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ORIGINAL CONTRIBUTION

Combined arginine and glutamine decrease release of de novo synthesized leukotrienes and expression of proinflammatory cytokines in activated human intestinal mast cells

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Abstract Purpose
Glutamine and arginine modulate inflammatory responses of epithelial cells and monocytes. Here, we studied the response of human mast cells to pharmacological doses of arginine and glutamine.
Methods Mast cells isolated from intestinal tissue were incubated with physiological doses of arginine (0.1 mmol/L) and glutamine (0.6 mmol/L) or with pharmacological doses of arginine (2 mmol/L) and glutamine (10 mmol/L) for 18 h. Following stimulation by IgE receptor cross-linking mast cell mediators were measured by enzymatic assay, ELISA, multiplex bead immunoassay, or real-time RT-PCR, and activation of intracellular signaling molecules was determined using proteome profiler array or immunoblotting.
Results We found that the combined challenge of mast cells with pharmacological doses of arginine and glutamine caused a decrease in induced release of de novo synthesized leukotriene C_4 but not of pre-stored β-hexosaminidase. Moreover, we found reduced expression of chemokines monocyte chemoattractant protein-1 (CCL2), macrophage inflammatory protein-1β (CCL4), IL-8 (CXCL8), and TNF in response to high doses of both amino acids. The anti-inflammatory effects of arginine and glutamine were associated with decreased activation levels of signaling molecules known to be involved in mast cell cytokine expression such as MAPK family members extracellular signal-regulated kinase, c-Jun N-terminal kinase, and p38, and the protein kinase B (Akt).
Conclusion Arginine and glutamine attenuate IgE-dependent human mast cell activation by decreasing lipid mediator release and expression of proinflammatory cytokines.

Keywords Arginine · Glutamine · Intestinal mucosa · Mast cell · Cytokine expression

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Introduction

Arginine and glutamine are considered conditionally essential amino acids especially in stages of metabolic stress in the gut [1, 2]. While the reports about beneficial effects of arginine by itself on the gut in states of disease are controversial, it has been shown that pharmacological doses of arginine in combination with glutamine exert protective effects, for example, in Crohn's disease (CD) by reducing the production of proinflammatory cytokines such as TNF, IL-6, and IL-8 (CXCL8) [3]. The reason for harmful effects of L-arginine in states of disease or injury is unclear, but is likely related to its function on nitric oxide (NO) and NO donor production [4, 5]. Beneficial and contra-indicated effects of NO are still under debate and probably a question of dosage and site of action [6].

In different human and murine immune cells as well as in animal models of colitis, glutamine exerts anti-inflammatory and gut-protecting effects by decreasing inflammatory cytokines like TNF and IL-8 (CXCL8) and intestinal damage as well as augmenting immune regulatory cytokines IL-2, IL-4, IL-10, and antigen-presenting complex HLA-DR on monocytes [7–13]. In human duodenal biopsies, glutamine decreased IL-6 and CXCL8 but augmented anti-inflammatory IL-10 production in inflamed and non-inflamed conditions [14, 15].

Mast cells are well known as critical effector cells in immediate-type allergic and other inflammatory reactions. They are capable of producing multiple cytokines and chemokines suggesting an important role in immunoregulation and host defense. Moreover, they release a number of preformed and de novo synthesized inflammatory mediators such as histamine, leukotrienes, proteases, and prostaglandins upon stimulation with IgE-dependent and IgE-independent agonists [16, 17]. In the gut, mast cells interact with nerves and epithelial cells to regulate various physiological processes such as intestinal motility, intestinal permeability, or ion and fluid secretion. Furthermore, mast cells are involved in intestinal inflammation, for example, in the course of allergic enteritis or inflammatory bowel disease (IBD) [17–19]. Moreover, increased levels of proinflammatory mediators such as TNF are found in mast cells of inflamed gut in IBD and are considered to be secreted mainly from mast cells [20].

However, our knowledge is limited regarding down-regulation of human mast cells in inflammatory settings. Here, we investigated the response of human intestinal mast cells to arginine and glutamine analyzing IgE-dependent release of inflammatory mediators and cytokines as well as underlying mechanisms.

Materials and Methods

Isolation and culture of human intestinal mast cells

Mature human mast cells were isolated from macroscopically normal surgery tissue specimen derived from patients who underwent bowel resection because of cancer as described in detail elsewhere [21]. Permission to conduct the study was obtained from the local ethical committee (Stuttgart, Germany). All persons gave their informed consent to their inclusion in the study. The distribution of male and female donors was approximately equal. After mechanic and enzymatic digestion of mucosal tissue, the obtained cell suspension was incubated overnight in culture medium (RPMI 1640 GlutaMax®I (Invitrogen/Gibco) supplemented with 10 % heat-inactivated fetal calf serum (Biochrom AG), 100 µg/mL streptomycin, 100 µg/mL gentamicin, 100 U/mL penicillin, and 0.5 µg/mL amphotericin B (all PAN Biotech GmbH)) and then enriched by magnetic cell separation of c-Kit-expressing cells using CD117 microbead kit after dead cell removal (both Miltenyi Biotec). The c-Kit$^+$ cells were cultured with 25 ng/mL rh stem cell factor (SCF) (PeproTech) at a density of 2×10^5 mast cells per mL in medium. Generally, after 2–3 weeks, mast cell purity increases to 95–100 %. Only cell preparations with purity >95 % were used for experiments. Mast cells isolated from independent surgical colon tissue specimens were analyzed separately in each experiment; altogether 12 independent surgical colon tissue specimens were used. Pure mast cells were cultured in the presence of SCF alone or in combination with 2 ng/mL rhIL-4 (PeproTech) for 10 days before being used in the described experiments. IL-4 is known to enhance IgE-dependent mediator release in tissue-derived human mast cells [22–24]. Cultured mast cells were fed once a week by exchanging half of the culture medium supplemented with cytokines.

Treatment with glutamine and arginine and stimulation of human intestinal mast cells

To examine effects of pharmacological (high) doses compared to physiological (low) doses of arginine and glutamine on human intestinal mast cells, the culture medium containing 1.15 mmol/L L-arginine and 2.06 mmol/L L-glutamine was completely removed. Mast cells were washed twice and cultured overnight at a density of 2×10^5 mast cells per mL in SILAC medium (SILAC RPMI 1640 without L-arginine, L-glutamine, L-lysine, PAA Laboratories GmbH) supplemented with 10 % heat-inactivated dialyzed fetal bovine serum (PAA Laboratories GmbH), 25 mmol HEPES, 100 µg/mL streptomycin, 100 µg/mL gentamicin, 100 U/mL penicillin, 0.5 µg/mL amphotericin B, 0.274 mmol/L L-lysine (PAA Laboratories GmbH). L-arginine (Arg) and L-glutamine (Gln) (PAA Laboratories GmbH) were added as follows: 0.1 mmol/L Arg and 0.6 mmol/L Gln (Arg^{low}/Gln^{low}), 2 mmol/L Arg and 0.6 mmol/L Gln (Arg^{high}/Gln^{low}), 0.1 mmol/L Arg and 10 mmol/L Gln Arg^{low}/Gln^{high}), 2 mmol/L Arg and 10 mmol/L Gln (Arg^{high}/Gln^{high}). The low doses of arginine and glutamine refer to physiological plasma concentrations, the high doses of arginine and glutamine were used according to previous findings [25–27]. Following incubation for 18 h, mast cells were activated by IgE receptor (FcεRI) crosslinking using treatment with 0.4 µg/mL myeloma IgE for 60 min and subsequent 1 µg/mL anti-human IgE for 10 min to determine phosphorylation of different signaling molecules or for 90 min to analyze cytokine expression and release of β-hexosaminidase and leukotrienes. Cell viability was monitored by measurement

of electrical impedance and was affected neither by combined high nor by combined low concentrations of L-arginine and L-glutamine. Numbers of mast cells determined by cell counting after staining with trypan blue and phenotype of mast cells examined under microscope using cytocentrifuge smears stained with May–Grünwald/Giemsa were unchanged after amino acid treatment.

β-hexosaminidase and leukotriene release

The amount of β-hexosaminidase in supernatants was examined by color enzyme assay [28]. The percent degranulation of mast cells was calculated with the following formula: (β-hexosaminidase activity in the supernatant fraction/total of β-hexosaminidase activity in the cellular and supernatant fraction) × 100. The concentration of leukotriene (LT) C_4 in supernatants was determined by ELISA kit (Assay Designs) according to the manufacturer's instructions.

RNA preparation and real-time RT-PCR

Total RNA was prepared from 1×10^5 mast cells using RNeasy mini kit (Qiagen). Real-time RT-PCR was performed with SYBR® Green PCR master mix (Applied Biosystems) as described [29]. The following specific sense and anti-sense primers were used: GAPDH: 5′-TGGTC TCCTCTGACTTCAAC-3′,5′-CCTGTTGCTGTAGCCAA ATT-3′, product size 128 bp; CCL2: 5′CTTCTGTGCC TGCTGCTCAT-3′, 5′-CGGAGTTTGGGTTTGCTTGTC-3′, product size 273 bp; CCL4: 5′-GCTAGTAGCTGC CTTCTGCTCTCC-3′, 5′CAGTTCCAGCTGATACACG TACTCC-3′, product size 238 bp; CXCL8: 5′-CTGA GAGTGATTGAGAGTGG-3′,5′-ACAACCCTCTGCACC CAGTT-3′, product size 122 bp; TNF: 5′-CAAGCCT GTAGCCCATGTTG-3′, 5′-AGAGGACCTGGGAGTAG ATG-3′, product size 161 bp.

Cytokine release

Cytokine release was measured by multiplex bead immunoassay (Luminex 100, Luminex Corporation, Austin, TX, USA) according to the manufactures' instructions.

Flow cytometry

For each labeling, 1×10^5 mast cells were washed twice (5 min, 400 g) and resuspended in PBS supplemented with 0.1 % BSA, 0.1 % sodium azide and 250 µg/mL rabbit IgG. Cells were incubated with PE anti-human FcεRI α-chain (Miltenyi Biotec) or PE-labeled isotype control. After incubation for 30 min, cells were washed, and analysis was performed using the FACSCanto (BD Bioscience, San Jose, USA).

Proteome profiler array

Relative phosphorylation levels of a broad array of signaling molecules were measured by Human Phospho-Kinase Array Kit (R&D System) according to manufacturer's instructions. The antigen–antibody complexes were visualized using electrochemiluminescence detection system (Fluor Chem, Biozym Scientific GmbH). The obtained signals were measured by bioimaging analyzer (Alpha Innotech Corporation).

Immunoblotting

To obtain whole-cell extracts, mast cells were lysed in extraction buffer containing 25 mM Tris–HCl pH7.5, 0.5 mM EDTA, 0.5 mM EGTA, 0.05 % Triton X-100, 10 mM β-mercaptoethanol, supplemented with the protease inhibitor cocktail Complete TM mini (Roche Diagnostics, Germany). Protein concentration was determined using Bio-Rad protein assay (Bio-Rad). Cell extracts (10–20 µg protein each) were separated on a 12 % SDS–polyacrylamide gel. Proteins were transferred onto Hybond-P polyvinylidene difluoride membranes (Amersham Biosciences) using a semidry electroblotter. Membranes were blocked with 2 % BSA in PBS containing 0.1 % tween overnight. Membranes were probed with anti-phospho-ERK (MAPK)-1/2 mAbs (Alexis, Lausen, Switzerland). The antigen–antibody complexes were visualized using electrochemiluminescence detection system as described by the manufacturer (Fluor Chem, Biozym Scientific GmbH, Germany). The obtained signals were measured by bioimaging analyzer (Alpha Innotech Corporation, San Leandro, CA, USA). The membranes were stripped following probing with anti-phospho-ERK1/2 mAb and probed second time with anti β-actin (Cell Signaling Technology) to normalize the obtained signals.

Statistics

Data from multiple experiments were expressed as mean +SEM. Paired t test was used to analyze differences between two groups. A value of $p < 0.05$ was considered to be statistically significant.

Results

Pharmacological doses of arginine and glutamine decrease the release of LTC_4, but not of β-hexosaminidase, in

human intestinal mast cells activated by FcεRI crosslinking.

Mast cells were pre-cultured in the presence of their growth factor SCF alone or combined with IL-4. Following pre-culture, mast cells were incubated initially for 18 h with combined physiological (low) doses of arginine and glutamine (0.1 mmol/L and 0.6 mmol/L, respectively) and with combined pharmacological (high) doses of arginine and glutamine (2 mmol/L and 10 mmol/L, respectively). The release of β-hexosaminidase was not affected by combined high doses of arginine and glutamine compared to low doses in IgE-activated human intestinal mast cells (Fig. 1a). In contrast to pre-stored β-hexosaminidase, overnight incubation with high doses of arginine and glutamine led to decreased release of de novo synthesized LTC_4 compared to low doses of both amino acids (Fig. 1b). This effect was observed in both mast cells pre-cultured with SCF alone or with SCF and IL-4, but was more pronounced in IL-4-treated cells. The analysis of IgE-dependent leukotriene release in response to normal medium concentrations containing 1 mmol/L arginine and 2 mmol/L glutamine in comparison with high and low doses of arginine and glutamine revealed that only pharmacological but not medium doses of arginine and glutamine showed significant differences to physiological doses of arginine and glutamine (Fig. 1c).

Arginine and glutamine reduce cytokine expression levels in human intestinal mast cells

Mast cells pre-cultured with IL-4 in addition to SCF and incubated with high doses of arginine and glutamine for 18 h showed reduced cytokine expression levels in response to stimulation by FcεRI crosslinking compared to mast cells incubated with combined low doses of arginine and glutamine. We found reduced mRNA levels for monocyte chemoattractant protein-1 (CCL2), macrophage inflammatory protein-1β (CCL4), TNF, and CXCL8 (Fig. 2). Surprisingly, in the absence of IL-4, we detected significant effects of high doses of arginine and glutamine on the expression of TNF, but not on CCL2, CCL4, and CXCL8 (Fig. 2). The release of CCL2, CXCL8, and TNF was also reduced after treatment with pharmacological doses of arginine and glutamine (Fig. 2e). However, the release of CCL4 was not changed. To examine if the observed anti-inflammatory effects of pharmacological doses of arginine and glutamine on IL-4 cultured mast cells depend on one amino acid alone, IL-4-cultured mast cells were additionally analyzed by treatment with either pharmacological doses of arginine or pharmacological doses of glutamine, respectively. However, only treatment with combined pharmacological doses of both amino acids led to significantly reduced mRNA levels for CCL2, CCL4, CXCL8, and TNF in mature human mast cells (Fig. 3).

Arginine and glutamine decrease phosphorylation levels of multiple signaling molecules in human intestinal mast cells stimulated by FcεRI crosslinking

To assess IgE-dependent signaling affected by arginine and glutamine, we first analyzed the expression of FcεRI in response to high versus low doses of arginine and glutamine. Mast cells pre-cultured with SCF and IL-4 were incubated for 18 h with combined high or combined low doses of arginine and glutamine, and FcεRI expression was examined by flow cytometry. We observed almost no effect

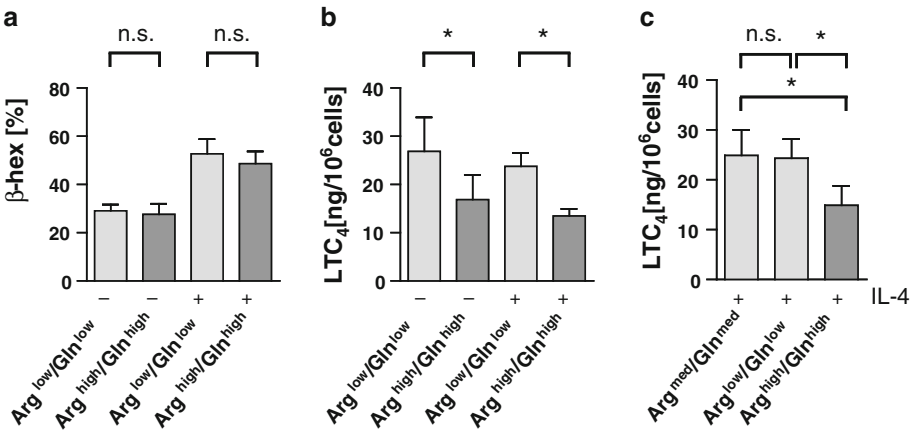

Fig. 1 IgE-mediated release of pre-stored β-hexosaminidase (β-hex) **(a)** and de novo synthesized LTC_4 **(b, c)** by human intestinal mast cells in response to arginine (Arg) and glutamine (Gln). Mast cells were cultured with (+) or without (−) IL-4 in addition to SCF for 10 days and incubated with combined physiological (low) doses of arginine and glutamine (0.1 mmol/L and 0.6 mmol/L, respectively) or combined doses of arginine and glutamine (1 mmol/L and 2 mmol/L, respectively) present in culture medium (med) versus combined pharmacological (high) doses of arginine and glutamine (2 mmol/L and 10 mmol/L, respectively) for 18 h and stimulated with IgE and anti-IgE for 90 min ($n = 5$); n.s. indicates no significant differences, *asterisk* indicates $p < 0.05$ for significant differences

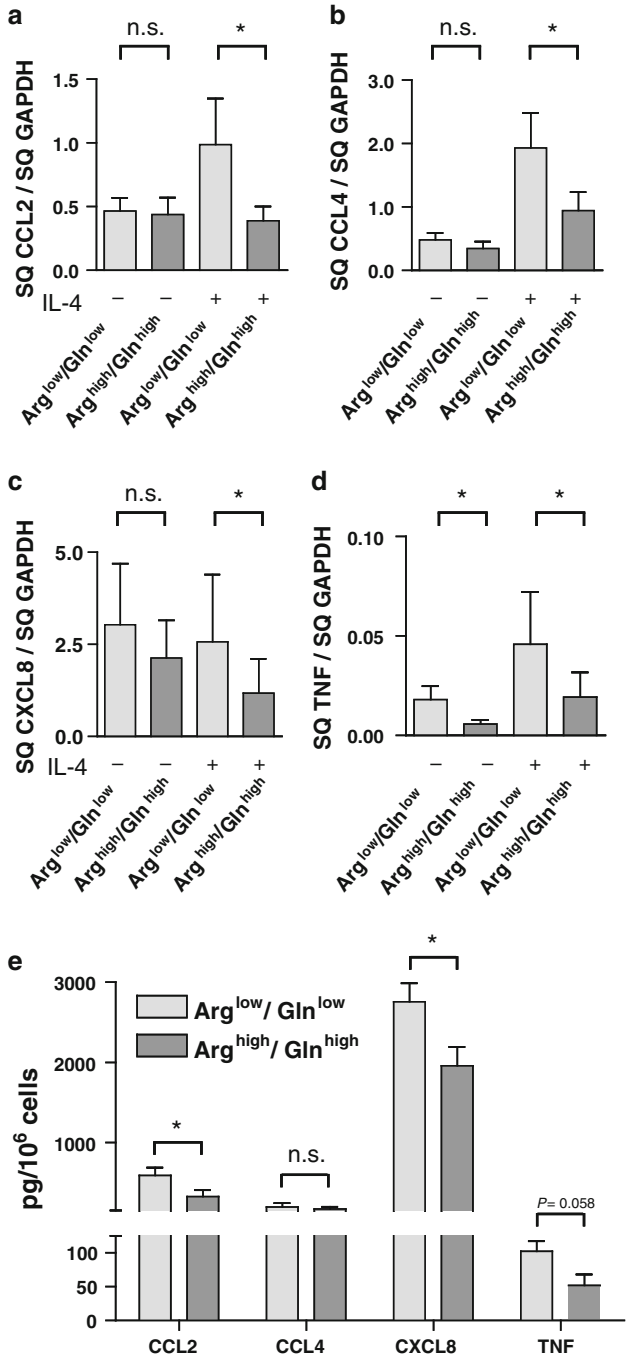

Fig. 2 Expression of mRNA for CCL2 **(a),** CCL4 **(b),** CXCL8 (**c**), and TNF (**d**) in human intestinal mast cells in response to high versus low doses of arginine (Arg) and glutamine (Gln). Mast cells were cultured with (+) or without (−) IL-4 in addition to SCF for 10 days, incubated with combined physiological (low) doses of arginine (Arg) and glutamine (Gln) (0.1 mmol/L and 0.6 mmol/L, respectively) or combined pharmacological (high) doses of arginine and glutamine (2 mmol/L and 10 mmol/L, respectively) for 18 h and stimulated with IgE and anti-IgE for 90 min ($n = 5$–6). **e** Corresponding release of CCL2, CCL4, CXCL8 ($n = 4$), and TNF ($n = 3$) by mast cells pre-cultured with IL-4 and stimulated by IgE and anti-IgE for 3 h; *n.s.* indicates no significant differences, *asterisk* indicates $p < 0.05$ for significant differences

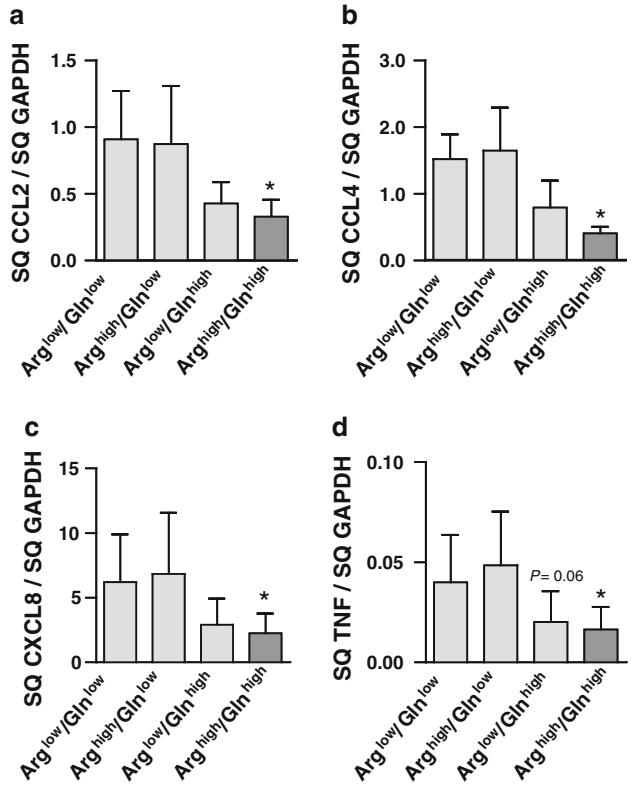

Fig. 3 Expression levels of mRNA for CCL2 **(a),** CCL4 **(b),** CXCL8 (**c**), and TNF (**d**) of human intestinal mast cells in response to arginine (Arg) and glutamine (Gln). Mast cells were cultured with IL-4 in addition to SCF for 10 days and incubated for 18 h with physiological (low) or pharmacological (high) doses of arginine and glutamine in the following combinations: 0.1 mmol/L Arg and 0.6 mmol/L Gln (Arg^{low}/Gln^{low}), 2 mmol/L Arg and 0.6 mmol/L Gln (Arg^{high}/Gln^{low}), 0.1 mmol/L Arg and 10 mmol/L Gln Arg^{low}/Gln^{high}), 2 mmol/L Arg and 10 mmol/L Gln (Arg^{high}/Gln^{high}). Mast cells were stimulated with IgE and anti-IgE for 90 min ($n = 6$); *asterisk* indicates $p < 0.05$ for significant differences to physiological (low) doses of arginine and glutamine (Arg^{low}/Gln^{low})

of high doses compared to low doses of arginine and glutamine (95 ± 4 % vs. 100 % of Δ mean fluorescence). Experiments using different concentrations of IgE and anti-IgE revealed a clear dose response, but no additional effect of high versus low doses of arginine and glutamine (not shown). Then, we measured activation of several signaling molecules involved in different signal cascades playing a role in inflammatory processes (Fig. 4). We have shown that mitogen-activated protein kinases (MAPK) such as extracellular signal-regulated kinase (ERK) and other serine/threonine kinases such as Akt are involved in cytokine expression in human intestinal mast cells [29, 30]. Mast cells pre-cultured with SCF and IL-4 were incubated for 18 h with combined high or combined low concentration of arginine and glutamine and subsequently stimulated by FcεRI crosslinking for 10 min. We found that pharmacological doses of arginine and glutamine resulted in

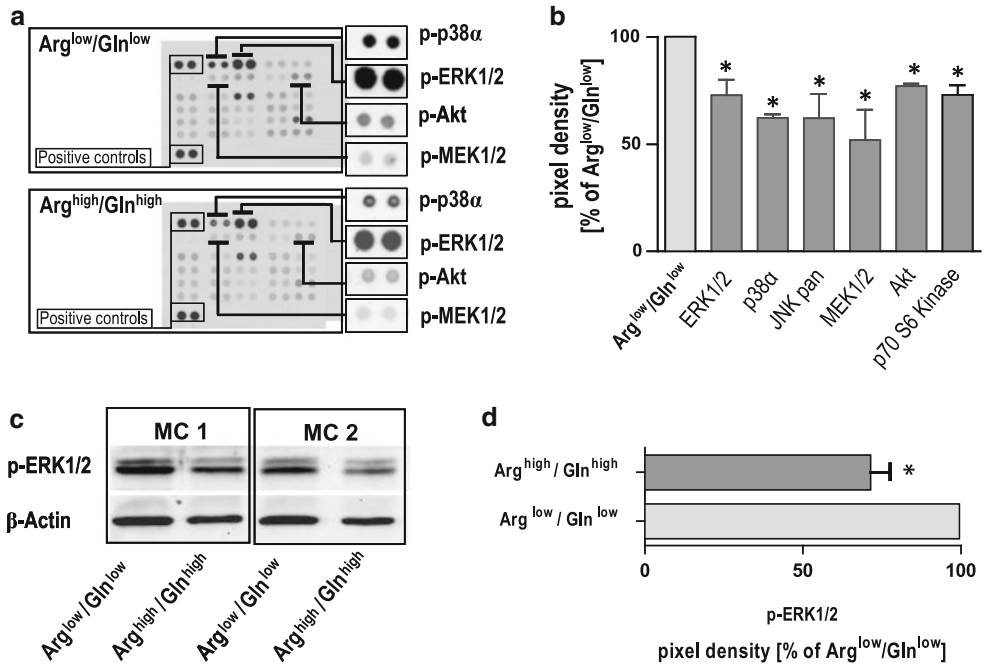

Fig. 4 Effect of high doses of arginine and glutamine on phosphorylation levels of signaling molecules in human intestinal mast cells. Representative array results (**a**) and the densitometric analysis (**b**) are shown. Mast cells were cultured with SCF and IL-4 for 10 days and incubated with combined physiological (low) doses of arginine (Arg) and glutamine (Gln) (0.1 mmol/L and 0.6 mmol/L, respectively) or combined pharmacological (high) doses of arginine and glutamine (2 mmol/L and 10 mmol/L, respectively) for 18 h and stimulated with IgE and anti-IgE for 10 min ($n = 4$). **b** Densitometric values were normalized using the positive controls of the different array membranes (**a**), and relative phosphorylation levels were calculated with the formula (phosphorylation level of Arg^{high}/Gln^{high})/(phosphorylation level of Arg^{low}/Gln^{low}) × 100; *asterisk* indicates $p < 0.05$ for significant differences of phosphorylation levels of signaling molecules in response to high doses of arginine and glutamine to low doses (Arg^{low}/Gln^{low}). **(c)** Corresponding Western blot analysis of ERK1/2 activation. Mast cells were cultured with SCF and IL-4 for 10 days and incubated with combined physiological (low) doses of arginine (Arg) and glutamine (Gln) or combined pharmacological (high) doses of arginine and glutamine for 18 h and stimulated with IgE and anti-IgE for 10 min. Two representative experiments are shown (MC 1, MC 2). **d** Densitometric analysis of activated ERK1/2 were normalized to the corresponding β-actin staining, and relative phosphorylation levels of ERK1/2 were calculated with the formula (phosphorylation level of Arg^{high}/Gln^{high})/(phosphorylation level of Arg^{low}/Gln^{low}) × 100 ($n = 4$). *Asterisk* indicates $p < 0.05$ for significant differences in response to high doses of arginine and glutamine to low doses

decreased activation levels of MAP kinase family member ERK1/2, c-Jun N-terminal kinase (JNK), p38 as well as MAPK kinase (MEK) 1/2 (range about 30–45 %) compared to cells treated with low doses of arginine and glutamine (Fig. 4). Further, activation of Akt and its downstream target p70 S6 kinase was attenuated by combined high doses of arginine and glutamine about 25 % (Fig. 4). In signal transducers and activators of transcription (STAT) family members, phosphorylation states were also slightly reduced by high doses compared to low of both amino acids.

Discussion

Glutamine and arginine have been reported to modulate inflammatory responses of several cell types such as epithelial cells [26], monocytes [11], and macrophages [12]. Effects of arginine and glutamine on mast cells have hitherto not been analyzed. Noteworthy, we could show here that human intestinal mast cells respond to the combined challenge with arginine and glutamine at pharmacological doses with decreased IgE-mediated release of leukotrienes as well as expression of multiple cytokines/chemokines such as CCL2, CCL4, CXCL8, and TNF. Starting point of our investigation was the observation that combined high doses of arginine and glutamine decreased TNF-, IL-6-, and CXCL8-production by biopsies from CD patients [3].

In the human intestinal epithelial cell line HCT-8, the reduced production of CXCL8 caused by pharmacological doses of glutamine (10 mmol/L) was accompanied by a down-regulated NF-kB activation, while the reduced production of CXCL8 caused by pharmacological doses of arginine (2 mmol/L) was mediated involving the NO pathway [26, 31]. We found decreased cytokine mRNA levels after treatment by combined pharmacological doses of arginine and glutamine (Figs. 2, 3). Maybe, glutamine is mainly responsible for the observed anti-inflammatory effects of combined pharmacological doses of arginine and

glutamine, although we did not detect statistical significance (Fig. 3). Arginine function in inflammation is discussed controversially. While it is able to decrease CXCL8 in epithelial cells, it exerted rather harmful effects in an experimental model of enterocolitis [26, 32]. The diverse effects of arginine are related to its distinct pathways. Here, we assume arginine was converted into polyamines by arginase rather than into NO by NO synthase.

We found that combined high doses of arginine and glutamine led to decreased activation of 16 out of 46 measured signaling molecules in human mast cells following priming with IL-4 and stimulation by FcεRI crosslinking, among the members of the MAPK family such as ERK, p38α, JNK pan, and MEK1/2 as well as the serine/threonine kinase Akt (Fig. 4). These findings are in line with our previous results showing that expression and release of multiple mast cell mediators such as LTC_4, CXCL8, and IL-6 in response to FcεRI crosslinking depend on activation of ERK, p38, or Akt [29]. Thus, our findings could explain the reduced expression of chemokines such as CXCL8 in response to high doses of arginine and glutamine since we have shown that both ERK and Akt are involved in CXCL8 expression [29]. Noteworthy, activation of Akt as well as enhanced activation of ERK in human intestinal mast cells were dependent on priming with IL-4, which might explain that arginine and glutamine affected CXCL8 expression in IL-4-primed mast cells, but not significantly in mast cells cultured without IL-4.

The reduced activation of NF-κB and p38 by combined high doses of arginine and glutamine in biopsies from CD patients was accompanied by a decreased production of TNF [3]. TNF is a key mediator in inflammatory process of IBD, and mast cells are reported to function as main TNF source in IBD [20]. Activation of p38 was found to be linked to TNF release by cultured biopsies from CD patients [33]. Interestingly, p38 phosphorylation was not affected by glutamine alone in biopsies from CD patients [3]. Therefore, it is tempting to speculate that combined pharmacological application of arginine and glutamine has an impact on mast cell function by decreasing TNF in part via p38. The reduced p38α activation might also contribute to reduced expression of other cytokines since p38 is well known to be involved in cytokine expression. For instance, we have shown that expression of the proinflammatory cytokine IL-6 is dependent on p38 in human intestinal mast cells [29].

The release of LTC_4 in activated mast cells was reduced by high doses of arginine and glutamine in both SCF- and IL-4-cultured mast cells (Fig. 1). Interestingly, the release of β-hexosaminidase was not affected (Fig. 1). Since we recently found that the release of LTC_4 is regulated in part by Akt, the reduced phosphorylation levels of Akt in response to arginine and glutamine could be a reason for the reduced release of LTC_4 following both culture conditions [29]. In contrast, β-hexosaminidase was not regulated by ERK1/2, p38, or Akt, respectively, that might explain why β-hexosaminidase release was not influenced by pharmacological concentrations of arginine and glutamine [29].

Kretzmann et al. measured a down-regulation of Akt phosphorylation levels in TNBS-induced colitis in response to glutamine [13]. Akt functions as a link of multiple signaling pathways involved in inflammation such as NF-κB/STAT and inducible NO synthase signaling [34, 35]. Other studies indicated that glutamine affected primarily the NF-κB pathway [3, 13, 31, 36]. These observations are in line with findings that NF-κB expression and activation are strongly enhanced in inflamed gut of patients with IBD [37] accompanied by an increased expression of IL-1, IL-6, and TNF [38]. However, we have not analyzed the NF-κB pathway in this study, but we found that in addition to MAPK and Akt, the activation of STATs was reduced by arginine/glutamine application. Interestingly, activation of STATs was found to be up-regulated in IBD [39, 40]. The STAT3/5 pathway, known to mediate expression of genes involved in different cellular processes [41], has been reported to lead to cyclooxygenase-2 and prostaglandin E_2 expression in epithelial cells [42]. However, effects of glutamine alone on STAT3 phosphorylation or STAT3-mediated prostaglandin production were not found [13, 14].

In summary, we have shown that combined pharmacological doses of arginine and glutamine attenuate mast cell activation by decreasing the release of de novo synthesized leukotrienes and expression of proinflammatory cytokines such as CCL2, CCL4, CXCL8, and TNF by affecting signaling pathways known to be involved in mast cell cytokine expression such as ERK, p38, and Akt.

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Conflict of interest The authors declare that they have no conflict of interests.

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