Glutamine Pretreatment Reduces IL-8 Production in Human Intestinal Epithelial Cells by Limiting IκBα Ubiquitination

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ABSTRACT Glutamine, the most abundant amino acid in the human body, plays several important roles in the intestine. Recent studies showed that glutamine regulates protein metabolism and intestinal inflammation among other mechanisms by reducing proinflammatory cytokine release. Because regulation of the inflammatory response was shown to be linked to proteolysis regulation, we hypothesized that glutamine pretreatment could act on IL-8 production in human intestinal epithelial cells through the regulation of inhibitor κB (IκB) ubiquitination. The HCT-8 cells were pretreated for 24 h with 0.6, 2, or 10 mmol/L glutamine. IL-8 concentration and IκB (free and ubiquitinated) expressions were assessed by ELISA and immunoblotting, respectively. A pretreatment with 10 mmol/L glutamine decreased IL-8 production under both basal and proinflammatory conditions (both $P < 0.05$). In the presence of a proteasome inhibitor (MG132), the ubiquitin-IκBα complex expression was not significantly modified by glutamine under basal conditions but decreased significantly under proinflammatory conditions ($P < 0.05$). After the addition of 10 mmol/L of glutamine, the free IκBα expression increased under basal and stimulated conditions (both $P < 0.05$). A glutamine pretreatment of 10 mmol/L did not affect ubiquitin expression or proteasome activity. This study indicates that glutamine pretreatment may reduce the intestinal inflammatory response by limiting the proteolysis of IκBα. J. Nutr. 136: 1461–1465, 2006.

KEY WORDS: • *glutamine* • *cytokine* • *IκBα* • *ubiquitin* • *intestine*

Glutamine, the preferred substrate for both enterocytes and other rapidly dividing cells, such as immune cells, was proposed to have beneficial effects on clinical outcomes in critically ill patients (1,2). These effects could be explained by the influence of glutamine on intestinal functions as described in animal models (3). Indeed, glutamine improved intestinal integrity by increasing cell proliferation (4,5), reducing intestinal epithelial cell apoptosis (6), or improving gut protein metabolism (7,8).

Glutamine also modulates the inflammatory response. For example, glutamine influenced cytokine production by various cell types in vitro (9,10). Plasma IL-8 concentration was decreased by parenterally administered glutamine in septic rats (11) and in patients with severe pancreatitis (12). Intestinal cytokine production was also modulated by glutamine in different models. In rats, glutamine decreased tumor necrosis factor-α (TNF-α)[2] and IL-8 production and improved 2,4,6-trinitrobenzene sulfonic acid–induced tissue lesions (13), whereas it restored IL-4 and IL-10 intestinal production during total parenteral nutrition (14,15). In addition, we reported previously that glutamine decreased basal and IL-1β–induced productions of IL-6 and IL-8 and increased IL-10 production by human duodenal mucosa (16–18). It was also reported that glutamine prevented nuclear factor (NF)-κB activation in intestinal epithelial cells (19). However, pathways by which glutamine modulates cytokine production have yet to be fully clarified.

In fact, glutamine may reduce inflammatory response by increasing IL-10 production, but also inducing heat shock protein (hsp) expression (20,21) or improving antioxidant status (6). In addition, glutamine may regulate proteolysis because we reported that enteral glutamine reduced the ubiquitin mRNA level in human duodenal mucosa (8). Moreover, the ATP-ubiquitin-dependent proteolytic pathway contributes to the regulation of the inflammatory response. Indeed, inhibitor of κB (IκB) ubiquitination allows the translocation of NF-κB in the nucleus and consequently the transcription of proinflammatory genes, including IL-8 (22). Liboni et al. (23) showed recently that the effect of induction of IL-8 production by glutamine depletion involved an increase in the IκB-ubiquitin complex in Caco-2 cells. Nevertheless the effects of glutamine supplementation on IκBα degradation have not yet been examined.

Thus, the aim of our study was to evaluate the effects of glutamine pretreatment on IκBα ubiquitination in human intestinal epithelial cells.

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[2] Abbreviations used: hsp, heat shock protein; IFN-γ, interferon-γ, IκB, inhibitor of κB; LPS, lipopolysaccharide; NF-κB, nuclear factor-κB; TNF-α, tumor necrosis factor-α.

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MATERIALS AND METHODS

Materials. The medium and products used for cell culture were obtained from Eurobio. Recombinant human IL-1β and MG132, an inhibitor of proteasome activity, were supplied by Sigma-Aldrich; recombinant human TNFα and recombinant interferon-γ (IFNγ) were from Tebu. Protein G-agarose, antibodies for IκBα (native form), and ubiquitin were obtained from Calbiochem. ELISA kits were from R&D Systems and Hyperfilm MP from Amersham Biosciences. HCT-8 cells, derived from human ileocecal epithelial adenocarcinoma, were obtained from the European Collection of Animal Cell Cultures.

Cell culture. The cells were seeded at a density of 12×10^3 cells/cm^2 into 21-cm^2 tissue culture dishes; they were grown in DMEM containing 2 mmol/L glutamine and supplemented with 10% heat-inactivated fetal bovine serum, 1% nonessential amino acid, and 1% antibiotics (0.1 U/L penicillin, 0.1 U/L streptomycin) as standard medium at 37°C in a water-saturated atmosphere with 5% CO_2. To study the influence of glutamine, glutamine-free and serum-free DMEM media were used.

Cells between passages 42 and 61 were used at confluence after 3 d of growth. Glutamine was added at various concentrations (0.6, 2, and 10 mmol/L) for 24 h and then cells were stimulated or not with a mixture of proinflammatory cytokines called cytomix (1 μg/L IL-1β, 20 μg/L TNFα, 10 μg/L IFNγ) as previously described (24–26). To study free and ubiquitinated IκBα, cells were treated 1 h before cytomix stimulation with the proteasome inhibitor MG132 at 20 μmol/L and then stimulated with or without cytomix for 30 min. At the end of the experiment, culture media were collected, filtered, immediately frozen in liquid nitrogen, and stored at −80°C until cytokine measurement. The cells were washed with PBS, then centrifuged at 1000 $\times$ *g* for 10 min, and the cell pellets stored at −80°C for protein measurement.

IL-8 assay. After a pretreatment with increasing concentrations of glutamine for 24 h, cells were stimulated or not with cytomix for 18 h. IL-8 concentration was measured with ELISA kits using a 96-well plate reader (Σ960 photometer, Metertech) at a wavelength of 450 nm (each sample was quantified in duplicate). The sensitivity was 13.9 ng/L for IL-8. Values were then normalized to total cellular protein assessed with the method of Lowry (27) using bovine serum albumin as a standard.

Immunoprecipitation of IκBα. After pretreatment with increasing concentrations of glutamine for 24 h, cells were stimulated or not with cytomix for 30 min. Then, cells were washed with ice-cold PBS and Buffer A (50 mmol/L HEPES, 150 mmol/L NaCl, 10 mmol/L EDTA, 10 mmol/L β-glycerophosphate, 100 mmol/L NaF, 2 mmol/L ortho-vanadate, pH 7.5). They were then lysed in lysis buffer (Buffer A supplemented with 1% Triton X-100, 1 mmol/L phenylmethyl sulfoxide, 20 μmol/L leupeptin, 2 nmol/L aprotinin) for 15 min at 4°C and centrifuged at 12,000 $\times$ *g* for 15 min. IκBα was immunoprecipitated in the supernatant by the addition of 1 μg of specific rabbit antibody preadsorbed on protein-A agarose for 2 h (28). Protein concentrations of cell extracts were measured by the method of Bradford (29).

Immunoblotting for IκBα and ubiquitin. Total proteins (25 μg) and immunoprecipitated proteins (60 μg) were separated on 4–12% Tris-Glycine resolving gels (Invitrogen) and transferred to a polyvinylidene fluoride membrane. The membrane was blocked for 1 h at room temperature with 5% (wt:v) nonfat dry milk in Tris-buffered saline (TBS; 10 mmol/L Tris, pH 8; 150 mmol/L NaCl) plus 0.05% (wt:v) Tween 20 followed by an overnight incubation at 4°C with anti-IκBα (1:1000) or with anti-ubiquitin (1:500) antibodies. After 3 washes in a blocking solution of 5% (wt:v) nonfat dry milk in TBS:0.05% Tween 20, a 1-h incubation with peroxidase-conjugated goat anti-rabbit IgG (1:3000) was performed. After 3 additional washes, immunocomplexes were revealed using the enhanced chemiluminescence detection system (Amersham Biosciences). Protein bands were quantified by densitometry using IRIS 5.10 software.

Proteasome activity. After being washed with cold PBS, cells were scraped into 200 μL of ice-cold lysis buffer containing 30 mmol/L Tris-HCl (pH 7.2), 1 mmol/L dithiothreitol, and 1% Triton X-100. Cells were placed on ice for 15 min and then centrifuged at 11,000 $\times$ *g* for 15 min at 4°C. Protein (20 μg) was incubated with 20 mmol/L Tris-HCl (pH 7.2), 0.5 mmol/L EDTA, 0.035% SDS, and 70 μmol/L of the fluorogenic proteasome substrate Suc-LLVY-MCA (Calbiochem) at 37°C for 1 h, as previously described (30). For each sample, incubation was also performed in the presence of a specific inhibitor, clasto-lactacystin β-lactone (10 μmol/L). Measurements of proteolysis (unquenched MCA peptide) were realized in a microtiter plate fluorometer Mithras LB 940 (Berthold Technologies) (excitation, 355 nm; emission, 460 nm). The activity values shown were derived by subtracting the fluorescence obtained in the presence of the specific inhibitor from the value obtained in its absence and expressed in relative fluorescent units/60 min.

Statistical analysis. Results (absolute values) are expressed as means ± SEM for the indicated number of independent experiments. Statistical analysis was performed using StatView 5.0 software (SAS Institute). When 2 conditions were compared, the statistical difference was calculated using a *t* test if comparable variances were observed. In other cases, the nonparametric Mann-Whitney test was used. When >2 conditions were compared, statistical analysis was performed using 1-way or 2-way ANOVA if comparable variances were observed. In others cases, a nonparametric test was used. Pairwise multiple comparisons were conducted using Student's *t* test as a post hoc test. The level of statistical significance was fixed at $P < 0.05$.

RESULTS

Effect of cytomix on IL-8 production and on free and ubiquitinated IκBα expression. The production of IL-8 was greater in the presence of cytomix compared with control cells (**Fig. 1***A*). Indeed, IL-8 production increased ~24-fold ($P < 0.05$). We then determined, using time courses, that the greatest degradation of IκBα occurred in HCT-8 cells at 30 min (data not shown) as was reported recently in Caco-2 cells (23). Free IκBα expression decreased ($P < 0.05$, Fig. 1*B*) and the ubiquitinated IκBα expression increased under proinflammatory conditions compared with control conditions ($P < 0.05$, Fig. 1C). Thus, we tested the effect of increasing concentrations of glutamine on these variables.

Effect of glutamine on IL-8 production in HCT-8 cells. Increasing concentrations of glutamine from 0.6 to 10 mmol/L decreased IL-8 production under basal (**Fig. 2***A*) and stimulated (Fig. 2*B*) conditions, both $P < 0.05$. Indeed, IL-8 production was significantly reduced by 10 mmol/L glutamine compared with 0.6 mmol/L glutamine under basal conditions (48% of 0.6 mmol/L glutamine) and compared with 0.6 and 2 mmol/L glutamine under stimulated conditions (68 and 73% of 0.6 and 2 mmol/L glutamine, respectively). To investigate whether the mechanism of glutamine-mediated alteration of IL-8 expression involved IκBα degradation, we evaluated free and ubiquitinated IκBα expression.

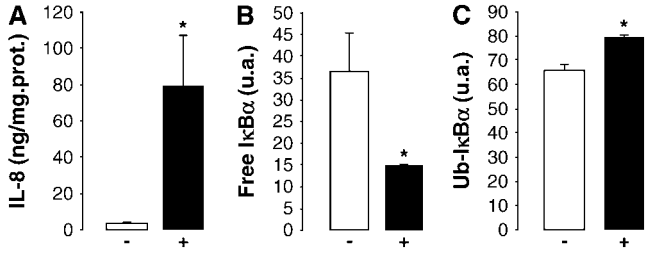

FIGURE 1 Effect of cytomix on IL-8 production, free IκBα, and ubiquitinated IκBα expression in HCT-8 cells. HCT-8 cells were cultured for 24 h with 0.6 mmol/L glutamine and then stimulated (+) or not (−) with cytomix (1 μg/L IL-1β, 20 μg/L TNFα, and 10 μg/L IFNγ). After 18 h of stimulation, IL-8 production (*A*) was measured in culture media by an ELISA assay. After 30 min of stimulation, the expression of free (*B*) and ubiquitinated (*C*) IκBα was evaluated by immunoprecipitation and immunoblotting. Values are means ± SEM, $n = 4$. *Different from unstimulated, $P < 0.05$.

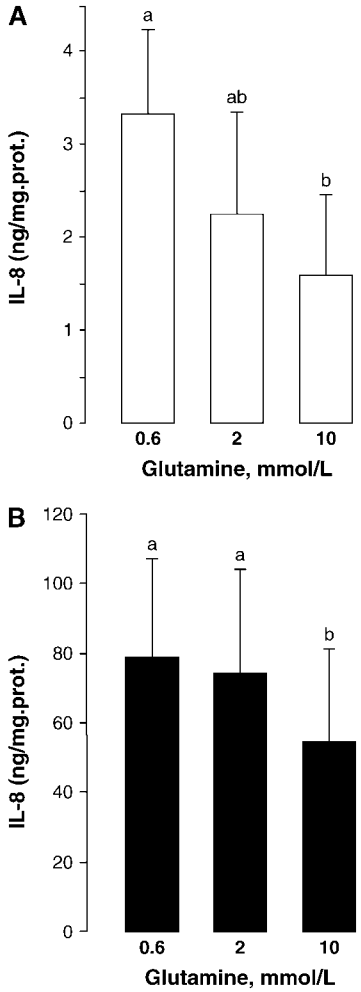

FIGURE 2 Effect of glutamine on IL-8 production in HCT-8 cells under basal and stimulated conditions. HCT-8 cells were pretreated with 0.6, 2, or 10 mmol/L glutamine for 24 h. Then, cells were cultured for 18 h in the presence of 0.6, 2, or 10 mmol/L glutamine, without (*A*) or with (*B*) cytomix (1 μg/L IL-1β, 20 μg/L TNFα, and 10 μg/L IFNγ). Values are means ± SEM, $n = 4$. Means without a common letter differ, $P < 0.05$.

Effect of glutamine on free and ubiquitinated IκBα expression. Glutamine affected ubiquitinated and free IκBα (**Fig. 3**). The ubiquitin-IκBα complex expression was not modified by glutamine under basal conditions ($P = 0.07$) but it was decreased under proinflammatory conditions ($P < 0.05$, Fig 3*B*). As a consequence, the cytomix-induced ubiquitin-IκBα increase was blunted in the presence of 10 mmol/L glutamine. On the other hand, 10 mmol/L glutamine significantly increased free IκBα expression under basal conditions, and this effect persisted in the presence of cytomix (Fig. 3C). Interestingly, the comparison of data from cytomix-treated and -untreated cells suggests that 10 mmol/L glutamine was required for a significant increase in IκBα expression under inflammatory conditions, whereas under basal conditions, 2 mmol/L glutamine was sufficient to have an effect.

Thus, glutamine supplementation decreased ubiquitinated IκBα expression and stimulated free IκBα expression while decreasing IL-8 production. To understand the mechanisms by which glutamine affects IκB ubiquitination, we assessed the influence of glutamine on free ubiquitin protein expression and proteasome activity.

Effect of glutamine pretreatment on ubiquitin protein expression and proteasome activity. Because ubiquitinated IκBα was studied 30 min after cytomix, a time that was likely too short to influence protein synthesis, ubiquitin protein expression and proteasome activity were assessed under basal conditions in response to glutamine pretreatment (0.6 and 10 mmol/L). Supplementation with 10 mmol/L glutamine did not modify ubiquitin protein expression or proteasome activity (data not shown).

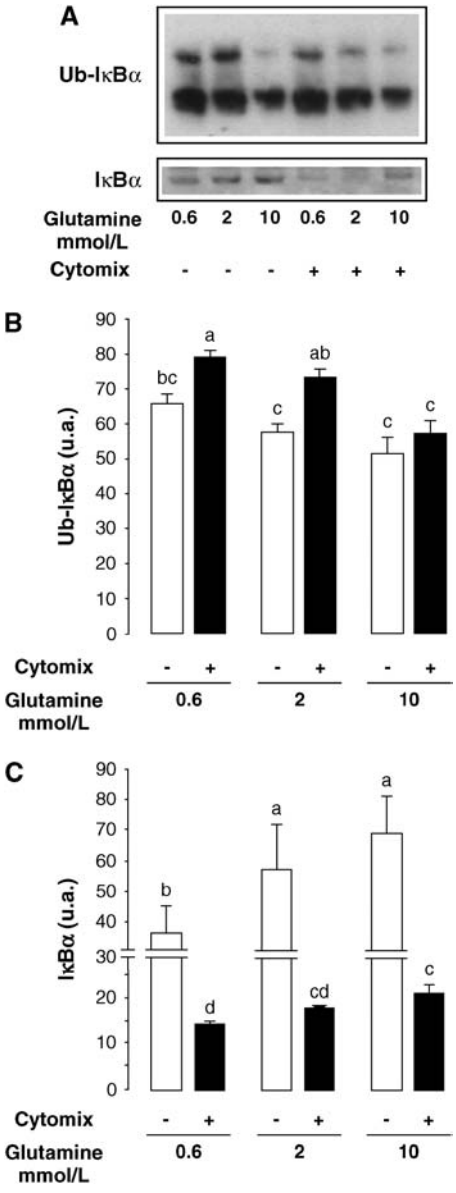

FIGURE 3 Effect of glutamine on free and ubiquitinated IκBα expression in HCT-8 cells. HCT-8 cells were pretreated with 0.6, 2, or 10 mmol/L glutamine for 24 h. One hour before stimulation, 20 μmol/L MG132 was added to the culture media. Then, cells were cultured for 30 min in the presence of 0.6, 2, or 10 mmol/L glutamine and without (open bars; −) or with (black bars; +) cytomix (1 μg/L IL-1β, 20 μg/L TNFα, and 10 μg/L IFNγ). As illustrated (*A*), ubiquitinated IκBα (*B*) and free IκBα (*C*) expression was evaluated in the cytosolic fraction using immunoprecipitation of IκBα followed by immunoblot with anti-ubiquitin or anti-IκBα antibodies. Values are means ± SEM, $n = 4$. Means without a common letter differ, $P < 0.05$.

DISCUSSION

In the present study, we showed that glutamine pretreatment reduced IL-8 production under both basal and proinflammatory

conditions in human intestinal epithelial HCT-8 cells. Our data also indicated that the ubiquitination process of IκB was involved in this effect.

The endogenous production of IL-8 by HCT-8 cells appeared minimal in the presence of 0.6 mmol/L glutamine, which is a physiologic plasma concentration (31). Indeed, recent evidence showed that glutamine deprivation enhanced IL-8 production after lipopolysaccharide (LPS) stimulation in Caco-2 cells (23,32,33). Under basal conditions, glutamine deprivation alone or associated with an inhibitor of glutamine synthetase, blocking de novo glutamine synthesis, did not increase IL-8 production (32). Under stimulated conditions, glutamine deprivation exacerbated the LPS response (23,32). On the other hand, glutamine supplementation was associated with a decrease in IL-8 intestinal production in experimental models in rats (13,34). We also showed previously that glutamine reduced proinflammatory cytokine production (IL-6, IL-8) by human duodenal mucosa (16–18) and others (32). In contrast, in previous studies, glutamine together with stimulation with cytomix did not affect IL-8 production in human intestinal epithelial cells (25,32). This discrepancy could be explained by different experimental procedures such as the dose of glutamine tested and the timing of supplementation of glutamine. Indeed, in our previous work (25), glutamine was not added 24 h before cytomix but at the same time as cytomix. Thus, pretreatment in the present study may be critical in eliciting a response to glutamine. In the present study, we compared 2 and 10 mmol/L glutamine to the physiologic plasma concentration (0.6 mmol/L), which was not examined in the earlier study (25). Thus, this wider range of concentrations allows a greater ability to demonstrate the effect of glutamine. These concentrations are relevant to in vivo studies because they mimic the concentrations that may be achieved locally during an enteral infusion of glutamine-supplemented diets.

The mechanisms by which glutamine modulates inflammatory cytokine production are still under debate. Indeed, glutamine may affect IL-8 production by the upregulation of anti-inflammatory and protective processes such as IL-10 production (18,35) or hsp expression (21,36–38). On the other hand, glutamine also affected the NF-κB pathway (19,23). Liboni et al. (23) showed that enhanced IL-8 production by glutamine deprivation was mediated by an increase in IκBα ubiquitination and then its degradation, whereas the influence of glutamine supplementation on this pathway remains unknown. In the present study, ubiquitinated IκBα and free IκBα expressions were increased and decreased, respectively, by cytomix as previously described (22,23). Glutamine pretreatment partially restored these expressions. In fact, ubiquitinated IκBα expression was reduced by 10 mmol/L glutamine; consequently, free IκBα expression was increased. In addition, glutamine reduced IL-8 production ~31%, whereas it increased ubiquitinated IκBα expression ~28%. These results suggest that glutamine may limit IL-8 production through the degradation pathway of IκBα in HCT-8 cells.

Moreover, we demonstrated that the ubiquitin protein and the proteasome activity do not seem to be involved in this effect because glutamine did not affect these variables. In Caco-2 cells, the phosphorylation mechanisms were also not involved in the effects of glutamine on IκB degradation (23). Thus, glutamine may influence IκBα ubiquitination by the regulation of specific ubiquitin-conjugating enzymes. IκB is ubiquitinated by the SCF-βTrCP ubiquitin-ligase complex and subsequently degradated by the proteasome (39). The influence of glutamine on these processes remains unknown. In addition, there are some differences between the effects of glutamine on cytokine-treated and -untreated cells; this may reflect the differential use of glutamine for metabolic or regulatory pathways according to the level of experimental stress. Further investigations are warranted to explore these mechanisms.

In conclusion, glutamine pretreatment decreases IL-8 production under both basal and proinflammatory conditions in human intestinal epithelial HCT-8 cells, through the regulation of IκB ubiquitination.

LITERATURE CITED

1. Coeffier M, Dechelotte P. The role of glutamine in intensive care unit patients: mechanisms of action and clinical outcome. Nutr Rev. 2005;63:65–9.
2. Déchelotte P, Hasselmann M, Cynober L, Allaouchiche B, Coëffier M, Hecketsweiler B, Merle V, Mazerolles M, Samba D, et al. L-Alanyl-L-glutamine dipeptide-supplemented total parenteral nutrition reduces infectious complications and glucose intolerance in critically ill patients: the French controlled randomised double-blind multicenter study. Crit Care Med. 2006;34:598–604.
3. Souba WW. Nutritional support. N Engl J Med. 1997;336:41–8.
4. Higashiguchi T, Hasselgren PO, Wagner K, Fischer JE. Effect of glutamine on protein synthesis in isolated intestinal epithelial cells. JPEN J Parenter Enteral Nutr. 1993;17:307–14.
5. Scheppach W, Dusel G, Kuhn T, Loges C, Karch H, Bartram HP, Richter F, Christl SU, Kasper H. Effect of L-glutamine and n-butyrate on the restitution of rat colonic mucosa after acid induced injury. Gut. 1996;38:878–85.
6. Evans ME, Jones DP, Ziegler TR. Glutamine prevents cytokine-induced apoptosis in human colonic epithelial cells. J Nutr. 2003;133:3065–71.
7. Bouteloup-Demange C, Claeyssens S, Maillot C, Lavoinne A, Lerebours E, Dechelotte P. Effects of enteral glutamine on gut mucosal protein synthesis in healthy humans receiving glucocorticoids. Am J Physiol Gastrointest Liver Physiol. 2000;278:G677–81.
8. Coeffier M, Claeyssens S, Hecketsweiler B, Lavoinne A, Ducrotte P, Dechelotte P. Enteral glutamine stimulates protein synthesis and decreases ubiquitin mRNA level in human gut mucosa. Am J Physiol Gastrointest Liver Physiol. 2003;285:G266–73.
9. Yassad A, Lavoinne A, Bion A, Daveau M, Husson A. Glutamine accelerates interleukin-6 production by rat peritoneal macrophages in culture. FEBS Lett. 1997;413:81–4.
10. Yaqoob P, Calder PC. Cytokine production by human peripheral blood mononuclear cells: differential sensitivity to glutamine availability. Cytokine. 1998; 10:790–4.
11. Lin MT, Saito H, Furukawa S, Fukushima R, Kazuhiko F, Lee PH, Chang KJ, Chen WJ. Alanyl-glutamine enriched total parenteral nutrition improves local, systemic, and remote organ responses to intraperitoneal bacterial challenge. JPEN J Parenter Enteral Nutr. 2001;25:346–51.
12. de Beaux AC, O'Riordain MG, Ross JA, Jodozi L, Carter DC, Fearon KC. Glutamine-supplemented total parenteral nutrition reduces blood mononuclear cell interleukin-8 release in severe acute pancreatitis. Nutrition. 1998;14:261–5.
13. Ameho CK, Adjei AA, Harrison EK, Takeshita K, Morioka T, Arakaki Y, Ito E, Suzuki I, Kulkarni AD, et al. Prophylactic effect of dietary glutamine supplementation on interleukin 8 and tumour necrosis factor alpha production in trinitrobenzene sulphonic acid induced colitis. Gut. 1997;41:487–93.
14. Fukatsu K, Lundberg AH, Kudsk KA, Hanna MK, Johnson CD, Wu Y, Wilcox HG, Zarzaur BL. Modulation of organ ICAM-1 expression during IV-TPN with glutamine and bombesin. Shock. 2001;15:24–8.
15. Kudsk KA, Wu Y, Fukatsu K, Zarzaur BL, Johnson CD, Wang R, Hanna MK. Glutamine-enriched total parenteral nutrition maintains intestinal interleukin-4 and mucosal immunoglobulin A levels. JPEN J Parenter Enteral Nutr. 2000;24: 270–4.
16. Coeffier M, Miralles-Barrachina O, Le Pessot F, Lalaude O, Daveau M, Lavoinne A, Lerebours E, Dechelotte P. Influence of glutamine on cytokine production by human gut in vitro. Cytokine. 2001;13:148–54.
17. Coeffier M, Marion R, Leplingard A, Lerebours E, Ducrotte P, Dechelotte P. Glutamine decreases interleukin-8 and interleukin-6 but not nitric oxide and prostaglandins E(2) production by human gut in-vitro. Cytokine. 2002;18:92–7.
18. Coeffier M, Marion R, Ducrotte P, Dechelotte P. Modulating effect of glutamine on IL-1beta-induced cytokine production by human gut. Clin Nutr. 2003;22: 407–13.
19. Moon M, Pritts T, Salzman A, Fischer J, Hasselgren PO. Glutamine prevents LPS-induced NF-κB activation in human intestinal epithelial cells [abstract]. Gastroenterology. 1998;114:A1410–1.
20. Wischmeyer PE, Riehm J, Singleton KD, Ren H, Musch MW, Kahana M, Chang EB. Glutamine attenuates tumor necrosis factor-alpha release and enhances heat shock protein 72 in human peripheral blood mononuclear cells. Nutrition. 2003;19:1–6.
21. Coeffier M, Le Pessot F, Leplingard A, Marion R, Lerebours E, Ducrotte P, Dechelotte P. Acute enteral glutamine infusion enhances heme oxygenase-1 expression in human duodenal mucosa. J Nutr. 2002;132:2570–3.
22. Jobin C, Haskill S, Mayer L, Panja A, Sartor RB. Evidence for altered regulation of I kappa B alpha degradation in human colonic epithelial cells. J Immunol. 1997;158:226–34.

23. Liboni KC, Li N, Scumpia PO, Neu J. Glutamine modulates LPS-induced IL-8 production through IkappaB/NF-kappaB in human fetal and adult intestinal epithelium. J Nutr. 2005;135:245–51.

24. Marion R, Coeffier M, Leplingard A, Favennec L, Ducrotte P, Dechelotte P. Cytokine-stimulated nitric oxide production and inducible NO-synthase mRNA level in human intestinal cells: lack of modulation by glutamine. Clin Nutr. 2003; 22:523–8.

25. Marion R, Coeffier M, Gargala G, Ducrotte P, Dechelotte P. Glutamine and CXC chemokines IL-8, Mig, IP-10 and I-TAC in human intestinal epithelial cells. Clin Nutr. 2004;23:579–85.

26. Marion R, Coeffier M, Lemoulan S, Gargala G, Ducrotte P, Dechelotte P. L-Arginine modulates CXC chemokines in the human intestinal epithelial cell line HCT-8 by the NO pathway. Biochimie. 2005;87:1048–55.

27. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951;193:265–75.

28. Baldwin AS Jr. The NF-kappa B and I kappa B proteins: new discoveries and insights. Annu Rev Immunol. 1996;14:649–83.

29. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 1976;72:248–54.

30. Yin L, Laevsky G, Giardina C. Butyrate suppression of colonocyte NF-kappa B activation and cellular proteasome activity. J Biol Chem. 2001;276: 44641–6.

31. Souba WW, Pan M, Stevens BR. Kinetics of the sodium-dependent glutamine transporter in human intestinal cell confluent monolayers. Biochem Biophys Res Commun. 1992;188:746–53.

32. Huang Y, Li N, Liboni K, Neu J. Glutamine decreases lipopolysaccharide-induced IL-8 production in Caco-2 cells through a non-NF-kappaB p50 mechanism. Cytokine. 2003;22:77–83.

33. Liboni K, Li N, Neu J. Mechanism of glutamine-mediated amelioration of lipopolysaccharide-induced IL-8 production in Caco-2 cells. Cytokine. 2004;26:57–65.

34. Li N, Liboni K, Fang MZ, Samuelson D, Lewis P, Patel R, Neu J. Glutamine decreases lipopolysaccharide-induced intestinal inflammation in infant rats. Am J Physiol Gastrointest Liver Physiol. 2004;286:G914–21.

35. Aosasa S, Wells-Byrum D, Alexander JW, Ogle CK. Influence of glutamine-supplemented Caco-2 cells on cytokine production of mononuclear cells. JPEN J Parenter Enteral Nutr. 2003;27:333–9.

36. Wischmeyer PE. Glutamine and heat shock protein expression. Nutrition. 2002;18:225–8.

37. Uehara K, Takahashi T, Fujii H, Shimizu H, Omori E, Matsumi M, Yokoyama M, Morita K, Akagi R, Sassa S. The lower intestinal tract-specific induction of heme oxygenase-1 by glutamine protects against endotoxemic intestinal injury. Crit Care Med. 2005;33:381–90.

38. Chow A, Zhang R. Glutamine reduces heat shock-induced cell death in rat intestinal epithelial cells. J Nutr. 1998;128:1296–301.

39. Chen ZJ. Ubiquitin signalling in the NF-kappaB pathway. Nat Cell Biol. 2005;7:758–65.