stain was used to detect collagen deposition of lung tissues. The sections were incubated in Weigert's iron hematoxylin solution for 5 min at room temperature and rinsed with distilled water. The sections were

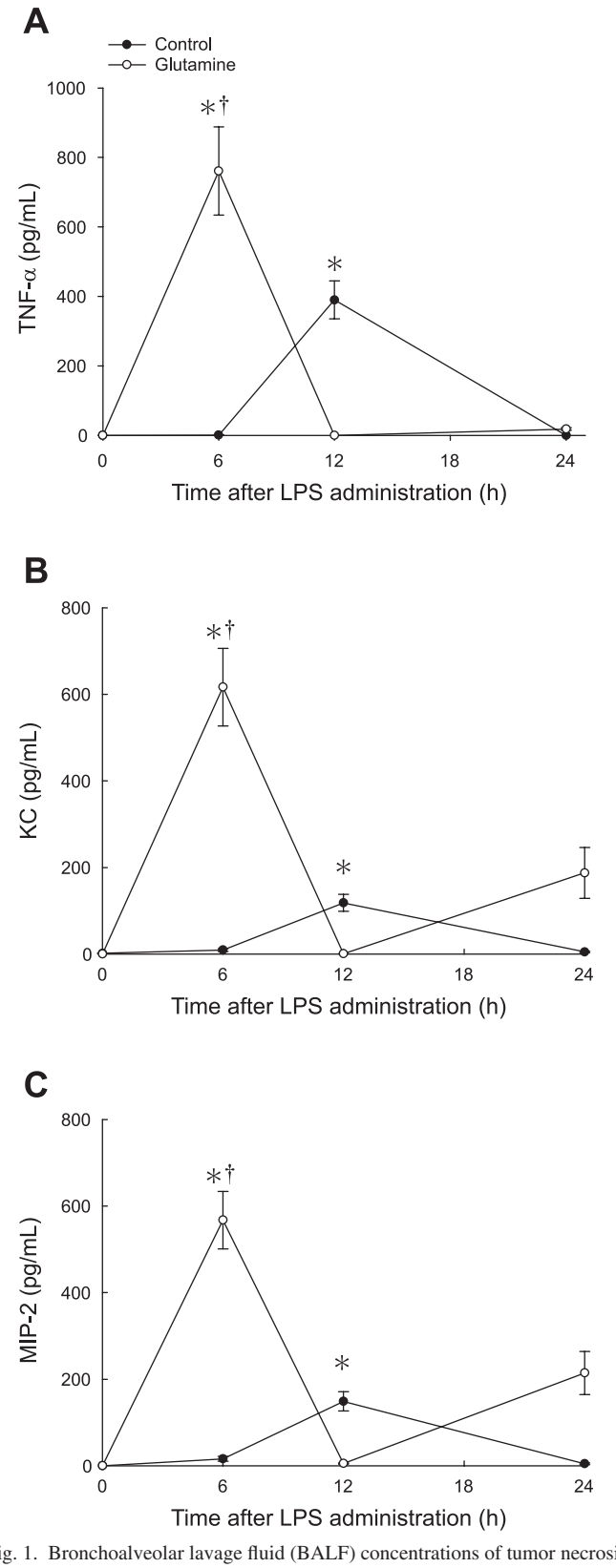


Fig. 1. Bronchoalveolar lavage fluid (BALF) concentrations of tumor necrosis factor (TNF)-α (*A*), keratinocyte-derived chemokine (KC; *B*), and macrophage inflammatory protein (MIP)-2 (*C*) at different time points after lipopolysaccharide (LPS) administration ($n = 10$). Results are means ± SD. *Significantly different from other time points in same group ($P < 0.05$). †Significantly different from peak value of control group ($P < 0.05$).

then incubated with Biebrich scarlet-acid fuchsin solution for 2 min, followed by a distilled water rinse. Sections were placed in a phosphotungstic acid solution for 5 min and then rinsed with water. After that, the sections were placed in aniline blue solution (Sigma) for 2.5 min, followed by a distilled water rinse. The sections were then placed in 1% acetic acid for 1 min, followed by 95% alcohol, two changes of absolute alcohol, and two changes of xylene. The sections were mounted under coverslips with a xylene-soluble medium (Sigma). Positive staining for collagen fiber around bronchioles was photographed at ×20 magnification, and five randomly selected fields per section were captured for quantification of collagen accumulation around alveolar space by the software. The staining areas of cytoplasm and air space were determined by calculating the color pixel values within each image, and the areas of collagen around alveolar space were calculated by following formula: total area − air space − cytoplasm areas (μm^2).

Statistical analysis. All data are shown as means ± SD. All statistical analyses were performed with SAS v10 software. After normality was tested by bell shape, symmetry of distribution, and kurtosis, all groups demonstrated normal distribution. Different time courses and treatments were analyzed by two-way analysis of variance (ANOVA) using Duncan's test. Body weights, areas of the interalveolar septum, and collagen accumulation around alveolar space between groups were analyzed by Student's *t*-test, and the survival rate was evaluated by the Kaplan-Meier method. A *P* value of <0.05 was considered significant.

RESULTS

Body weights. There were no differences between the two groups in initial body weights and body weights after the experimental diets were fed for 10 days (control 16.52 ± 0.48 vs. Gln 16.77 ± 0.26 g, $P > 0.05$).

Cytokine levels in BALF. TNF-α, KC, and MIP-2 levels reached peaks at 6 h after LPS in the Gln group, which was earlier than in the control group. All these cytokines reached

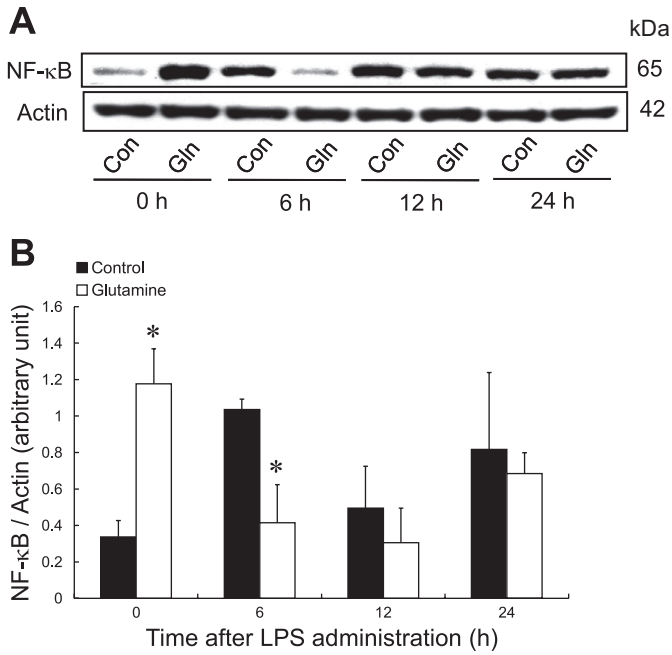


Fig. 2. Western blot analysis of nuclear factor (NF)-κB protein in lungs from control and glutamine (Gln) groups at different time points. *A*: representative Western blot photographs. Equal loading of proteins is illustrated by β-actin bands. *B*: mean ± SD values of densitometric analysis for 5 different observations. *Significantly different from control group ($P < 0.05$).

peaks at 12 h in the control group. The peak values of the cytokines in the Gln group were higher than those in the control group (Fig. 1).

NF-κB expression in lung. Expression of NF-κB was higher at 0 h but lower at 6 h in the Gln group than in the control group ($P < 0.05$). There were no differences in NF-κB expressions at 12 and 24 h between the two groups (Fig. 2).

Neutrophil numbers in BALF. The percentage of neutrophils among WBC was significantly higher at 12 h than at other time points in the control group, while the highest neutrophil percentage in the Gln group was at 6 h after LPS (Fig. 3*A*). The absolute neutrophil number was higher at 6 h and lower at 12 h in the Gln group than in the control group (Fig. 3*B*). Compared with the control group and other time points in the Gln group, the total WBC counts were highest at 12 h in the Gln group (Fig. 3*C*).

TBARS concentration in lung homogenates. Compared with the Gln group, the TBARS levels at 12 h and 24 h were significantly lower than those at 0 and 6 h in the control group. The Gln-treated group had the lowest levels at 6 h, which returned to levels comparable to 0 h at 12 and 24 h (Fig. 4).

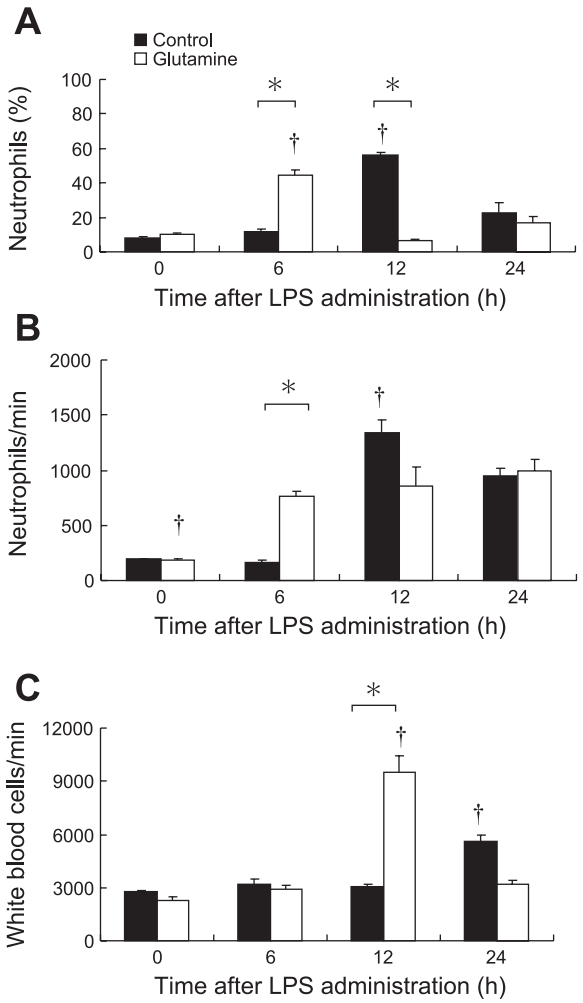


Fig. 3. Percentage of neutrophils (*A*), neutrophil counts (*B*), and white blood cell counts (*C*) in BALF ($n = 10$). Results are means ± SD. *Significantly different between 2 groups at same time point ($P < 0.05$). †Significantly different from other time points in same group ($P < 0.05$).

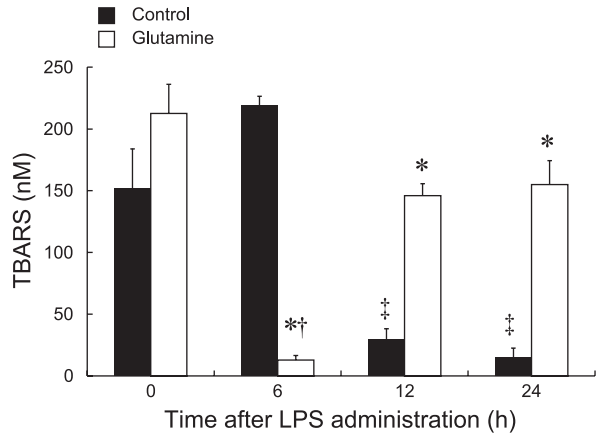


Fig. 4. Concentrations of thiobarbituric acid-reactive substances (TBARS) in lung homogenates ($n = 10$). Results are means ± SD. *Significantly different between 2 groups at same time point ($P < 0.05$). †Significantly lower than other 3 time points in the Gln group. ‡Significantly different from 0 h and 6 h time points in the control group.

Histopathological aspects of the lung. Histopathological findings of the lung tissues with H & E staining showed that the pulmonary architecture was normal at 0 h in both groups (Fig. 5, *A* and *B*). However, at 24 h (Fig. 5, *C* and *D*) and 2 wk (Fig. 5, *E* and *F*) after LPS, destruction of alveolar structures and thickening of the septal space were extensively observed in the Gln group, whereas interstitial inflammation was not so obvious in the control group (as indicated by arrows in Fig. 5). The areas of interalveolar space between groups at each time point are quantified in Fig. 5*G*, and the areas were higher in the Gln group at 24 h ($P = 0.035$) and at 2 wk ($P = 0.009$) than in the control group. Masson's trichrome staining showed that after LPS had been induced for 2 wk collagen accumulation around the alveolar space (Fig. 6*B*) was more severe than that of the control group (Fig. 6*A*), and the quantification result is shown in Fig. 6*E* ($P = 0.0165$). Also, collagen accumulation around bronchioles in the Gln group (Fig. 6*D*) was more obvious than that of the control group (Fig. 6*C*).

Survival rates. No differences in survival rates were observed between the two groups at 2 wk after ALI. The survival rates at the time point of 2 wk were 93.3% in the control group and 73.3% in the Gln group ($P = 0.2048$).

DISCUSSION

In this study, we provided oral Gln supplementation for 10 days before ALI was induced. Although it is unlikely that patients would have pretreatment with a high dose of Gln before developing pneumonia, this model may occur through the course of hospitalization. Because enteral immune-modulating formulas are commonly used in nutrition support for critically ill patients, preventive use of a Gln-supplemented diet may be recommended for these patients.

Cytokines are a diverse group of biologically active proteins that are instrumental in the evolution of ALI. TNF-α and the CXC chemokine family are important cytokines associated with pulmonary inflammation that mediate neutrophil recruitment (29). TNF-α is produced primarily by mononuclear phagocytes and may prime neutrophils in response to specific activating factors that enhance respiratory bursts, release reactive oxygen metabolites, and produce phagocytosis (29). A

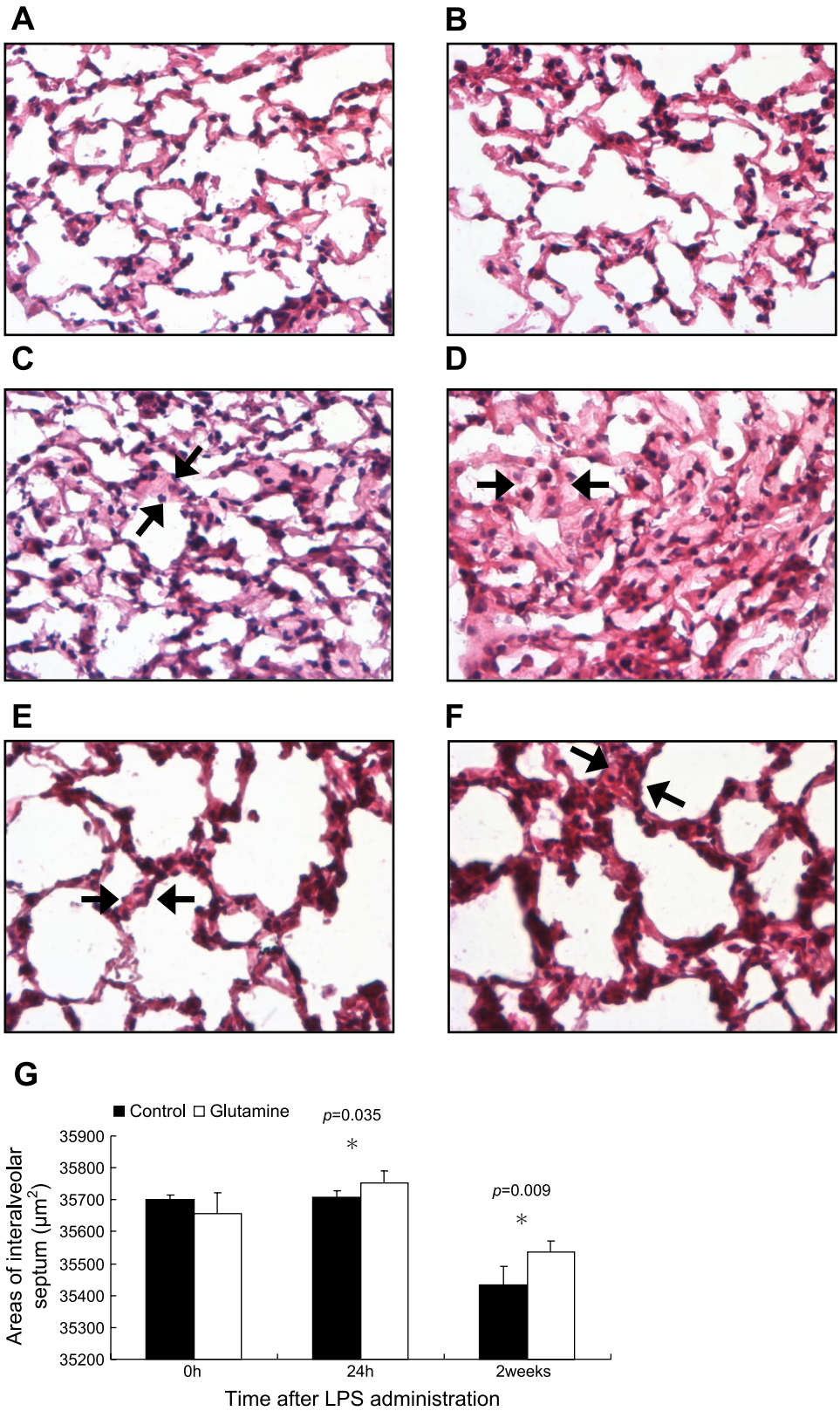
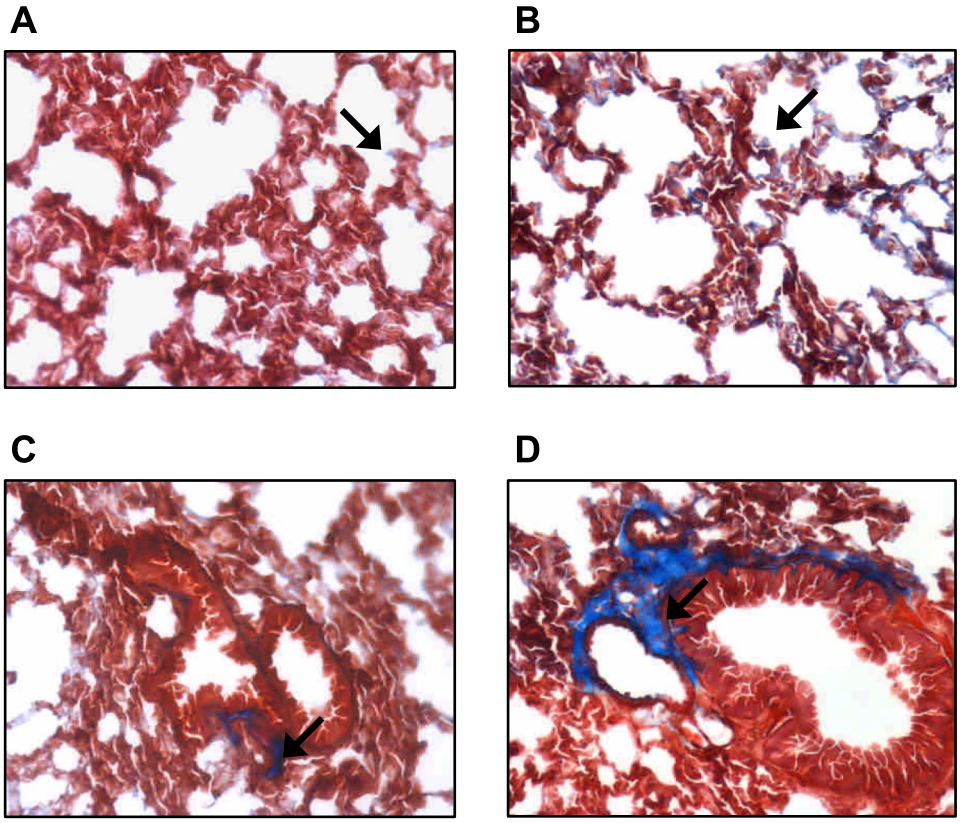


Fig. 5. Hematoxylin and eosin staining of lung tissues from mice at 0 h in control (*A*) and Gln (*B*) groups, 24 h in control (*C*) and Gln (*D*) groups, and 2 wk in control (*E*) and Gln (*F*) groups after LPS administration. Magnification ×40. Arrows indicated the thickening of the septal space. *G*: quantification of interalveolar space areas between groups ($n = 5$). Interalveolar space areas were assessed with Image-Pro Plus software. Staining intensity for nucleus, air space, and total area were determined by calculating the pixel values within each image, and the areas of the interalveolar septum were calculated with the formula described in MATERIALS AND METHODS. *Significant difference between the 2 groups ($P < 0.05$).

previous study showed that the magnitude of TNF correlated with a higher incidence and severity of ARDS and mortality (18). KC and MIP-2 are murine homologs of the human CXC chemokine family. CXC chemokines have been found to play important roles in mediating neutrophil infiltration in the lung parenchyma and pleural space in response to endotoxin and bacterial challenge. Previous studies by Frevert et al. (11, 12) revealed that rats passively immunized with neutralizing KC



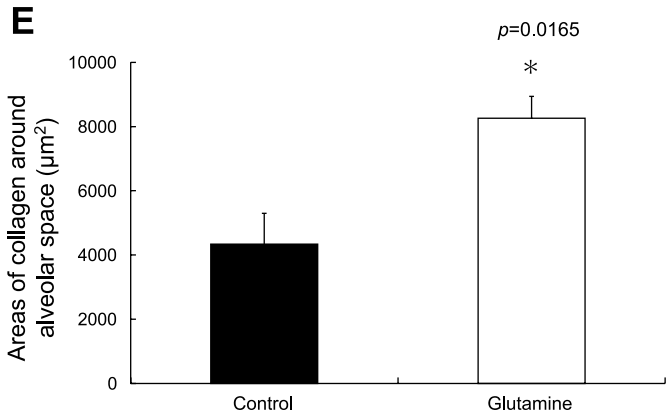


Fig. 6. Masson's trichrome staining of alveolar tissues from mice in control (*A*) and Gln (*B*) groups and staining of bronchioles from mice in control (*C*) and Gln (*D*) groups after LPS administration for 2 wk. Magnification ×20. *E*: quantification of collagen accumulation around alveolar space between groups ($n = 5$). Cytoplasm stains red; blue staining of collagen indicates the presence of fibrosis as indicated by arrows. Areas of collagen around alveolar space were assessed with Image-Pro Plus software. Details of Masson's trichrome staining and the calculating formula are described in MATERIALS AND METHODS. *Significant difference between the 2 groups ($P < 0.05$).

antibodies before intratracheal LPS administration exhibited reduced neutrophil accumulation within the lung. Greenberger et al. (13) found that neutralization of MIP-2 attenuated neutrophil recruitment and bacterial clearance in murine *Klebsiella* pneumonia. In this study, we found that the Gln group had the highest TNF-α, KC, and MIP-2 concentrations at 6 h after LPS, and the peak levels in the Gln group occurred before those of the control group. These results indicate that Gln supplementation promotes inflammatory cytokine production at an earlier stage of ALI. Since neutrophils and macrophages produce CXC chemokines in response to LPS, this finding suggests that inflammatory cells are recruited at an earlier phase of ALI after Gln administration. These results are comparable to the higher neutrophil number at 6 h after LPS as determined in the BALF in the Gln group observed in this study. NF-κB is sequestered in the cytoplasm in an inactive state. NF-κB is activated by a variety of external stimulants, including LPS and cytokines. Binding of LPS to Toll-like receptor triggers signaling transduction, and activation of NF-κB represents the terminal step in the signaling pathway, which triggers the transcription of genes involved in the inflammatory response (20) and plays an important role in ALI caused by LPS (9). In this study, we found that the changing pattern of NF-κB expression was comparable to that of the chemokines, but the time points of NF-κB expression preceded the production of chemokine. This result may indicate that LPS is involved in the activation of NF-κB, and NF-κB is responsible for at least one of the signaling pathways related to the translation of the CXC. We only measured total NF-κB in lung homogenates; the dynamic changes in cytoplasmic and nuclear movements cannot be stated for certain. However, we did observe that Gln supplementation resulted in higher NF-κB

expression than that in the control group, and CXC possibly in response to NF-κB at an earlier phase of ALI.

ALI results in the generation of reactive oxygen species and increased oxidative stress in the lungs (7). In this study we measured TBARS in the lungs to evaluate lipid peroxidation between groups. TBARS are derivative products from oxidation and thus can be treated as a marker for assessing the extent of oxidative stress in the body (25). We found that, compared with the TBARS levels at 0 h and 6 h, the control animals had lower levels at 12 h and 24 h. This might be explained by the protective effect of endogenous antioxidants generated on eliminating free radicals at the later stage of ALI. Gln is the main fuel for rapidly proliferating cells. Various studies have shown that Gln has antioxidant effects under conditions of metabolic stress. Hong et al. (15) found that pretreatment with Gln-supplemented nutrition protects the liver during hepatic injury by preserving glutathione (GSH) stores. An in vitro study by Babu et al. (2) also found that Gln can prevent liver damage possibly mediated via GSH synthesis. GSH is a major antioxidant and acts as a vital component in host defense. Gln was found to be rate limiting for GSH synthesis, and the availability of Gln is critical for generating GSH stores (31). In this study, we found that lipid peroxides were significantly lower in the early phase, whereas these products were higher in the later stage of ALI in the Gln group than in the control group. A study by Castell et al. (4) found that the addition of Gln increased the respiratory bursts of human polymorphonuclear neutrophils stimulated with mitogen. We speculated that GSH derived from Gln effectively eliminated superoxide anions in the early stage (at 6 h after LPS) but failed to remove the excessive reactive oxidants generated by neutrophils in the later phase of ALI. Because Gln activates and delays the spontaneous apoptosis of neutrophils, this may consequently lead to more superoxide anions accumulated in the late stage of ALI as observed at 12 and 24 h after LPS, thus aggravating damage to the lungs. Since GSH levels were not measured in this study, whether GSH is involved in the changes of TBARS in the lung must be clarified.

The results of the histopathological findings are consistent with our speculation that damage to the lungs was more severe in the Gln group. Collagen deposition and thickening of the septal space in lung tissues as observed by staining 2 wk after ALI were much greater in the Gln group, indicating that more severe lung injury occurred when Gln was administered. A previous study also showed that Gln is required for the protein synthesis of extracellular matrix including collagen and α-actin (24). However, our findings differed from the results found by other investigators (21, 27, 28, 34). Their studies showed that Gln exerts an anti-inflammatory effect and prevents the occurrence of lung injury. In their study, lung injury was induced by cecal ligation and puncture (CLP) or intravenous LPS injection. CLP is a model with live multiplying bacteria in the blood, the abdomen is the site of injury, and intravenous LPS injection results in systemic endotoxin circulation. Since Gln activates and promotes the phagocytic activities of immune cells, the inflammatory reaction may be attenuated when Gln is administered in their model, thus preventing injury to remote organs. In our study, LPS was applied directly to the lungs. LPS is an endotoxin of gram-negative bacteria. When LPS was administered, neutrophils were recruited to the lung. Although no bacteria were eliminated, neutrophils that infiltrate the lungs express proinflammatory cytokines and contribute to the oxidant-induced injury of tissues (26). Activation of neutrophils and delay of apoptosis exerted by Gln may aggravate damage to the lungs. Different models used in various studies might have distinct results among the animals used. The mechanisms responsible for the adverse effects of Gln in ALI require further investigation.

In summary, the present study showed that dietary Gln administration resulted in higher inflammatory cytokine production and more neutrophils being recruited in the early stage of LPS-induced ALI. Also, a larger amount of lipid peroxides accumulated in the lungs in the Gln group. These results are consistent with the histopathological findings that the alveolar septae were thickened and collagen deposition in lung tissues was greater in the Gln group. These findings suggest that Gln supplementation causes greater oxidative stress to the lungs and may consequently result in more severe lung damage in the model of ALI induced by LPS. Although administration of LPS alone might not reflect the whole complexity of ALI, infections with gram-negative bacteria and exposure to their predominant pathogenic component still play an important role in the development and outcome of ALI. This study provides basic information and may imply that Gln supplementation in ALI should be carefully evaluated.

GRANTS

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