Glutamine Modulates Phenotype and Stimulates Proliferation in Human Colon Cancer Cell Lines

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ABSTRACT

Glutamine supplementation has been advocated for patients requiring parenteral nutritional support. However, the direct effect of glutamine on neoplastic cells is poorly understood. We therefore investigated the effects of glutamine on the proliferation, differentiation, and cell-matrix interactions of two human colon carcinoma cell lines (Caco-2 and SW620) adapted to glutamine-free media. Doubling times were calculated by logarithmic transformation of serial cell counts. Alkaline phosphatase, cathepsin C (dipeptidyl peptidase), lactase, and isomaltase expression (markers of differentiation) were assayed by digestion of synthetic substrates. Adhesion to matrix proteins was assessed by colorimetric quantitation of toluidine blue staining of adherent cells. Surface expression of Caco-2 receptors for matrix proteins (integrins) was studied by biotinylation and immunoprecipitation with specific antibodies. Glutamine (1-10 mM) dose-dependently stimulated Caco-2 proliferation on all matrices studied with maximal effect at 7 mM. For instance, Caco-2 doubling time on collagen IV decreased by 57 ± 0.2% (SE) (P < 0.001). Glutamine inhibited the expression of all four digestive enzymes with maximal inhibition ranging from 10 to 40% (P < 0.05 for all). Adhesion to matrix proteins was markedly diminished (51 ± 1%, P < 0.01) by glutamine (5 mM) treatment, correlating with decreased α2 and β1 integrin subunit surface expression. Glutamine had similar effects on SW620 cells, stimulating proliferation, inhibiting digestive enzyme expression, and diminishing both adhesion and integrin surface expression. Glutamine supplementation modulates the phenotype of at least two human colon carcinoma cell lines, increasing proliferation, decreasing differentiation, and decreasing adhesion to matrix proteins in association with decreased integrin expression. Although the mechanisms of these effects await elucidation, such characteristics would appear to predict more aggressive tumor behavior and raise the possibility that nutritional supplementation with glutamine may be deleterious in patients with cancer.

INTRODUCTION

Attempts to refine nutritional supplementation have recently focused on the amino acid glutamine. Both a primary metabolic fuel and a precursor for nucleotide synthesis in rapidly dividing enterocytes (1-3), glutamine is trophic and protective for the intestinal mucosa (4-8). Indeed, parenteral glutamine as a component of total parenteral nutrition has proved effective in supporting the jejunal and colonic mucosal mass in several models of chemotherapeutic injury (4, 9-11). Such observations have stimulated suggestions that glutamine supplementation might facilitate the nutritional management of the malmourished or fasting cancer patient (12). However, the effects of nutritional supplementation on the malignancy itself are poorly understood. Acceleration of tumor growth and metastasis during nonspecific nutritional repletion have been reported previously in fasting rats (13). The effects of supplementation with glutamine, which is trophic for normal intestinal mucosa, might be of particular concern in the management of patients with colonic malignancies.

We therefore studied the effects of glutamine supplementation on the phenotype of the well-differentiated human Caco-2 cell line, derived from a malignant colonic adenocarcinoma, and previously widely used as a model for phenotypic regulation (14-16), and human SW620 cell line, derived from a metastatic human colon adenocarcinoma and morphologically less well differentiated (17). The Caco-2 cells were adapted over ten passages to growth in cell culture medium with a low glutamine concentration (0.11 mM) and then supplemented with physiologically relevant glutamine concentrations during each experiment. SW620 cells are conventionally cultured without supplemental glutamine and were similarly glutamine supplemented for experimental studies.

Specifically, we investigated the effects of glutamine supplementation on Caco-2 and SW620 proliferation, differentiation, and cell-matrix interactions, since aggressive neoplasms exhibit alterations in each of these characteristics. Caco-2 proliferation was studied by quantitation of cell doubling time by logarithmic transformation of direct cell counts. Digestive brush border enzyme activity was chosen as a marker of cell differentiation and was studied using digestive enzyme assays for alkaline phosphatase, cathepsin C (dipeptidylpeptidase), lactase, and isomaltase. Cell adhesion to extracellular matrix substrates, specifically type IV collagen and type I collagen, was assessed using a cell attachment assay and quantitated colorimetrically by toluidine blue staining of fixed cells. Since we then observed that glutamine supplementation resulted in decreased cell adhesion, we also studied Caco-2 α1, α2, and β1 integrin surface expression to seek a mechanism for this effect.

MATERIALS AND METHODS

Cells. The Caco-2 cells used for the studies represented a clonal subpopulation selected for a highly differentiated state (18). The cells were maintained at 37°C in 5% CO2 and adapted to a growth medium without supplemental glutamine over ten passages. This consisted of Dulbecco’s minimal essential medium supplemented with 10% fetal bovine serum, 10 μg/ml transferrin (Boehringer Mannheim Corp., Indianapolis, IN), 1 mM pyruvate, 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, 100 units/ml penicillin G, and 0.1 mg/ml streptomycin. Although this medium was not supplemented with exogenous glutamine, glutamine derived from the fetal bovine serum resulted in glutamine levels of 0.11 mM in unsupplemented culture medium (assay courtesy of Roche Laboratories, Raritan, NJ). All experiments were performed on the cells within eight passages. 1-GLU-Glutamine used for these studies was obtained from Sigma Chemical Co. (St. Louis, MO) and was freshly added to culture medium before each experiment to augment the baseline glutamine concentration by 1-10 mM. Additional confirmatory studies utilized Caco-2 cells not adapted to glutamine-free growth and compared cell phenotype in conventional media (2 mM glutamine) with media supplemented to 10 mM glutamine.

The SW620 cells used for these studies were obtained from the American Tissue Culture Collection (ATCC CCL 227) and were propagated in 10% fetal bovine serum, 45% RPMI, and 45% Dulbecco’s modified Eagle’s medium. Studies were performed on these cells within eight passages.

For studies of the effects of glutamine in the absence of cell proliferation, cells were either pretreated with 20 μg/ml mitomycin C (Sigma) for 2 h (14)
or cultured with 30 mM hydroxyurea (Sigma), which we have also observed to maximally inhibit cell proliferation (data not shown).

Matrix Proteins. Type IV collagen was purified from lacthyritic Engelbreth-Holm-Swarm tumors (19). Type I collagen was isolated and purified from rat skin as described previously (20). Bacteriological plastic dishes (Falcon, Oxnard, CA) were precoated with saturating concentrations of matrix substrates as described previously (20).

Antibodies. Antibody to the α1 integrin subunit was a generous gift from Dr. M. Hemler (Dana-Farber Cancer Institute, Boston, MA) (21). Antibody to the α2 subunit was purchased from GIBCO-BRL (Gaithersburg, MD). Antibody to β1 subunit was a generous gift from Dr. C. Buck (Wistar Institute, Philadelphia, PA) (22).

Assays. Proliferation was quantitated by serial direct cell counts. After trypsinization, five million cells were plated in triplicate in 35-mm bacteriological plastic dishes precoated with type I or type IV collagen and allowed to adhere for 1 h. This resulted in 80–90% adhesion. One-half of the dishes were then washed with phosphate-buffered saline, fixed in 10% formalin, and stained with hematoxylin for counting of adherent cells. The cells in the second group of dishes were treated with media supplemented with glutamine (0–10 mM) for 48 h and then washed, fixed, and stained. The number of cells per high power field was counted in ten random fields per dish and doubling time was calculated by logarithmic transformation. Caco-2 DNA synthesis was also quantitated after methanol:acetic acid (3:1) fixation as trichloroacetic acid-precipitable and 0.1 M NaOH-soluble Caco-2 [3H]thymidine uptake. We have previously validated this technique in Caco-2 cells by comparison with direct cell counting of nuclei labeled with [3H]thymidine and stained with photographic emulsion after fixation (18). Assessment of the relative fraction of the cell population in G0-G1, S, or G2-M phases of mitosis was measured by flow cytometry using a Coulter Epics Profile flow cytometer (Coulter Electronics, Hialeah, FL).

For assays of digestive enzyme activity, cells were plated on bacteriological plastic dishes (Falcon), coated with matrix substrates, and allowed to grow to confluence. Cells were then treated with a glutamine-supplemented medium (0–10 mM) for 48 h and lysed on ice for 1 h in phosphate-buffered saline supplemented with calcium and magnesium and containing 0.5% Triton X-100 and 0.35 M NaCl. After separation of insoluble lipid components by centrifugation, the supernatants were assayed for protein (BCA assay; Pierce, Rockford, IL) and samples were diluted to equal protein content. Assays of enzyme activity were then performed by digestion of synthetic substrates (23, 24).

Cell lysate alkaline phosphatase was assayed by digestion of p-nitrophenyl phosphate disodium hexahydrate (Sigma 104) reagent (Sigma) in 100 mM glycine buffer (pH 10.0). After incubation at 37°C for 1 h, the reaction was stopped with 1 M NaOH. Each sample was measured spectrophotometrically at 410 nm against a reagent blank, and enzyme activity was calculated by interpolation against simultaneously assayed enzyme standards. Cathepsin C was similarly quantitated by digestion of alanyl-p-nitroanilide (Sigma) in 114 mM Tris buffer (pH 7.0) for 4 h at 37°C. The reaction was stopped with 1 M acetic buffer (pH 4.2) and samples were measured spectrophotometrically at 380 nm. Standard dilution curves for alkaline phosphatase and dipeptidylpeptidase were performed simultaneously with each assay using synthetic enzymes. Isoamylase and lactase were measured by incubating the cell lysate with 0.056 M isomaltooligosaccharide (Sigma) or lactose solution (Sigma) for 6 h at 37°C followed by 1 h incubation with glucose oxidase in Tris buffer (pH 7.0) solution. Samples were measured against reagent blanks at 420 nm and standard glucose dilution curves were performed with each experiment.

Conditions for all digestive enzyme assays were initially calibrated by assay of serial dilutions of known standards and all experimental assays were then performed within the range of linear response (data not shown). Results were standardized during each experiment against known standards and expressed as enzyme activity in IU/μg of protein.

Adhesion studies were performed either by counting cells per ×20 power field in an Olympus CK-2 microscope or by a cell attachment assay described by GIBCO-BRL. Briefly, cells were grown in T-25 culture plastic flasks, and after confluence was achieved the cells were treated with glutamine-supplemented medium (0–10 mM) for 48 h. Thereafter the cells were trypsinized, cell number was calculated using a hemocytometer, and cells were plated on either 60-mm matrix-precoated plastic dishes or 96-well enzyme-linked immunosorbent assay plates precoated with matrix substrates. After 1 h, nonadherent cells were gently washed away with phosphate-buffered saline and adhesion was quantitated. For 60-mm dish studies, the number of cells in at least 30 random high power fields was counted microscopically per dish after fixation with 10% formalin and staining with hematoxylin. For the 96-well enzyme-linked immunosorbent assay plates utilized in the cell attachment assay, adherent cells were fixed in 10% formalin, stained with 1% toluidine blue in 10% formalin and incubated overnight. The cells were lysed in 2% SDS and absorbance was measured at 600 nm. Standard dilution curves were performed with each experiment to verify that the assay was being performed in a linear part of the curve.

For investigations of integrin surface pool expression, cells were cultured on type I collagen to 80% confluence in control medium and then cultured for an additional 48 h in control medium or medium supplemented with 5 mM glutamine. Surface biotinylation and immunoprecipitation for α1, α2, and β1 integrin subunits was performed in suspended cells by the protocol recommended by the supplier of the α2 antibody (GIBCO-BRL). Immunoprecipitations were resolved under nonreducing conditions by SDS-polyacrylamide gel electrophoresis using a 6% resolving gel, 3.5% stacking gel, electrobotted to nitrocellulose, and visualized using the Western Blot 228 stabilized substrate for alkaline phosphatase (Promega, Madison, WI). Results were quantitated densitometrically using a Microtek IIXE ScanMaker and an IBM-based densitometric software package (SigmaScan/Image; Jandel Scientific).

Statistics. Statistical analysis was performed using unpaired t test or ANOVA at 95% confidence where appropriate. Data are reported as mean ± SE. Band intensity after immunoprecipitation and SDS-polyacrylamide gel electrophoresis for integrin studies was normalized to the intensity of the relevant integrin subunit band in control lanes after densitometric quantitation for purposes of graphic depiction but was analyzed by paired t test.

RESULTS

Glutamine Supplementation Increases Caco-2 and SW620 Proliferation. Caco-2 cell proliferation was studied on two extracellular matrix protein substrates, type IV collagen and type I collagen. Supplementation of the culture medium with increasing concentrations of glutamine (0–10 mM) dose-dependently decreased Caco-2 doubling time on both type IV (Fig. 1) and type I collagen (Fig. 2), with a maximal effect at 7 mM of 56.6 ± 2% for collagen IV and 43.4 ± 1.8% on collagen I, respectively. The observed change was significant for all concentrations studied by ANOVA (n = 30, P < 0.01). These results were confirmed by quantitating [3H]thymidine uptake in Caco-2 cells grown on collagen I. Glutamine supplementation dose-dependently stimulated [3H]thymidine uptake. For instance, glutamine supplementation (7 mM) increased [3H]thymidine uptake by 12 ± 2.1% (n = 12, P < 0.02 by t test; data not shown).

Both unsupplemented Caco-2 cells and cells supplemented with 10 mM glutamine remain diploid by flow cytometry. Although the proliferation of the Caco-2 cells was virtually ablated when the cells were cultured in 0.1% serum media (with absolute cell number increasing from 16.2 ± 1.25 cells/high power field to 17 ± 4.2 cells/high power field after 24 h), the ability of glutamine supplementation to stimulate Caco-2 proliferation was preserved. For instance, in the same experiment, supplementation with 10 mM glutamine in medium with 0.1% serum increased cell proliferation as compared to control (23.4 ± 8.5 cells/high power field for the glutamine-treated cells versus 17 ± 4.2 cells/high power field for the untreated cells; n = 30, P < 0.05).

The proliferation of SW620 cells was also dose dependently stimulated by glutamine, achieving statistical significance at 1 mM and a maximal effect at 7 mM. The doubling time of the SW620 cells in one study was 38.2 ± 0.7 h in unsupplemented medium, 25.9 ± 0.9 h in medium supplemented with 1 mM glutamine, and 23.8 ± 0.1 h in medium supplemented with 7 mM glutamine (n = 40, all P < 0.001).

3 The abbreviations used are: SDS, sodium dodecyl sulfate; ANOVA, analysis of variance.

4 Unpublished data.
The Effects of Glutamine Supplementation on Caco-2 Proliferation Are Specific for Glutamine. In order to determine whether the effects of glutamine supplementation described above might occur after supplementation with any amino acid, we compared the effects of supplementation with equimolar (10 mM) concentrations of L-glutamine, D-glutamine, glutamic acid, asparagine, and glycine. Indeed, while some stimulation was observed with each amino acid studied, the effects of both L-glutamine and D-glutamine were significantly greater than those of these other amino acids (n = 30, P < 0.01; Fig. 3).

Glutamine Supplementation Decreases Caco-2 and SW620 Digestive Enzyme Activity. Digestive enzyme activity was quantitated to assess cell differentiation. The activity of alkaline phosphatase, cathepsin C, lactase, and isomaltase was studied in cell lysates.

Glutamine supplementation resulted in a dose-dependent decrease in the activity of all four enzymes studied. For instance, the decrease in alkaline phosphatase activity associated with glutamine supplementation achieved statistical significance at 3 mM (ANOVA, n = 6, P < 0.05) and was maximal (35 ± 1.7% decrease) at 10 mM (Fig. 4). Cathepsin C activity was decreased in a similar fashion, achieving statistical significance at 3 mM and having maximal effect (53 ± 2.5%) at 7 mM (Fig. 5). Studies of isomaltase and lactase activity yielded similar results. Glutamine supplementation (7 mM) caused a statistically significant (n = 6, P < 0.05) decrease in disaccharidase activity in Caco-2 cells (Fig. 6).

In confirmatory parallel studies, the effects of glutamine were also studied on alkaline phosphatase and cathepsin C activity in the parent line of Caco-2 cells not adapted to glutamine-free growth and in the SW620 cell line which does not require glutamine supplementation. As compared with the 2 mM glutamine supplementation normally utilized to culture the parental Caco-2 cell line, supplementation with 10 mM glutamine decreased alkaline phosphatase and cathepsin C activities by 43.1 ± 1.0 and 43.2 ± 0.6%, respectively (n = 4, P < 0.001). Glutamine supplementation of the SW620 culture medium similarly inhibited the expression of digestive enzymes. Alkaline phosphatase and cathepsin C expression were decreased by 76.24 ± 1.9 and 38.74 ± 2.8%, respectively after 8 mM glutamine supplementation (n = 6, P < 0.001).7

We confirmed that the effects of glutamine supplementation on cell differentiation did not reflect only an inhibition of cell proliferation by quantitating changes in Caco-2 digestive brush border activity in response to glutamine supplementation when proliferation was blocked by two different inhibitors of DNA synthesis, mitomycin C and hydroxyurea. Glutamine supplementation decreased both alkaline phosphatase and cathepsin C activity in Caco-2 cells when proliferation was blocked by each of these agents (Fig. 7).
Glutamine Supplementation Causes Decreased Expression of Integrin Surface Pools. Since glutamine supplementation appeared to inhibit Caco-2 adhesion to the extracellular matrix substrates, we further hypothesized that this phenomenon might be mediated by the effects of glutamine on Caco-2 integrin expression. To investigate glutamine modulation of cell surface integrin pools, lysed cells were immunoprecipitated with α1, α2, and β1 integrin subunit-specific antibodies. Indeed, glutamine supplementation substantially decreased the surface expression of all three integrin subunits studied. Specifically, α1 integrin surface expression decreased by 73 ± 16.4%, α2 by 47 ± 5.6%, and β1 by 50 ± 12.3% (Fig. 11). Similar decreases in α1, α2, and β1 integrin subunit surface expression were observed in the SW620 cell line after glutamine supplementation (data not shown).

DISCUSSION

Malnutrition is common in cancer patients and the need to treat such malnutrition would seem equally obvious. However, while nutritional supplementation may benefit these patients, it may also...
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Because some of these effects may benefit patients undergoing extensive surgical resections, chemotherapy, or radiation therapy, glutamine has been advocated as a supplement to both total parenteral nutrition and enteral diets in order to preserve the mass and integrity of the intestinal mucosa (12, 31). Enteral feeding formulas containing free glutamine are currently in widespread clinical use. These include Impact (Sandoz Corp., Minneapolis, MN), Vivonex Plus (Sandoz), and Alitra Q (Ross Laboratories, Columbus, OH). The relative instability of glutamine in solution has previously impeded the development of glutamine-supplemented parenteral formulations. However, parenteral formulas containing glutamine dipeptides, such as alanylglutamine or glycylglutamine, have now been developed. These exhibit effects similar to those of free glutamine in animals (27, 28) and humans (32) and are currently in investigational use in humans (33).

The effects of glutamine on neoplastic cell proliferation and phenotype are less clear, however. We therefore studied the effects of glutamine supplementation on the proliferation, differentiation, and adhesion to the underlying matrix of two cell lines derived from human colon cancers. These are characteristics which may contribute to malignant behavior when altered in neoplastic cells. This study demonstrates, in at least two colon carcinoma cell lines, that glutamine supplementation can exert three effects on some colonic tumor cells: (a) glutamine stimulates proliferation; (b) glutamine supplementation decreases brush border enzyme activity, suggesting a loss of cellular differentiation; (c) glutamine profoundly inhibits adhesion to extracellular matrix substrates, possibly by decreasing surface expression of the α1, α2, and β1 integrin subunits.

Both the cell lines utilized in these studies are well characterized lines (17, 18) while the Caco-2 cell line in particular has previously been widely utilized to study phenotypic regulation in vitro (14–16).

The glutamine concentrations studied (1–10 mM) are consistent with glutamine concentrations used parenterally or enterally in animals (9, 34, 35) and in enteral formulas used currently in humans (Impact, Vivonex Plus, Alitra Q). In the latter, the concentration of free glutamine can be as high as 100 mM, a dose that appears to have no adverse effect in humans (36). Measured glutamine concentrations in vivo in humans have ranged from 0.6 to 0.9 mM in serum to as much as 20 mM in the muscles (3). Glutamine levels in the milieu of the intestinal mucosa are likely to be variable and to be heavily influenced by the patient’s diet. The glutamine concentrations used in experimental total parenteral nutrition solutions in humans have utilized glutamine concentrations of up to 40 mM (5.9 g l-glutamine/liter)

support the tumor (13, 19, 25). In particular, manipulating the nutritional balance to maintain the intestinal mucosa during perioperative fasting might also stimulate the growth of residual cancer after resection.

Glutamine is an important metabolic fuel and a precursor of nucleotide synthesis in rapidly dividing enterocytes (1–3). As a component of parenteral nutrition, glutamine has been reported to support gastrointestinal mucosal mass during starvation (4, 9, 26, 27), sepsis (28), chemotherapy (2, 10), or radiation (12) and to enhance mucosal hyperplasia after massive intestinal resection in animals (7). Enteral administration glutamine may decrease the incidence of upper gastrointestinal bleeding associated with stress ulceration in rats (6). Glutamine may also stimulate intestinal immune cell function by preserving the ability of intestinal immune tissue to produce secretory IgA, decreasing bacterial adherence to enterocytes and bacterial translocation (29, 30).

Fig. 9. Effect of glutamine supplementation on adhesion of nonproliferating Caco-2 cells. The cells were grown in T-25 bacteriological plastic flasks to confluence and then treated for 48 h in the absence and presence of glutamine supplementation. Cells were counted using a hemocytometer, cell number was equalized by dilution, and the cells were seeded on the enzyme-linked immunosorbent assay plates precoated with saturating amounts of collagen I. After 1 h, the plates were washed, adherent cells were fixed and stained with toluidine blue. The toluidine blue was then resolubilized and adherent cell number was measured colorimetrically. Cell adhesion was significantly decreased for all glutamine concentrations studied compared to controls (cells cultured without supplemental glutamine). Results are expressed as a percentage of adherent cells without glutamine supplementation (mean ± SE (bars), n = 12; *, P < 0.01 by ANOVA).

Fig. 10. Effect of glutamine supplementation on adhesion of proliferating Caco-2 cells. The cells were grown in T-25 bacteriological plastic flasks to confluence in order to block cell proliferation, the cells were cultured after pretreatment with 20 μM mitomycin C (MIT) or during continuous treatment with 30 μM hydroxyurea (HU) and then treated for 48 h in the absence and presence of glutamine supplementation (10 mM). After 1 h of incubation, the plates were washed, adherent cells were fixed and stained with hematoxylin, and cell number per high power field was counted directly under a microscope. The cells supplemented with glutamine (■) exhibited decreased adhesion to type IV collagen as compared with control cells cultured without supplemental glutamine (□). The decrease in enzyme activity was statistically significant for each [mean ± SE (bars), n = 12; *, P < 0.01 by ANOVA]. Cell adhesion was significantly decreased by glutamine supplementation compared to cells cultured without supplemental glutamine [mean ± SE (bars), n = 20; *, P < 0.001]. This effect was preserved for cells cultured in 0.1% serum media [mean ± SE (bars), n = 20; *, P < 0.001]. CON, control (proliferating cells); MIT, mitomycin; HU, hydroxyurea; 0.1% FBS, 0.1% fetal bovine serum media.

Fig. 11. Effect of glutamine supplementation on Caco-2 integrin subunit surface expression. The cells were cultured on the collagen IV matrix substrate in the absence (□) and presence (■) of glutamine supplementation (5 mM). After surface biotinylation, the cells were lysed, and α1, α2, and β1 integrin subunits were immunoprecipitated using subunit-specific antibodies. The integrins were resolved by SDS-polyacrylamide gel electrophoresis, transferred to nitrocellulose by electroblootting, and visualized by avidin-biotin staining. Results were quantitated using densitometry, and integrin subunit surface pool size for each unit was expressed as a percentage of its own control (without glutamine supplementation). All three integrin pools (α1, α2, and β1) decreased significantly in response to glutamine [mean ± SE (bars), n = 4; *, P < 0.01 by ANOVA].
(33). To simulate conditions characteristic of the intestinal lumen in fasting patients, we adapted a Caco-2 cell subclone to a culture medium with a low glutamine concentration (0.1 mM) derived from supplementation of the medium with 10% fetal bovine serum. Additional studies further confirmed that glutamine supplementation retains its ability to alter the Caco-2 phenotype even in 0.1% serum, suggesting that the effects described above do not require high concentrations of serum-derived growth factors as cofactors. The SW620 cell line does not require glutamine supplementation in cell culture and thus provides a control to confirm that the results presented here do not reflect the effects of our adaptation of the Caco-2 line to medium without supplemental glutamine, as does the observation that increasing glutamine supplementation from 2 mM to 10 mM in the parental Caco-2 line exerts similar effects on digestive enzyme expression.

The mechanism of the effects described here is as yet unclear. The observation that both L-glutamine and its nonmetabolizable isomer mo-glutamine exert similar effects on Caco-2 proliferation suggests that the effects of glutamine are not likely to represent only those of a metabolic fuel. Given that glutamine stimulates cell proliferation, the possibility might be raised that the effects of glutamine supplementation on other aspects of the phenotype of the colon carcinoma cell lines studied might reflect only changes in the average age or maturity of the more rapidly dividing cell pool. However, the studies described above demonstrate that the effects of glutamine supplementation on Caco-2 phenotype are preserved even when cell proliferation is inhibited by two different agents.

It might also be hypothesized that some of these results could be explained by the rapid degradation of supplemental glutamine in the media. However, the expected degradation rate of glutamine under the conditions studied is approximately 10%/day (37). Our dose-response studies demonstrated statistically significant differences between in the effects of 1 mM glutamine and higher concentrations from 3 to 10 mM. Since all studies were performed within 24–48 h of glutamine supplementation, we would therefore expect a 20% loss of glutamine due to degradation and metabolism. Thus, glutamine degradation, although a factor in vitro as well as in vivo, is not sufficient to explain the present results.

Thus, while extrapolation from cell culture studies to the in vivo situation must always be cautious (38), these observations suggest the possibility that glutamine supplementation may exert similar effects on some colonic neoplasms in vivo and furthermore that supplementation with glutamine may exert more of a trophic effect in this regard than supplementation with other amino acids.

Indeed, the finding that glutamine supplementation stimulates the proliferation of colonic tumor cell lines is consistent with previous observations in animal studies. Although a trophic effect of glutamine supplementation on tumor growth could not be demonstrated in sarcoma-bearing rats (39), increased cellular proliferation has previously been observed in vitro after glutamine supplementation in hematopoietic, gastric, pancreatic, and breast cancer cell lines (40, 41). In fact, it has been suggested that glutamine might be a preferential fuel for tumors (39, 42). Acivicin, an inhibitor of glutamine metabolism has been investigated recently as a potential antitumor agent (43).

In addition to stimulating proliferation, glutamine supplementation appeared to interfere with the expression of differentiated characteristics by these cell lines. The expression of brush border digestive enzymes is commonly utilized as a marker of cell differentiation (44). We studied the expression of four different enzymes: a phosphatase (alkaline phosphatase), a protease (cathepsin C), and two disaccharidases (isomaltase and lactase), and found them to be dose dependently inhibited by glutamine supplementation. Although rapidly proliferating cells might be expected to be routinely less differentiated, this relationship appears to be more complex. Epidermal growth factor, e.g., appears to promote both proliferation and cell differentiation in vitro in the Caco-2 cell line.4

These results are consistent with the observations of Jacobs et al. (26) in rats given diets supplemented with glutamine (2 g/100 ml). These investigators documented a trend toward decreasing mucosal disaccharidases (sucrase, lactase, maltase) expression (cell differentiation) with glutamine supplementation, although the statistical significance of this observation could not be established due to the variability intrinsic to the more complicated in vivo experimental system. At the same time, an increase in cell differentiation was observed after administration of a trophic agent (epidermal growth factor). The promotion of a more anaplastic phenotype by glutamine supplementation was also suggested in tumor-bearing (MCA sarcoma) rats where glutamine caused increase in the ratio of aneuploid to diploid cells (39).

The loss of digestive enzyme expression may be an important marker for the differentiated state, but it is not crucial to contribute to aggressive tumor behavior. Changes in cell adhesion to the underlying matrix, however, are likely to be critical in tumor invasion and metastasis. Because the complexity of in vivo extracellular matrix precludes ready analysis, we chose to study the effects of glutamine supplementation on cancer cell-matrix interactions using purified matrix proteins. Type IV collagen is the major collagen in the basement membrane to which colonocytes normally adhere and which contains early colonic dysplasias and neoplasias. The interstitial matrix beneath the basement membrane is rich in type I collagen. Cell adhesion to each of these matrix proteins may therefore be important in the processes of tumor invasion and metastasis. Glutamine supplementation appears to inhibit Caco-2 adhesion to both type I and type IV collagen. Since we have previously demonstrated that both the α1β1 and the α2β1 integrin heterodimers expressed by Caco-2 cells can bind these proteins (15), it seems likely that the marked decrease in Caco-2 α1, α2, and β1 integrin surface pools associated with glutamine supplementation may contribute to the decreased adhesion we have observed. Although extrapolation from a cell line in culture to in vivo tumors is risky, it does not seem unreasonable to postulate that if a similar decrease in integrin expression and cell adhesion to matrix proteins occurs in vivo, this may contribute to a higher propensity of these cancer cells for invasion and metastasis.

The modulatory effect of glutamine on adhesion to matrix substrates and integrin expression by the human colon cancer cell lines studied here did not correspond with the changes in Caco-2 migration over extracellular matrix substrates. These sets of observations are not inconsistent since epithelial sheet migration is a complex process involving integrin organization, cytoskeletal rearrangements, cell membrane changes, and other processes beyond simple cell adhesion (14, 15, 44–46).

In summary, glutamine supplementation profoundly alters the phenotype of at least two human colon cancer cell lines. To the extent to which in vivo colon cancers resemble the Caco-2 and SW620 cell lines, these data suggest that glutamine supplementation may stimulate neoplastic proliferation; promote a more anaplastic, less differentiated phenotype; and interfere with integrin-mediated cell adhesion to matrix. Such changes might be postulated to predict more aggressive tumor behavior in vivo. Nutritional supplementation with glutamine may be therefore be deleterious in patients with colon cancer. This possibility is of considerable concern since glutamine supplementation has been recommended (12, 31) and is currently used (33) in fasting cancer patients requiring nutritional support or undergoing chemotherapy or radiation therapy, many of whom may have residual cancer. Further studies are warranted to elucidate the effect of glutamine supplementation on colonic neoplasms in vivo.
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