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ORIGINAL ARTICLE

Glutamine Deprivation Causes Hydrogen Peroxide-induced Interleukin-8 Expression via Jak1/Stat3 Activation in Gastric Epithelial AGS Cells

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Background: The Janus kinase (Jak)/Signal transducers of activated transcription (Stat) pathway is an upstream signaling pathway for NF-κB activation in *Helicobacter pylori*-induced interleukin (IL)-8 production in gastric epithelial AGS cells. *H. pylori* activates NADPH oxidase and produces hydrogen peroxide, which activates Jak1/Stat3 in AGS cells. Therefore, hydrogen peroxide may be critical for IL-8 production via Jak/Stat activation in gastric epithelial cells. Glutamine is depleted during severe injury and stress and contributes to the formation of glutathione (GSH), which is involved in conversion of hydrogen peroxide into water as a cofactor for GSH peroxidase.
Methods: We investigated whether glutamine deprivation induces hydrogen peroxide-mediated IL-8 production and whether hydrogen peroxide activates Jak1/Stat3 to induce IL-8 in AGS cells. Cells were cultured in the presence or absence of glutamine or hydrogen peroxide, with or without GSH or a the Jak/Stat specific inhibitor AG490.
Results: Glutamine deprivation decreased GSH levels, but increased levels of hydrogen peroxide and IL-8, an effect that was inhibited by treatment with GSH. Hydrogen peroxide induced the activation of Jak1/Stat3 time-dependently. AG490 suppressed hydrogen peroxide-induced activation of Jak1/Stat3 and IL-8 expression in AGS cells, but did not affect levels of reactive oxygen species in AGS cells.
Conclusions: In gastric epithelial AGS cells, glutamine deprivation increases hydrogen peroxide levels and IL-8 expression, which may be mediated by Jak1/Stat3 activation. Glutamine supplementation may be beneficial for preventing gastric inflammation by suppressing hydrogen peroxide-mediated Jak1/Stat3 activation and therefore, reducing IL-8 production. Scavenging hydrogen peroxide or targeting Jak1/Stat3 may also prevent oxidant-mediated gastric inflammation.
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Key Words: Glutamine, Hydrogen peroxide, Janus kinase, Interleukin-8, Signal transducers of activated transcription

INTRODUCTION

Interleukin (IL)-8 is the major activator of neutrophil extravasation into the gastric mucosa, which is an important aspect of gastric inflammation.[1] Elevated levels of IL-8 are found in gastric tissues from the patients with *Helicobacter pylori* infection.[2] Previously, we showed that Janus kinase (Jak)/Signal transducers of activated transcription (Stat) signaling is a prerequisite for NF-κB activation, leading to IL-8 production in *H. pylori*-infected gastric epithelial AGS cells.[3] *H. pylori* infection activates NADPH oxidase and produces reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2) that activate Jak/Stat signaling in AGS cells.[4] H_2O_2 activates the oxidant-sensitive transcription factor NF-κB and induces the expression of IL-8 in gastric epithelial cells.[5,6] H_2O_2, not superoxide or nitric oxide, activates Jak2, Stat1, and Stat3 in various types of cells, including fibroblasts and the A431 adenocarcinoma cell lines.[7] Therefore, by activating Jak/Stat H_2O_2 may have a critical role in IL-8 expression. IL-8 increases in the gastric tissues of cancer patients.[8,9] IL-8 is also reported to be a promoter of angiogenesis[10] and acts as an autocrine growth factors for colon carcinoma cells.[11] Therefore, IL-8 may be an important mediator of gastric inflammation and carcinogenesis.

Glutathione (GSH) is composed of three amino acids, cysteine,

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glycine, and glutamate. After cellular uptake, glutamine is converted to glutamic acid, which is a precursor of GSH. Previously, we demonstrated that glutamine deprivation increases ROS levels and induces IL-8 expression by activating NF-κB in ataxia telangiectasia fibroblasts.[12] In Caco cells, glutamine deprivation increases IL-8 production after treatment with lipopolysaccharide.[13] In human intestinal cells, glutamine supplementation reduces IL-6 levels by suppressing NF-κB activation.[14,15] Glutamine supplementation also reduces inflammation and foveolar hyperplasia in *H. pylori*-infected mice.[16] The same study found that body weight gain, food consumption, *H. pylori* colonization, and serum immunoglobulin G did not differ in *H. pylori*-infected mice fed supplemental glutamine compared with mice fed a control diet. Therefore, glutamine deprivation may induce IL-8 expression mediated by H_2O_2-stimulated inflammatory signaling in gastric epithelial cells.

In the present study, we investigated whether glutamine deprivation induces IL-8 production by increasing H_2O_2 levels and whether H_2O_2 directly activates Jak1/Stat3 to induce IL-8 expression in gastric epithelial AGS cells.

MATERIALS AND METHODS

1. Cell culture

The human gastric epithelial AGS cell line (American Type Culture Collection, Manassas, VA, USA) was cultured in RPMI 1640 medium (GIBCO, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (GIBCO), 100 U/mL penicillin, and 100 μg/mL streptomycin, with or without 2 mM glutamine, (Sigma, St. Louis, MO, USA) at 37°C in a humidified atmosphere of 5% CO_2 and 95% air.

2. Experimental protocol

In the first set of experiments, cells were cultured in the absence or presence of glutamine (2 mM) with or without GSH (10 mM) for 12 hours (for measurement of IL-8 mRNA levels) or 24 hours (for measurement of the levels of GSH, H_2O_2, and IL-8 in the medium). In the second set of experiments, cells were cultured in the presence or absence of H_2O_2 (100 μM) with or without a Jak/Stat specific inhibitor AG490 (40 μM) for 30 minutes (for measurement of ROS levels), 60 minutes (for measurement of Jak1/Stat3 activation), 4 hours (for measurement of IL-8 mRNA levels), and 12 hours (for measurement of IL-8 levels in the medium).

3. Determination of intracellular glutathione and hydrogen peroxide levels in the medium

Cells were washed with ice-cold PBS, harvested by scraping into PBS, and homogenized in 300 μL of extraction buffer (0.1% Triton X-100 and 0.6% sulfosalicylic acid in KPE [0.1 M potassium phosphate buffer with 5 mM EDTA disodium salt, pH 7.5]). GSH in the acid-soluble supernatant was analyzed with an enzyme assay using a microplate reader.[17,18] GSH content was expressed as nmole/mg protein, determined by the Bradford assay (Bio-Rad Laboratories, Hercules, CA, USA). The level of H_2O_2 in the medium was determined by modification of the ferrithiocyanate method and expressed as nmole/mL.[19]

4. Determination of intracellular reactive oxygen species levels

Cells were loaded with 10 μg/mL of 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA; Invitrogen, Carlsbad, CA, USA) and incubated in 5% CO_2 and 95% air at 37°C for 30 minutes. DCF fluorescence was measured using a VICTOR X5 multilabel plate reader (PerkinElmer, Boston, MA, USA) at excitation and emission wavelengths of 485 and 520 nm, respectively.

5. Western blot analysis for Jak1, p-Jak1, Stat3 and p-Stat3

Cells were trypsinized, washed, and then homogenized in Tris-HCl (pH 7.4) buffer containing 1% NP-40 and protease inhibitor cocktail (Boehringer Mannheim, Indianapolis, IN, USA). The protein concentration of each sample was determined by Bradford assay (Bio-Rad Laboratories). Total cell extracts (50 μg) isolated from the cells were loaded per lane, separated by 6% SDS polyacrylamide gel electrophoresis under reducing conditions and transferred onto nitrocellulose membranes (Amersham Inc., Arlington Heights, IL, USA) by electroblotting. After blocking using 3% nonfat dried milk in TBS-T for 2 hours, the membrane was incubated with polyclonal antibodies for Jak1 (1:500 dilution, cat. no. 3332; Cell Signaling, Beverly, MA, USA), Stat3 (1:500 dilution, cat. No. 06-596; Upstate Biotechnology, Lake Placid, NY, USA), phospho-Jak1 (1:500 dilution, cat. no. sc-16773; Santa Cruz Biotechnology, Santa Cruz, CA, USA) and phospho- Stat3 (1:500 dilution, cat. no. 9131; Cell Signaling) diluted in TBS-T containing 3% nonfat dried milk at 4°C overnight. After washing with TBS-T, the immunoreactive proteins were visualized by using goat anti-rabbit secondary antibodies (1:2,000 dilution, cat. no. sc-2004; Santa Cruz Biotechnology) conjugated to horseradish peroxidase, which was followed by enhanced chemilumine-

scence (Santa Cruz Biotechnology).

6. Real-time PCR analysis of interleukin-8

Total RNA in cells was isolated by TRI reagent (Molecular Research Center Inc., Cincinnati, OH, USA). Total RNA was converted to cDNA by reverse transcription process using a random hexamer and virus reverse transcriptase (Promega, Madison, WI, USA) using the following conditions: 23°C for 10 minutes, 37°C for 60 minutes, and 95°C for 5 minutes. cDNA was used for real-time PCR with human specific primers for IL-8 and β-actin. The sequences of the IL-8 primers were 5′-ATGACT TCCAAGCTGGCCGTGGCT-3′ (forward primer) and 5′-TCTCAGCC CTCTTCAAA AACTTCT-3′ (reverse primer), which gave a 297 bp PCR product. For β-actin, the forward primer was 5′-ACCAA CTGGGACGACATGGAG-3′ and the reverse primer was 5′-GTGAGG ATCTTCATGAGGTAGTC-3′, which gave a 349 bp PCR product. Real-time PCR for quantifying IL-8 gene expression was conducted using a Light Cycler (Roche Applied Sciences, Indianapolis, IN, USA). cDNA was added to SYBR Green real-time PCR Master Mix (Toyobo Co., Osaka, Japan) containing 10 pg/mL of forward and reverse primers for IL-8 and was amplified using a Light Cycler. For PCR amplification, the cDNA was amplified using 40 cycles, with denaturation at 95°C for 15 seconds, annealing at 60°C for 15 seconds, and extension at 72°C for 45 seconds. The β-actin gene was amplified in the same reaction to serve as the reference gene.

7. ELISA for interleukin-8 level

IL-8 levels in the medium were determined by ELISA kits (Invitrogen) according to the manufacturer's instructions.

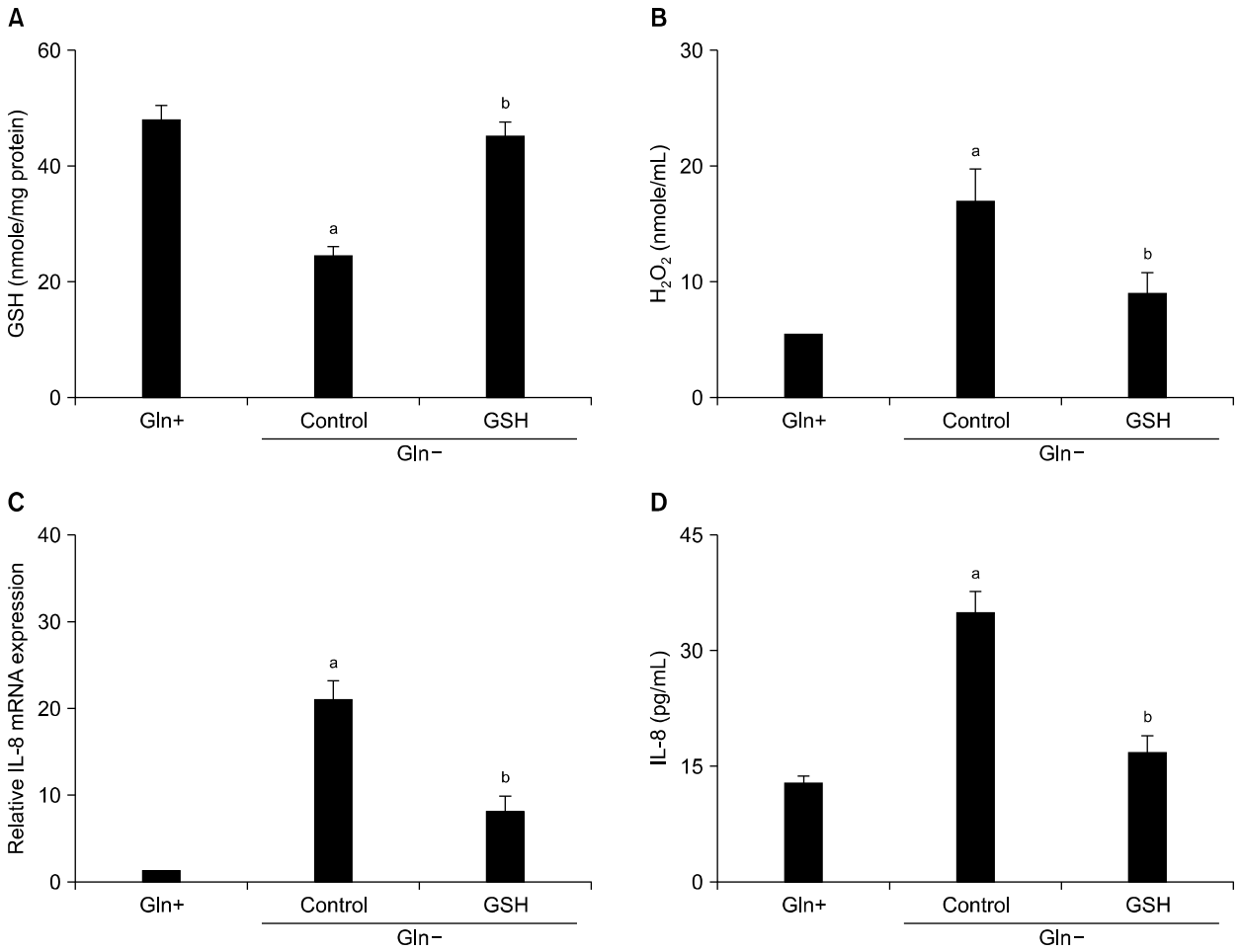

Figure 1. Levels of glutathione (GSH), H_2O_2, and interleukin (IL)-8 expression in AGS cells cultured in the absence or presence of glutamine, with or without GSH. The cells were cultured in the absence or presence of glutamine (2 mM) with or without GSH (10 mM) for 12 hours (for measurement of IL-8 mRNA levels; C) or 24 hours (for measurement of levels of GSH, H_2O_2, and IL-8 in the medium; A, B, and D). Values are expressed as mean ± SE of four different experiments. Gln+, cells cultured in the presence of glutamine; Gln−, cells cultured in the absence of glutamine. $^{a}P < 0.05$ vs. Gln+; $^{b}P < 0.05$ vs. Gln− control.

8. Statistical analysis

Statistically significant differences were determined using one-way ANOVA and Newman-Keul's test. All values are expressed as mean ± SE of four different experiments. A value of $P < 0.05$ was considered statistically significant.

RESULTS

As shown in Figure 1A and 1B, glutamine deprivation decreased intracellular GSH levels and increased hydrogen peroxide levels in the medium, an effect that was inhibited by treatment with GSH. mRNA and protein levels of IL-8 were higher in the cells cultured in the absence of glutamine than in cells cultured in the presence of glutamine. Therefore, GSH inhibited the glutamine deprivation-induced increase in IL-8 levels in AGS cells (Fig. 1C and 1D).

To evaluate whether H_2O_2 induces the activation of Jak1/Stat3 and IL-8 expression, cells were cultured in the absence or presence of H_2O_2, with or without AG490. Prior to the experiment, phospho-specific and total forms of Jak1/Stat3 were determined during 90 minutes-culture. As shown in Figure 2A, H_2O_2 induced phosphorylation of Jak1 and Stat3 time-dependently. Total forms of Jak1 and Stat3 were not changed by H_2O_2 treatment. Figure 2B shows that H_2O_2-induced activation of Jak1/Stat3 was inhibited by AG490 treatment at 60 minutes-culture. H_2O_2-induced mRNA and protein expression of IL-8 was lower in AG490-treated cells than in non-treated cells (Fig. 2D and 2E). However, the H_2O_2-induced increase in ROS levels was not affected by AG490 treatment (Fig. 2C).

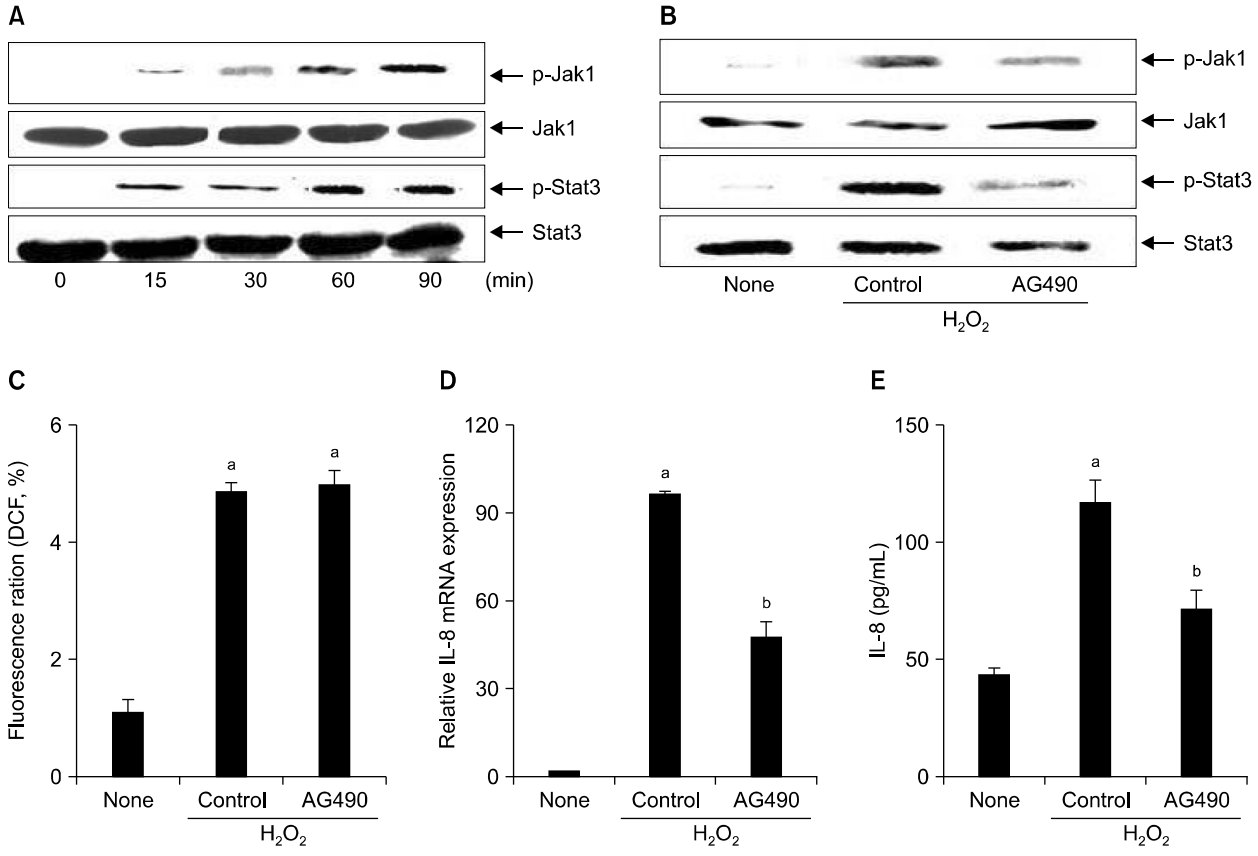

Figure 2. Levels of Janus kinase 1 (Jak1)/Signal transducers of activated transcription 3 (Stat3) activation, reactive oxygen species (ROS), and interleukin (IL)-8 expression in AGS cells cultured in the absence or presence of H_2O_2, with or without AG490. Cells were cultured in the presence or absence of H_2O_2 (100 μM) with or without AG490 (40 μM) for 30 minutes (for measurement of ROS levels; C), 60 minutes (for measurement of Jak1/Stat3 activation; B), 4 hours (for measurement of IL-8 mRNA levels; D), and 12 hours (for measurement of IL-8 levels in the medium; E). Prior to the experiment, time-dependent activation of Jak1/Stat3 was determined during 90 minutes-culture (A). Values are expressed as mean ± SE of four different experiments. $^aP < 0.05$ vs. none (cells cultured in the absence of H_2O_2); $^bP < 0.05$ vs. control (cells cultured in the presence of H_2O_2).

DISCUSSION

In the present study, we found that glutamine deprivation induces IL-8 production by increasing H_2O_2 levels, and that H_2O_2 activates Jak1/Stat3 to induce IL-8 expression in gastric epithelial AGS cells. These results suggest that glutamine supplementation suppresses oxidative stress-mediated gastric inflammation and carcinogenesis through maintenance of GSH levels in the cells and thus, suppressing H_2O_2-mediated activation of Jak/Stat and IL-8 expression.

H_2O_2 has been reported to cause activation of Jak/Stat in astrocytes[20] and periodontal ligament cells.[21] Following activation, Stat3 dimmers translocate into the nucleus where they selectively bind to γ-interferon activation sequence (GAS) element in the IL-8 promoter and induce IL-8 transcription.[22] In *H. pylori*-infected human gastric mucosal cells, the expression of IL-8 was shown to be mediated by ROS, including H_2O_2.[23] Therefore, reducing H_2O_2 levels by treatment with GSH or glutamine supplementation may inhibit gastric inflammation.

Glutamine is a conditionally essential nutrient since it is depleted from muscle stores during severe injury, illness, or stress.[24-26] In cells, glutamine is broken down into glutamate and used in the synthesis of the antioxidant GSH. Therefore, glutamine supplementation can be expected to increase GSH levels, an effect has been confirmed in rats with breast cancer.[27] In human intestinal mucosa, glutamine deprivation increases ROS levels[28,29] and reduces the production of IL-6 by inhibiting NF-κB activation.[30,31] The anti-inflammatory activity of glutamine is suggested to be attributable to its inhibition of prostaglandin synthesis,[32] but the use of glutamine in the synthesis of GSH, which reduces H_2O_2 levels, may also be important for preventing oxidant-mediated inflammation. In relation to carcinogenesis, glutamine administration has been shown to markedly alleviate oxidative/nitrosative stress, normalize SOD activity, increase levels of total GSH and block NO overproduction, but it does not reduce angiogenesis induced by hypertension in gastric tissues.[33] In contrast, glutamine promotes ovarian cancer cell proliferation, by increasing the activity of glutaminase and glutamate dehydrogenase through modulation of the mTOR/S6 and MAPK pathways, leading to cell proliferation.[34] A recent clinical study has shown that supplements of glutamine, eicosapentaenoic acid, and branched-chain amino acids can help maintain nutrition status, decrease the complications and improve compliance of esophageal cancer patients receiving concurrent chemo-radiotherapy and gastric cancer patients receiving postoperative adjuvant chemotherapy.[35]

In myc-expressing human renal cell carcinoma cells, cells use glutamine more efficiently than glucose.[36] Therefore, pharmacologic inhibition of glutamine metabolism is suggested as a potential therapeutic approach for the treatment of renal cell carcinoma. Glutamine also influences the signaling pathways involving the oncogenes myc and k-ras and the tumor suppressors p53 and sirt 4.[37] Since glutamine could stimulate proliferation of some cancer cells, more studies should be performed to fully understand these effects before glutamine is used for cancer therapy.

In conclusion, we show that reducing H_2O_2 levels by glutamine supplementation inhibits the expression of the inflammatory cytokine IL-8 through the suppression of Jak1/Stat3 activation in gastric epithelial cells. Thus, glutamine may prevent gastric inflammation as well as oxidant-mediated carcinogenesis. The inhibition of H_2O_2-induced activation of Jak1/Stat3 and Il-8 expression by glutamine suggests that it could be used as a chemo-preventive nutrient. In addition, preventing Jak1/Stat3 activation or scavenging excess H_2O_2 may be beneficial for preventing oxidant-mediated gastric inflammation.

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CONFLICTS OF INTEREST

No potential conflicts of interest were disclosed.

REFERENCES

1. Craig PM, Territo MC, Karnes WE, Walsh JH. Helicobacter pylori secretes a chemotactic factor for monocytes and neutrophils. Gut 1992;33:1020-3.
2. Fan XG, Chua A, Fan XJ, Keeling PW. Increased gastric production of interleukin-8 and tumour necrosis factor in patients with Helicobacter pylori infection. J Clin Pathol 1995;48:133-6.
3. Cha B, Lim JW, Kim H. Jak1/Stat3 is an upstream signaling of NF-κB activation in Helicobacter pylori-induced IL-8 production in gastric epithelial AGS cells. Yonsei Med J 2015;56:862-6.
4. Cha B, Lim JW, Kim KH, Kim H. 15-deoxy-D12,14-prostaglandin J2 suppresses RANTES expression by inhibiting NADPH oxidase activation in Helicobacter pylori-infected gastric epithelial cells. J Physiol Pharmacol 2011;62:167-74.
5. Kim Y, Seo JH, Kim H. β-Carotene and lutein inhibit hydrogen peroxide-induced activation of NF-κB and IL-8 expression in gastric epithelial AGS cells. J Nutr Sci Vitaminol (Tokyo) 2011;57: 216-23.

6. Hiramatsu Y, Satho T, Irie K, Shiimura S, Okuno T, Sharmin T, et al. Differences in TLR9-dependent inhibitory effects of H(2)O(2)-induced IL-8 secretion and NF-kappa B/I kappa B-alpha system activation by genomic DNA from five Lactobacillus species. Microbes Infect 2013;15:96-104.
7. Simon AR, Rai U, Fanburg BL, Cochran BH. Activation of the JAK-STAT pathway by reactive oxygen species. Am J Physiol 1998;275:C1640-52.
8. Chung HW, Jang S, Kim H, Lim JB. Combined targeting of high-mobility group box-1 and interleukin-8 to control micrometastasis potential in gastric cancer. Int J Cancer 2015;137:1598-609.
9. Liao J, Wen S, Cao L, Zhou Y, Feng Z. Effect of eradication of Helicobacter pylori on expression levels of FHIT, IL-8 and P73 in gastric mucosa of first-degree relatives of gastric cancer patients. PLoS One 2015;10:e0124576.
10. Srivastava SK, Bhardwaj A, Arora S, Tyagi N, Singh AP, Carter JE, et al. Interleukin-8 is a key mediator of FKBP51-induced melanoma growth, angiogenesis and metastasis. Br J Cancer 2015;112:1772-81.
11. Brew R, Erikson JS, West DC, Kinsella AR, Slavin J, Christmas SE. Interleukin-8 as an autocrine growth factor for human colon carcinoma cells in vitro. Cytokine 2000;12:78-85.
12. Kim MH, Kim A, Yu JH, Lim JW, Kim H. Glutamine deprivation induces interleukin-8 expression in ataxia telangiectasia fibroblasts. Inflamm Res 2014;63:347-56.
13. 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.
14. Chu CC, Hou YC, Pai MH, Chao CJ, Yeh SL. Pretreatment with alanyl-glutamine suppresses T-helper-cell-associated cytokine expression and reduces inflammatory responses in mice with acute DSS-induced colitis. J Nutr Biochem 2012;23:1092-9.
15. Remick DG. Interleukin-8. Crit Care Med 2005;33:S466-7.
16. Hagen SJ, Ohtani M, Zhou JR, Taylor NS, Rickman BH, Blackburn GL, et al. Inflammation and foveolar hyperplasia are reduced by supplemental dietary glutamine during Helicobacter pylori infection in mice. J Nutr 2009;139:912-8.
17. Jang SH, Lim JW, Morio T, Kim H. Lycopene inhibits Helicobacter pylori-induced ATM/ATR-dependent DNA damage response in gastric epithelial AGS cells. Free Radic Biol Med 2012;52:607-15.
18. Jang SH, Lim JW, Kim H. Mechanism of beta-carotene-induced apoptosis of gastric cancer cells: involvement of ataxia-telangiectasia-mutated. Ann N Y Acad Sci 2009;1171:156-62.
19. Tietze F. Enzymic method for quantitative determination of nanogram amounts of total and oxidized glutathione: applications to mammalian blood and other tissues. Anal Biochem 1969;27:502-22.
20. Park SJ, Kim HY, Kim H, Park SM, Joe EH, Jou I, et al. Oxidative stress induces lipid-raft-mediated activation of Src homology 2 domain-containing protein-tyrosine phosphatase 2 in astrocytes. Free Radic Biol Med 2009;46:1694-702.
21. Lee YS, Bak EJ, Kim M, Park W, Seo JT, Yoo YJ. Induction of IL-8 in periodontal ligament cells by H(2)O (2). J Microbiol 2008;46:579-84.
22. Gharavi NM, Alva JA, Mouillesseaux KP, Lai C, Yeh M, Yeung W, et al. Role of the Jak/STAT pathway in the regulation of interleukin-8 transcription by oxidized phospholipids in vitro and in atherosclerosis in vivo. J Biol Chem 2007;282:31460-8.
23. Zaidi SF, Ahmed K, Yamamoto T, Kondo T, Usmanghani K, Kadowaki M, et al. Effect of resveratrol on Helicobacter pylori-induced interleukin-8 secretion, reactive oxygen species generation and morphological changes in human gastric epithelial cells. Biol Pharm Bull 2009;32:1931-5.
24. Fan YP, Yu JC, Kang WM, Zhang Q. Effects of glutamine supplementation on patients undergoing abdominal surgery. Chin Med Sci J 2009;24:55-9.
25. Roth E. Nonnutritive effects of glutamine. J Nutr 2008;138:2025S-31S.
26. 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.
27. Thurman RG, Ley HG, Scholz R. Hepatic microsomal ethanol oxidation. Hydrogen peroxide formation and the role of catalase. Eur J Biochem 1972;25:420-30.
28. Humbert B, Nguyen P, Martin L, Dumon H, Vallette G, Maugère P, et al. Effect of glutamine on glutathione kinetics in vivo in dogs. J Nutr Biochem 2007;18:10-6.
29. Todorova VK, Harms SA, Kaufmann Y, Luo S, Luo KQ, Babb K, et al. Effect of dietary glutamine on tumor glutathione levels and apoptosis-related proteins in DMBA-induced breast cancer of rats. Breast Cancer Res Treat 2004;88:247-56.
30. Coëffier M, Marion R, Leplingard A, Lerebours E, Ducrotté P, Déchelotte 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.
31. Coëffier M, Miralles-Barrachina O, Le Pessot F, Lalaude O, Daveau M, Lavoinne A, et al. Influence of glutamine on cytokine production by human gut in vitro. Cytokine 2001;13:148-54.
32. Jain P, Khanna NK. Evaluation of anti-inflammatory and analgesic properties of L-glutamine. Agents Actions 1981;11:243-9.
33. Marques C, Licks F, Zattoni I, Borges B, de Souza LE, Marroni CA, et al. Antioxidant properties of glutamine and its role in VEGF-Akt pathways in portal hypertension gastropathy. World J Gastroenterol 2013;19:4464-74.
34. Yuan L, Sheng X, Willson AK, Roque DR, Stine JE, Guo H, et al. Glutamine promotes ovarian cancer cell proliferation through the mTOR/S6 pathway. Endocr Relat Cancer 2015;22:577-91.
35. Cong M, Song C, Zou B, Deng Y, Li S, Liu X, et al. Impact of glutamine, eicosapntemacnioc acid, branched-chain amino acid supplements on nutritional status and treatment compliance of esophageal cancer patients on concurrent chemoradiotherapy and gastric cancer patients on chemotherapy. Zhonghua Yi Xue Za Zhi 2015;95:766-9.
36. Shroff EH, Eberlin LS, Dang VM, Gouw AM, Gabay M, Adam SJ, et al. MYC oncogene overexpression drives renal cell carcinoma in a mouse model through glutamine metabolism. Proc Natl Acad Sci U S A 2015;112:6539-44.
37. Kim H. Glutamine as an immunonutrient. Yonsei Med J 2011;52:892-7.