

ORIGINAL PAPER

Glutamine preferentially inhibits T-helper type 2 cell-mediated airway inflammation and late airway hyperresponsiveness through the inhibition of cytosolic phospholipase A₂ activity in a murine asthma model

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Clinical and Experimental Allergy

Summary

Background The non-essential amino acid, L-glutamine (Gln), is abundant in the human body. Gln exhibits beneficial effects on endotoxin shock through the inhibition of cytosolic phospholipase A₂ (cPLA₂) activity. cPLA₂ has been reported to be implicated in the pathogenesis of asthma, but the effects of Gln on asthma have not yet been defined.

Objective To investigate the effects of Gln on allergic bronchial inflammation and airway hyperresponsiveness (AHR), and to determine the possible action mechanisms of Gln in a murine model of asthma.

Methods cPLA₂ phosphorylation was assessed by immunoprecipitation and Western blotting. Smears of bronchoalveolar lavage cells were stained with Diff-Quik solution for differential cell counting. Airway levels of the proteins [T-helper type-1 (Th1) and Th2 cytokines, and mucin] were measured by ELISA. mRNA expression of cytokines was assessed by real-time RT-PCR. AHR was assessed as a change in airway resistance (RL). Histological studies were performed to assess the levels of mucin and pulmonary inflammation.

Results Systemic Gln administration inhibited cPLA₂ phosphorylation and its enzymatic activity in the lungs. Additionally, Gln effectively suppressed the key features of Th2-dependent asthmatic features, such as airway eosinophilia, mucus formation, and airway type 2 cytokine production, as well as late AHR.

Conclusion Gln was found to be effective in the suppression of Th2-dependent phenotypes and late AHR, and this effect of Gln appeared to be at least partially attributable to its ability to suppress cPLA₂ activity in the airway. Our results suggest that clinical use of Gln for patients with asthma may be beneficial.

Keywords airway hyperresponsiveness, asthma, cPLA₂, glutamine, Th2

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Introduction

The non-essential amino acid, L-glutamine (Gln), is abundant in plasma [1], and Gln is an energy substrate for most cells [2, 3]. Consequently, Gln plays an important role in nitrogen- and carbon-skeleton exchange among different tissues, where this amino acid fulfils many different

physiological functions [4]. Extensive research in animal models of endotoxin shock, including severe injury models, has demonstrated that supplementation with Gln improves survival, enhances immune and gut barrier function, decreases bacteraemia, and inhibits gut mucosal atrophy [5, 6]. Clinical trials in human subjects have also demonstrated that Gln treatment decreases infectious complications, shortens hospital stay, and decreases hospital costs in a number of patient populations [7]. Regarding the molecular mechanism of Gln, it has been reported that increasing the oral availability of Gln could promote

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the T cell-driven, cell-mediated immune response [8]. We have shown previously that Gln protects animals from endotoxin shock through its inhibition of cytosolic phospholipase A₂ (cPLA₂) activity [9].

Bronchial asthma is characterized by reversible airway obstruction in response to allergens, chronic eosinophilic airway inflammation, and non-specific airway hyperresponsiveness (AHR). cPLA₂ has been reported to be implicated to play an important role in the pathogenesis of asthma [10–12]. cPLA₂ specifically releases arachidonic acid from the *sn*-2 position of glycerophospholipids to provide the precursor to various eicosanoids, such as leucotrienes (LTs), prostaglandins, and platelet-activating factor, which have been shown to induce airway eosinophilia and bronchoconstriction [12–15].

These findings led us to investigate the effects of Gln on bronchial inflammation and AHR in a murine model of asthma. The results indicated that systemic Gln administration effectively suppressed the key features of asthma that were induced by T-helper type 2 (Th2) cells, and also suppressed late AHR, both of which were associated with its ability, at least in part, to inhibit cPLA₂ activity in the airway.

Materials and methods

Animals

Specific pathogen-free female BALB/c mice were purchased from the Korean Institute of Chemistry Technology (Daejeon, Republic of Korea), housed in a laminar flow cabinet, and maintained on standard laboratory chow *ad libitum*. Mice were 7–8 weeks old at the start of each experiment. All experimental animals used in this study were cared for under a protocol approved by the Institutional Animal Care and Use Committee of the Chonbuk National University Medical School.

Reagents

L-Gln (Biotechnology performance certified, G-8540) was purchased from Sigma Chemical Co. (St Louis, MO, USA). Gln was dissolved in Hartmann's solution (30 mg/mL), and 750 mg/kg (15 mg/mouse) of Gln was administered i.p. 10 min after the beginning of the second airway challenge [9]. As an amino acid control, the same amount of alanine (Sigma Chemical Co.) was administered i.p.

Immunization and challenge

Mice were immunized and challenged as described previously [12]. In brief, mice were immunized i.p. with 20 µg of ovalbumin (OVA) (grade V from Sigma Chemical Co.) plus 1.0 mg aluminium hydroxide adjuvant (ImjectR Alum; Pierce, Rockford, IL, USA) on day 0, and with OVA

alone without alum on day 10. The immunized mice were exposed to aerosolized OVA for 20 min on day 17 (2.5% at first aerosolization) and day 24 (5.0% at second aerosolization). Control animals received the same immunization and the first OVA airway challenge, but were exposed to aerosolized saline instead of OVA for the second airway challenge.

Bronchoalveolar lavage

Bronchoalveolar lavage (BAL) was performed at the time indicated after the second airway challenge, as described previously [12].

Immunoprecipitation and Western blotting

Cell lysates were prepared in radioimmunoprecipitation assay buffer (0.1% SDS, 1% IGEPAL, 0.5% sodium deoxycholate, and 1 mM phenylmethylsulphonyl fluoride). Equal amounts of cell lysate were incubated with a mouse anti-cPLA₂ antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) with gentle rocking for 1 h at 4 °C, followed by the addition of protein A/G-agarose (Sigma Chemical Co.), and the mixtures were incubated overnight at 4 °C. After three washes with hypotonic buffer (10 mM HEPES, 1.5 mM MgCl₂, 0.5 mM KCl, 0.2 mM PMSF, and 0.5 mM DTT), the remaining pellet was separated on 10% SDS polyacrylamide gel under reducing conditions, and was then transferred onto a Protran nitrocellulose membrane (Schleicher & Schuell, Keene, NH, USA). Membranes were blocked for 2 h at room temperature in 5% skim milk in tris-buffered saline (TBS), followed by overnight incubation with anti-phospho-cPLA₂ (Cell Signaling, Beverly, MA, USA) or anti-cPLA₂. Blots were washed for 15 min with three changes of TBS-0.05% Tween 20 solution, followed by a 1-h incubation at room temperature with the HRP-conjugated anti-mouse antibody or anti-rabbit IgG antibody. Blots were washed again for 15 min and finally developed in the ECL Western detection reagents (Amersham-Pharmacia Biotech, Piscataway, NJ, USA).

Assay of cytosolic phospholipase A₂ activity

For the assay of cPLA₂ activity, mice lungs were minced and sonicated in a buffer (50 mg tissue/mL) containing 50 mM Tris-Cl, pH 7.5, 1 mM EDTA, 150 mM NaCl, and protease inhibitors using a tip-type sonicator (Branson Sonifier, Branson Ultrasonics Corp., CT, 20% amplitude, 3 min), followed by centrifugation for 20 min at 1000 g. The supernatants were fractionated by passaging 20 times with a 26 G needle and ultracentrifuged at 1 000 000 g for 1 h. cPLA₂ activity of the supernatants was assayed as described previously [12].

Real-time reverse transcriptase-polymerase chain reaction

RNA was prepared as described previously [16]. Reverse transcription was performed using 0.1 µg of total RNA in 10 µL of reaction mixture (Promega, Madison, WI, USA) containing oligo(dT) and avian myeloblastosis virus RT. PCR was performed with the Rotor-Gene 3000 System (Corbett Research, Morklake, Australia) programmed as 95 °C for 15 min, followed by 40 cycles of 95 °C for 20 s, 60 °C for 20 s, and 72 °C for 20 s, using the SYBR Green PCR Master Mix Reagent Kit (Qiagen, Valencia, CA, USA). The primers used were as follows: IL-4, 5'-ACAGGAG AAGGGACGCCAT-3' and 5'-GAAGCCCTACAGACGAGCT CA-3'; IL-5, 5'-AGCACAGTGGTGAAAGAGACCTT-3' and 5'-TCCAATGCATAGCTGGTGATT-3'; IL-12, 5'-CAGAAG CTAACCATCTCCTGGTTT-3' and 5'-TCCGGAGTAATTTGG TGCTTCACAC-3'; IL-13, 5'-GGAGCTGAGCAACATCACA CA-3' and 5'-GGTCCT GTAGATGGCATTGCA-3'; IFN-γ, 5'-CCTAGCTCTGAGACAATGAACGCT-3' and 5'-TGCCAGT TCCTCCAGATATCCAAG-3'; and β-actin, 5'-CTGAAGTC ACCCATGAACATGGC-3' and 5'-CAGAGCAGTAATCTCCT TCTGCA-3'. The relative levels of mRNA were calculated using the standard curve generated from sequential cDNA dilutions. The mean cycle threshold (C_t) values from quadruplicate measurements were used to calculate the gene expression, with normalization to β-actin as an internal control. Calculations of the relative levels of gene expression were conducted using the complementary computer software (Corbett Research) using a standard curve.

Determination of late airway hyperresponsiveness

Late AHR was defined as a change in airway function after challenge with aerosolized methacholine via the airways, as described elsewhere [17, 18]. Data regarding airway resistance (RL) were continuously collected after each methacholine challenge. Maximum values of RL were selected to express a change in airway function, which was represented as the per cent change from baseline after saline aerosol treatment.

Cytokine assay

The levels of IL-4, IL-5, IL-12, IL-13, and IFN-γ were quantified in BAL fluid using an ELISA performed according to the protocol of the manufacturer (IL-4 and IL-5; Endogen, Inc., Woburn, MA, USA; IL-12, and IL-13; R&D Systems Inc., Minneapolis, MN, USA). The sensitivities for IL-4, IL-5, IL-12, and IL-13 assays were 5, 5, 2, and 1.5 pg/mL, respectively.

Mucin analysis

Sections of fixed, embedded lung tissues were cut at 5 µm, placed on glass slides, and deparaffinized. Tissue samples

were then stained with alcian blue/periodic acid-Schiff (AB/PAS) and counterstained with haematoxylin and eosin (H&E). Mucin was also quantified using the mucin-binding lectin, Jacalin (Calbiochem, La Jolla, CA, USA), as described elsewhere [19, 20].

Histological studies

Lungs were fixed with 10% formalin, and the tissues were embedded in paraffin. Sections (5 µm) were stained with H&E for light microscopic examinations.

Measurement of glutamine

Samples from the plasma, lung tissues, and BAL fluids were treated with chloroform (CHCl_3) to degrade proteins and centrifuged at 20 000 g for 30 min. The aqueous phase was transferred into 1.6 mL cryovials and stored at -80 °C for later analysis. Amino acid concentrations were determined using a high-performance liquid chromatography (HPLC) (Waters Corporation; model 2690, Milford, MA, USA) methodology with a Tag C18 column and a fluorescence detector (Waters Corporation; model 474).

Statistical analysis

Data are expressed as mean $\pm$ SD. Statistical comparison was performed using one-way ANOVA, followed by the Fisher test. Significant differences between the groups were determined using the unpaired Student's *t*-test. A value of $P < 0.05$ was accepted as an indication of statistical significance.

Results

Glutamine inhibits cytosolic phospholipase A_2 phosphorylation and enzymatic activity in the airway

We first examined the effect of Gln on cPLA₂ phosphorylation and enzymatic activity of lungs in response to the second airway allergen challenge. cPLA₂ phosphorylation (Fig. 1a) and enzymatic activity (Fig. 1b) were increased significantly after inhalation of OVA compared with the levels after saline inhalation. The increased cPLA₂ phosphorylation and enzymatic activity were significantly reduced by the administration of Gln. Treatment of alanine as an amino acid control inhibited neither cPLA₂ phosphorylation (Fig. 1a) nor enzymatic activity (Fig. 1b).

Glutamine inhibits recruitment of neutrophils and eosinophils into the airway

OVA-immunized and OVA-challenged animals showed significant increases in the numbers of BAL macrophages, neutrophils, and eosinophils at 12, 24, and 48 h after the

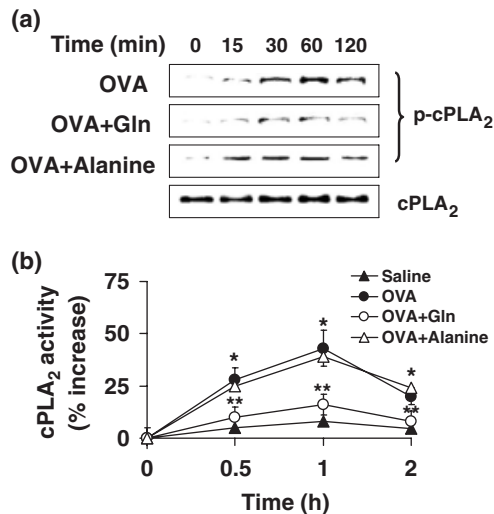


Fig. 1. Effect of Gln on cPLA₂ phosphorylation (a) and enzymatic activity (b) in the airway. Gln or alanine (750 mg/kg, 15 mg/mouse) was given i.p. 10 min after the beginning of the second airway challenge. Lungs were removed at the indicated times after the second OVA challenge. Data represent the mean $\pm$ SE of three separate experiments, and each included five animals per group. * P < 0.01 vs. saline; ** P < 0.05 vs. OVA. OVA, ovalbumin; cPLA₂, cytosolic phospholipase A₂; Gln, glutamine.

second OVA challenge compared with saline-challenged controls (Fig. 2a). Administration of Gln significantly suppressed the recruitment of neutrophils and eosinophils by more than 40–50% and 80–90%, respectively, but did not suppress macrophage recruitment. Treatment of alanine failed to inhibit recruitment of neutrophils and eosinophils into the airway (data not shown). Histological examination of lungs from OVA-sensitized mice revealed a pleiomorphic peribronchial and perivascular infiltrate consisting of eosinophils, lymphocytes, and neutrophils after OVA challenge (Fig. 2b), which was not observed in the saline-treated group. Gln-, but not alanine- treated mice showed significantly less allergic inflammation around airways and vessels (Fig. 2b).

Glutamine inhibits airway T-helper type 2 cytokine and mucus production

The effects of Gln on the production of proteins and mRNA expression of Th2 cytokines (IL-4, IL-5, and IL-13) were examined. The levels of Th2 cytokines in BAL fluids were significantly increased by airway challenge with OVA when compared with the saline-challenged control

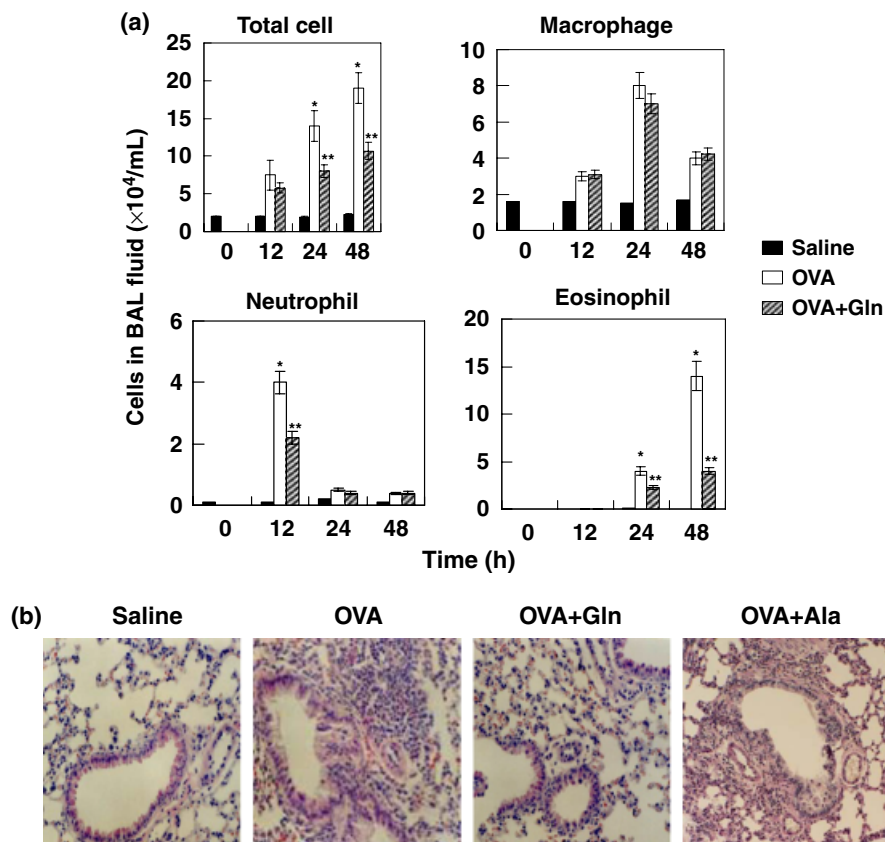


Fig. 2. Effect of Gln on total and differential cellular components of BAL (a) at the indicated time-points and on pathologic changes in lung tissues (b) at 48 h after the second airway challenge. Data represent the mean $\pm$ SE of three to five separate experiments, and each included five animals per group. * P < 0.01 vs. saline; ** P < 0.05 vs. OVA. OVA, ovalbumin; BAL, bronchoalveolar lavage; Gln, glutamine; Ala, alanine.

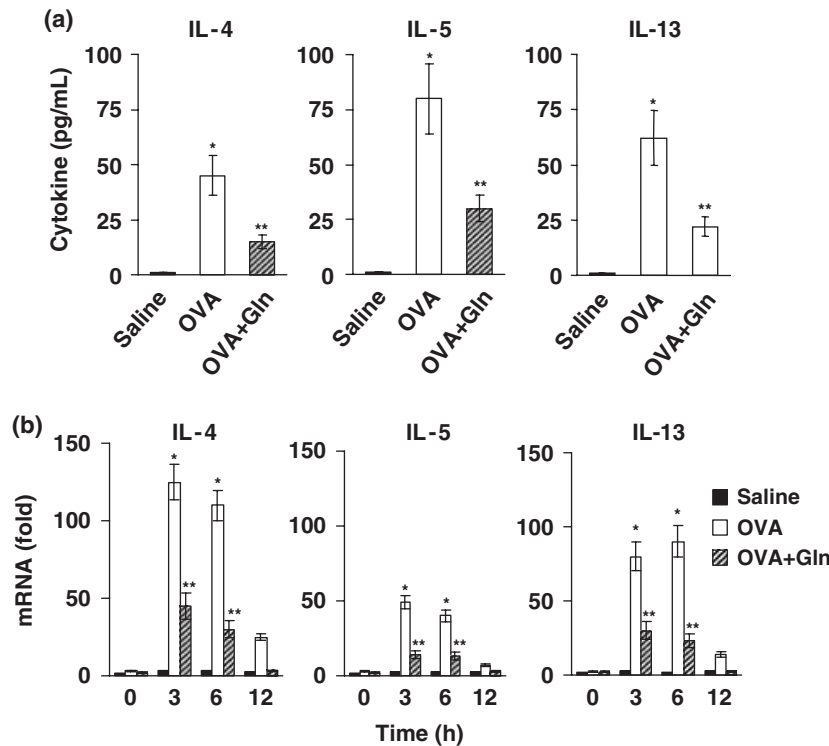


Fig. 3. Effect of Gln on the protein levels (a) and mRNA expression (b) of Th2 cytokines. (a) All cytokines in BAL fluids were measured 24 h after the second airway challenge. (b) Lungs were removed at the indicated time-points after the second airway challenge. Data represent the mean $\pm$ SE of three separate experiments, and each included five animals per group. * P < 0.01 vs. saline; ** P < 0.05 vs. OVA. OVA, ovalbumin; BAL, bronchoalveolar lavage; Gln, glutamine; Th2, T-helper type 2.

group (Fig. 3a). The administration of Gln significantly suppressed the concentrations of each of the Th2 cytokines examined. Treatment of alanine failed to inhibit Th2 cytokine levels in BAL fluids (data not shown). Gln inhibited the mRNA expression of Th2 cytokines (Fig. 3b) in a manner similar to the effect of Gln on protein levels. We also examined the effect of Gln on the expression of Th1 cytokines such as IFN- γ and IL-12 in the airways. However, the proteins and mRNA expression levels were undetectable under our experimental conditions (data not shown).

Goblet cell hyperplasia is frequently observed in the airways of asthmatic patients and in animal models of allergic asthma, and mucus plugging has long been recognized as a major factor contributing to mortality associated with acute severe asthma [21, 22]. To determine whether Gln treatment also affects airway mucus production, we compared PAS-stained sections of lungs 2 days after the second OVA challenge. Numerous PAS-positive goblet cells were present in the bronchi and bronchioles of OVA-immunized and OVA-challenged animals, but not in control mice, and in some instances, bronchial lumens were filled with mucus (Fig. 4a). In contrast, the number of mucus-containing epithelial cells in the airways of Gln-treated mice appeared to be markedly reduced, and little or no mucus was present in the bronchial lumens. In

addition to PAS-staining, BAL levels of mucin were also determined by ELISA. Consistent with the findings using PAS staining, BAL mucin levels were increased in OVA-immunized and OVA-challenged animals, and Gln treatment significantly reduced BAL mucin levels (Fig. 4b). Alanine failed to reduce mucus production (Figs 4a and b).

Glutamine inhibits late, but not early, airway hyperresponsiveness

Based on the findings that our murine model of asthma exhibits a biphasic increase in AHR, in which the peaks of early and late AHR occur at 1 and 10 h, respectively, after the second airway allergen challenge [12], the effect of Gln on the development of early and late AHR was examined. At both time-points, the dose-response curve of RL was shifted to the left in OVA-sensitized and OVA-challenged mice compared with that of control mice (Figs 5a and b). Additionally, the RL caused by administration of methacholine (at doses from 2.5 to 50 mg/mL) increased significantly in the OVA-sensitized and OVA-challenged mice compared with the controls. Neither Gln nor alanine affected the dose-response curve of RL at 1 h (Fig. 5a), whereas the dose-response curve of RL was slightly shifted to the left in Gln-treated mice at 10 h (Fig. 5b), indicating that Gln significantly inhibits the development

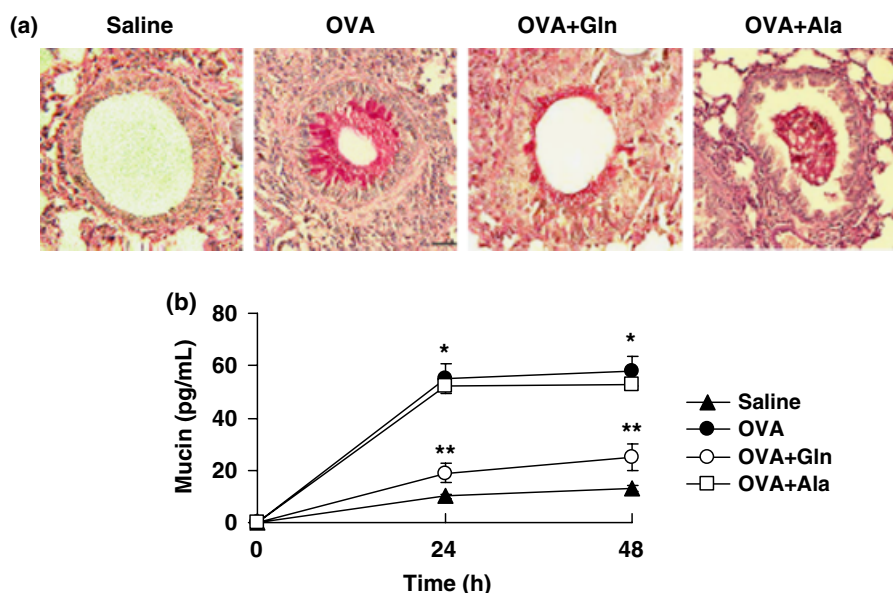


Fig. 4. Effect of Gln on airway mucus production. (a) Lungs were removed 48 h after the second airway challenge and stained with PAS. (b) BAL levels of mucus were determined by ELISA at the indicated time-points. Data represent the mean $\pm$ SE of two separate experiments, and each included five animals per group. * P < 0.01 vs. saline; ** P < 0.05 vs. OVA. OVA, ovalbumin; BAL, bronchoalveolar lavage; Gln, glutamine; Ala, alanine.

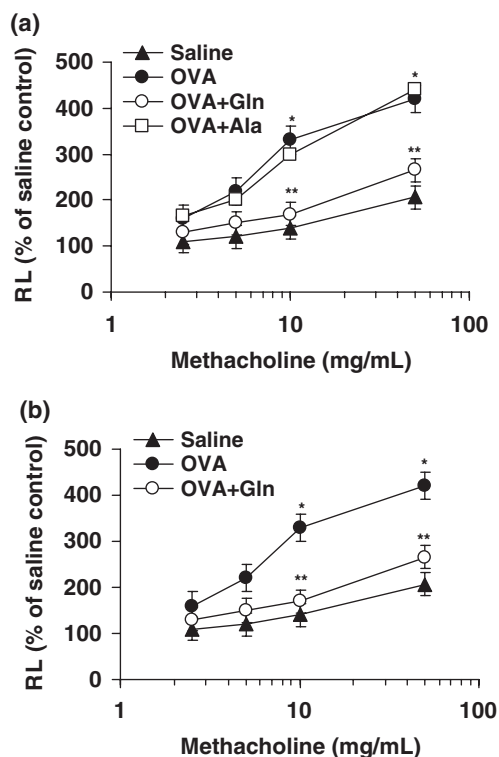


Fig. 5. Effect of Gln on the development of early and late AHR. Early (a) and late AHR (b) were determined 1 and 10 h after the second airway challenge, respectively. Data represent the mean $\pm$ SE of three separate experiments, and each included five animals per group. * P < 0.01 vs. saline group; ** P < 0.01 vs. OVA. OVA, ovalbumin; Gln, glutamine; Ala, alanine; AHR, airway hyperresponsiveness.

of late AHR only. Treatment of alanine failed to inhibit late AHR (data not shown).

Glutamine injection increases glutamine levels in the plasma and lungs

Gln injection rapidly increased Gln plasma levels. Gln reached a peak in the circulation within 5 min (33-fold increase compared with the basal value), but rapidly declined thereafter (Fig. 6a). Gln injection also significantly increased Gln levels in the lung tissues (threefold increase) (Fig. 6b) as well as in BAL fluids (sixfold increase) (Fig. 6c), although the amounts increased were very small compared with those in the plasma. We could not observe any decrease in Gln levels in the whole lung tissues after induction of asthma (data not shown).

Discussion

Based on the observations that Gln has the ability to suppress cPLA₂ activity [9], we have investigated the effect of Gln on the development of asthma features in a murine model of asthma for the first time. We found that systemic administration of Gln significantly inhibited allergen challenge-induced cPLA₂ phosphorylation and its enzymatic activity, as is the case for LPS [9].

Various eicosanoids generated by the action of cPLA₂ have been shown to induce airway eosinophilia and bronchoconstriction [12–15]. Inhibition of cPLA₂ prevents both eosinophilic infiltration and airway

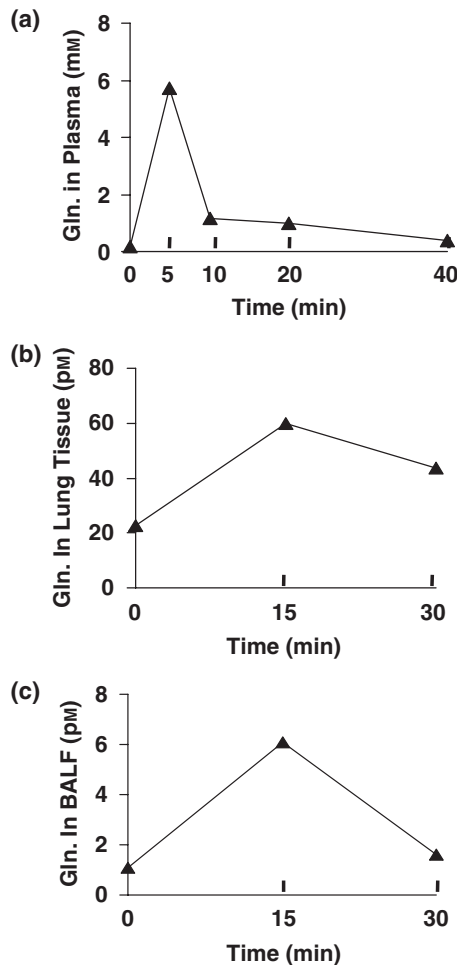


Fig. 6. Gln injection increases the levels of Gln in the plasma, lung tissues, and BAL fluids. Gln was measured from plasma (a), lung tissues (b), and BAL fluids (c) at the indicated time-points after Gln injection. Data are representative of two separate experiments, and each included three animals per group. OVA, ovalbumin; Gln, glutamine; BAL, bronchoalveolar lavage.

hyperresponsiveness [10–12]. In addition, many investigators have also reported an association between cPLA₂ metabolites and Th2 response in asthmatic reactions. These include LTE₄-mediated recruitment of eosinophils into asthmatic airways [23], inhibition of eosinophil and dendritic cell influx into airways in human asthma with a cysteinyl LT receptor antagonist [24, 25], and reduced AHR, pulmonary eosinophilic infiltration, and goblet cell hyperplasia with mucus plugging by 5-lipoxygenase inhibition [26, 27]. Taken together, these findings suggest tight links between cPLA₂ activity, Th2-dependent asthmatic phenotypes, and late AHR.

In this study, as seen with the cPLA₂ inhibitor, Gln-suppressed Th2 cell-dependent asthmatic phenotypes such as increased Th2 cytokine levels in the airway, mucus production, and airway eosinophilia, and the development of late AHR. These findings suggest that the suppression

in Th2 cell-dependent asthmatic phenotypes and late AHR can be attributed, at least in part, to Gln-induced inhibition of cPLA₂ phosphorylation and activity. This theory was furthered by the observation that a cPLA₂ inhibitor, AACOCF₃, exhibited an inhibitory effect similar to that of Gln, i.e. AACOCF₃ inhibited Th2-dependent asthmatic phenotypes and late AHR, but did not affect early AHR (data not shown).

Gln is abundant in the human body. Gln can be produced in sufficient quantities under stable conditions, but becomes a limiting amino acid during metabolic stresses, which are associated with higher susceptibility to infections and impairment of the immune response [28–30]. Therefore, it is likely that a decrease in the cellular concentration of Gln occurs during asthmatic reactions, resulting in increases in cPLA₂ phosphorylation and activity. Supplementation with Gln corrects such abnormalities.

A number of studies have shown that parenteral administration of Gln as a component of supportive nutritional therapy is safe and improves clinical outcomes in critically ill patients [7, 31–33]. In this regard, the clinical use of Gln for patients with asthma may be more beneficial than the use of other compounds or biological agents.

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References

- 1 Häussinger D. Hepatic Glutamine transport and metabolism. *Adv Enzymol* 1998; **72**:43–86.
- 2 Fox RE, Hopkins IB, Cabacungan ET, Tildon JT. The role of glutamine and other alternate substrates as energy sources in the fetal rat lung type II cell. *Pediatr Res* 1996; **40**:135–41.
- 3 Encarnacion S, Calderon J, Gelbard AS, Cooper AJ, Mora J. Glutamine biosynthesis and the utilization of succinate and glutamine by *Rhizobium etli* and *Sinorhizobium meliloti*. *Microbiology* 1998; **144**:2629–38.
- 4 Kovacevic Z, McGivan JD. Mitochondrial metabolism of glutamine and glutamate and its physiological significance. *Physiol Rev* 1983; **63**:547–605.
- 5 Newsholme EA, Crabtree B, Ardawi MSM. Glutamine metabolism in lymphocytes: its biochemical, physiological, and clinical importance. *Q J Exp Physiol* 1985; **70**:473–89.
- 6 Wischmeyer PE, Kahana M, Wolfson R, Ren H, Musch MM, Chang EB. Glutamine reduces cytokine release, organ damage, and mortality in a rat model of endotoxemia. *Shock* 2001; **16**:398–402.
- 7 Griffiths RD, Jones C, Palmer TE. Six-month outcome of critically ill patients given glutamine supplemented parenteral nutrition. *Nutrition* 1997; **13**:295–302.

- 8 Kew S, Wells SM, Yaqoob P, Wallace FA, Miles EA, Calder PC. Dietary glutamine enhances murine T-lymphocyte responsiveness. *J Nutr* 1999; **129**:1524–31.
- 9 Kim YS, Kim GY, Kim JH *et al*. Glutamine inhibits lipopolysaccharide-induced cytoplasmic phospholipase A₂ activation and protects against endotoxin shock in mouse. *Shock* 2006; **25**:290–4.
- 10 Uozumi N, Kume K, Nagase T *et al*. Role of cytosolic phospholipase A₂ in allergic response and parturition. *Nature* 1997; **390**: 618–22.
- 11 Myou S, Sano H, Fujimura M *et al*. Blockade of eosinophil migration and airway hyperresponsiveness by cPLA₂-inhibition. *Nat Immunol* 2001; **2**:145–9.
- 12 Choi IW, Kim S, Kim YS *et al*. TNF- α induces the late-phase airway hyperresponsiveness and airway inflammation through cytosolic phospholipase A₂ activation. *J Allergy Clin Immunol* 2005; **116**:537–43.
- 13 Fischer AR, McFadden CA, Frantz R *et al*. Effect of chronic 5-lipoxygenase inhibition on airway hyperresponsiveness in asthmatic subjects. *Am J Respir Crit Care Med* 1995; **152**:1203–7.
- 14 Fuller RW, Dixon CM, Dollery CT, Barnes PJ. Prostaglandin D₂ potentiates airway responsiveness to histamine and methacholine. *Am Rev Respir Dis* 1986; **133**:252–4.
- 15 Jones GL, Saroea HG, Watson RM, O'Byrne PM. Effect of an inhaled thromboxane mimetic (U46619) on airway function in human subjects. *Am Rev Respir Dis* 1992; **145**:1270–4.
- 16 Choi IH, Kim YS, Kim DK *et al*. Platelet-activating factor-mediated NF- κ B-dependency of a late anaphylactic reaction. *J Exp Med* 2003; **198**:145–51.
- 17 Eum SY, Maghni K, Hamid Q, Campbell H, Eidelman DH, Martin JG. Involvement of the cysteinyl-leukotrienes in allergen-induced airway eosinophilia and hyperresponsiveness in the mouse. *Am J Respir Cell Mol Biol* 2003; **28**:25–32.
- 18 Takeda K, Hamelmann E, Joetham A *et al*. Development of eosinophilic airway inflammation and airway hyperresponsiveness in mast cell-deficient mice. *J Exp Med* 1997; **186**:449–54.
- 19 Corry DB, Rishi K, Kanellis J *et al*. Decreased allergic lung inflammatory cell egression and increased susceptibility to asphyxiation in MMP2-deficiency. *Nat Immunol* 2002; **3**: 347–53.
- 20 Drouin SM, Corry DB, Hollman TJ, Kildsgaard J, Wetsel RA. Absence of the complement anaphylatoxin C3a receptor suppresses Th2 effector functions in a murine model of pulmonary allergy. *J Immunol* 2002; **169**:5926.
- 21 Kay AB. Asthma and inflammation. *J Allergy Clin Immunol* 1991; **87**:893.
- 22 Saetta M, Di Stefano A, Rosina C, Thiene G, Fabbri LM. Quantitative structural analysis of peripheral airways and arteries in sudden fatal asthma. *Am Rev Respir Dis* 1991; **143**:138.
- 23 Laitinen LA, Laitinen A, Haahtela T, Vilkkla V, Spur BW, Lee TH. Leukotriene E₄ and granulocytic infiltration into asthmatic airways. *Lancet* 1993; **341**:989–90.
- 24 Pizzichini E, Leff JA, Reiss TF *et al*. Montelukast reduces airway eosinophilic inflammation in asthma: a randomized, controlled trial. *Eur Respir J* 1999; **14**:12–8.
- 25 Parameswaran K, Liang H, Fanat A, Watson R, Snider DP, O'Byrne PM. Role for cysteinyl leukotrienes in allergen-induced change in circulating dendritic cell number in asthma. *J Allergy Clin Immunol* 2004; **114**:73–9.
- 26 Irvin CG, Tu YP, Sheller JR, Funk CD. 5-Lipoxygenase products are necessary for ovalbumin-induced airway responsiveness in mice. *Am J Physiol* 1997; **272**:L1053–8.
- 27 Henderson WR, Lewis JDB, Albert RK *et al*. The importance of leukotrienes in airway inflammation in a mouse model of asthma. *J Exp Med* 1996; **184**:1483–94.
- 28 Parry-Billings M, Evans J, Calder PC. Does glutamine contribute to immunosuppression after major burn? *Lancet* 1990; **336**:523–5.
- 29 Gore DC, Jahoor F. Glutamine kinetics in burn patients comparison with hormonally induced stress in volunteers. *Arch Surg* 1994; **129**:1318–23.
- 30 Newsholme EA. The possible role of glutamine in some cells of the immune system and the possible consequence for the whole animal. *Experientia* 1996; **52**:455–9.
- 31 Wischmeyer PE, Lynch J, Liedel J *et al*. Glutamine administration reduces Gram-negative bacteremia in severely burned patients: a prospective, randomized, double-blind trial versus isonitrogenous control. *Crit Care Med* 2001; **29**:2075–80.
- 32 Ziegler TR, Young LS, Benfell K *et al*. Clinical and metabolic efficacy of glutamine-supplemented parenteral nutrition after bone marrow transplantation. A randomized, double-blind, controlled study. *Ann Intern Med* 1992; **116**:821–8.
- 33 Novak F, Heyland DK, Avenell A, Drover JW, Su X. Glutamine supplementation in serious illness: a systematic review of the evidence. *Crit Care Med* 2002; **30**:2022–9.