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Glutamine as a Regulator of DNA and Protein Biosynthesis in Human Solid Tumor Cell Lines

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Objective

The transport of glutamine by six different human solid tumor-derived cell lines (*e.g.*, breast, colon, liver) was characterized and the impact of glutamine deprivation on rates of tumor cell proliferation and DNA and protein synthesis was assayed.

Summary Background Data

Glutamine is added routinely to cell culture media and its importance for cellular growth has been established. However, carrier-mediated glutamine transport by solid tumors has not been studied extensively, and the mechanisms by which glutamine contributes to cell growth regulation require further investigation.

Methods

In a panel of different human solid tumor-derived cells, sodium-dependent glutamine transport was characterized *in vitro* and rates of cell proliferation, protein and DNA synthesis, as well as thymidine transport, were correlated with glutamine concentrations in the culture media.

Results

In all cells, regardless of tissue origin, sodium-dependent glutamine transport was mediated almost exclusively by a single carrier. There was a range of Michaelis constants (Km) and maximal transport velocities (Vmax) for the glutamine transporter in each cell type, but the amino acid inhibition profiles were nearly identical, consistent with uptake by the System ASC family of transporters. Rates of cell growth, DNA and protein synthesis, and thymidine transport correlated with the glutamine concentration in the culture media, indicating the central role of this amino acid in regulating cellular proliferation.

Conclusions

These data indicate that glutamine transport by all solid tumors is mediated by the System ASC family of transporters. The variation in Km values suggests that some cancers may be better suited to survive in a low glutamine environment than others. The mechanism by which glutamine supports cell proliferation and regulates cell cycle kinetics involves its modulation of DNA and protein biosynthetic rates.

Malignant cells display uncontrolled rates of cellular proliferation and this process requires an increased supply of precursor amino acids to support key biosynthetic pathways. These substrates are obtained from the bloodstream and are translocated into the cytoplasm *via* specific membrane-bound transport proteins. In general, each transport system corresponds to a group of homogenous carrier proteins that reside in the plasma membrane. To support increased demands for amino acids, cancer cells must be endowed with very efficient transport systems. Indeed, malignant transformation is associated with the expression of highly efficient transporters to ensure that substrate availability does not become rate-limiting.[1,2] For example, human hepatoma cells transport glutamine at a rate 10 to 20 times faster than do normal hepatocytes.[2]

The uptake of glutamine by malignant cells exceeds that of other amino acids despite the fact that glutamine is not an essential amino acid. Eagle[3] first showed this avid requirement by determining concentrations of amino acids necessary to support cell proliferation in the Erlich ascites tumor cell. Glutamine requirements were tenfold higher than those for other amino acids. Although glutamine is used by tumor cells for the generation of energy, it also is used for protein and nucleotide biosyntheses. Normally, cells have access to an abundant supply of glutamine because the circulating concentration (600 μmol/L) is higher than that of any other amino acid. However, many fast-growing solid cancers outstrip their blood supply as they proliferate,[1,4] and intratumor blood glutamine levels may be reduced severalfold. This may lead to an impairment in cell growth and contribute to the clinical observation of central tumor necrosis.

Supraphysiologic concentrations of glutamine have been shown to stimulate the growth of colon cancers *in vitro,*[5] but the response to low glutamine concentrations similar to those observed clinically in advanced malignant disease or in the center of a poorly vascularized tumor has not been investigated. Given the importance of glutamine for tumor cell growth, we postulated that its availability plays a crucial role in modulating the rate of DNA and protein biosynthesis. In the present study, the uptake of glutamine by six different solid human cancers was characterized to determine if malignant cells share similarities in the mechanisms by which they obtain plasma glutamine. In addition, the effects of glutamine deprivation on rates of cell growth and the incorporation of leucine (an index of protein synthesis) and thymidine (a measure of DNA biosynthesis) into acid-insoluble material were studied.

The results show that glutamine transport by all solid tumors is mediated primarily by the System ASC family of transporters. Variations in carrier affinity (Km) for glutamine presumably distinguish transporter isozymes and suggest that some cancers may be better suited to survive in a low glutamine environment than others. Tumor cells with relatively lower affinity glutamine carriers tended to exhibit higher dependence on glutamine for growth, whereas those with higher affinity glutamine carriers could survive under conditions of greater glutamine impoverishment. Furthermore, the concentration of extracellular glutamine correlated directly with rates of DNA and protein synthesis and with thymidine transport rates, indicating that the mechanism by which glutamine supports cell proliferation relates to its regulation of key biosynthetic pathways.

MATERIALS AND METHODS

Chemicals

Radiolabeled amino acids (3H-L-glutamine and 3H-L-leucine) and 3H-thymidine were obtained from Amersham (Arlington Heights, IL). Cell culture media was from GIBCO/BRL (Grand Island, NY). Amino acids and all biochemicals were purchased from Sigma Chemical Inc. (St. Louis, MO), and fetal bovine serum was from Hyclone Laboratories (Logan, UT). Tissue culture plates were obtained from Costar Corporation (Cambridge, MA). The HT29 (colon cancer), HBL100, T47D (breast cancers), SK-Hep, and HepG2 (hepatomas) cells were obtained from ATCC (Rockville, MD). The KM12C (colon cancer) cells were provided by Dr. Isiah Fidler (MD Anderson Cancer Center, Houston, TX).

Cell Culture

Human cancer cell lines were grown in 75 cm^2 T-flasks at 37 C under a humidified atmosphere of 5% CO_2/95% air. The cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 2 mm L-glutamine (4 mm L-glutamine for HBL100 and T47D), 10% fetal bovine serum (FBS), 1000 units/mL penicillin and 1000 units/mL streptomycin, and 1 mg/L insulin. The culture medium was changed every 3 days until cells were confluent, at which point the cells were used for experiments. Dialyzed FBS (D-FBS) was used for the glutamine deprivation experiments.

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Cell Growth Measurements

Cells were seeded at a density of 1×10^5 cells/mL (1 mL/well) into 12-well tissue culture plates. After 24 hours, the culture medium was removed and changed to DMEM supplemented with 10% D-FBS plus glutamine (0–4 mm). Cells were detached from the plate with trypsin and quantified at days 0, 1, 3, and 5 (0, 1, 2, and 3 for SK-Hep) with a model ZM electronic cell counter (Coulter Electronics, Miami, FL). Cell growth in 2 mm glutamine (4 mm glutamine for HBL100 and T47D) was chosen as the control. In each of the cell lines, two different glutamine concentrations were determined in which cells could grow slowly or grow about half as fast as control, respectively, and these glutamine concentrations were used for subsequent experiments.

Glutamine Transport Measurement

Glutamine transport was measured by the cluster tray method of Gazzola et al.[6] Before the transport assays, the cells were rinsed twice with warm sodium-free Krebs-Ringer Phosphate Buffer (CholKRP, which was made by replacing the corresponding sodium salts with choline chloride and choline phosphate) to remove extracellular sodium and amino acids. After removal of CholKRP, the transport assay was initiated by transferring 0.25 mL of the uptake medium to 24-well trays. The transport of radiolabeled glutamine (5 μCi 3H-glutamine/mL) was performed for 1 minute at 37 C at 10 μmol/L unlabeled glutamine in both sodium Krebs-Ringers Phosphate (NaKRP) and CholKRP buffers. The transport reaction was terminated by rapidly discarding the uptake buffer and rinsing the cells three times with ice-cold buffer (2 mL/well/rinse). The wells containing the cells were allowed to dry and were solubilized in 200 μL of 0.2 N NaOH/0.2% sodium dodecyl sulfate solution. One hundred μL of the cell extract was neutralized with 10 μL 2 N hydrochloric acid and subjected to scintillation spectrophotometry. The remaining 100 μL in each well was used for the protein assay by the bicinchoninic acid protein method.[7]

The sodium-dependent glutamine transport values were obtained by subtracting the transport values in CholKRP from those in NaKRP. Saturable sodium-independent transport values were determined in CholKRP by subtracting the value in the presence of excess (10 mm) unlabeled glutamine from that in its absence. Transport velocities were expressed in units of picomoles per milligram of protein per minute.

Measurement of DNA and Protein Synthesis

For determination of DNA and protein synthesis, we measured the incorporation of 3H-thymidine and 3H-leucine, respectively, into acid-insoluble material. Cells grown in control glutamine concentrations were seeded in 24-well cluster trays (0.5 mL/well) at a density of 5×10^4/well. Based on cell growth curve, glutamine-deprived cells were seeded at different densities from 1.0×10^5/well to 2.5×10^5/well to control for density-dependent effects at each time point studied among three different glutamine concentration groups. However, for measurement of incorporation rates after 1 day of treatment, both control and glutamine-deprived cells were seeded at the density of 1.5×10^5/well. For all groups after 24 hours, the medium was removed and replaced with fresh DMEM plus 10% D-FBS and the appropriate concentration of glutamine (0 mm–4 mm).

Incorporation rates of 3H-thymidine and 3H-leucine into acid-insoluble material then were measured 1, 3, and 5 days (1, 2, and 3 days for SK-Hep). After removing the culture medium, the cells were incubated for 2.5 hours at 37 C in glutamine-free DMEM supplemented with 10% D-FBS and the corresponding glutamine concentrations in the presence of 3H-thymidine or 3H-leucine (1 μCi/mL). The assay was terminated after 2.5 hours, when the cells were washed twice with phosphate-buffered saline and fixed by washing three times with ice-cold 10% trichloroacetic acid. Thereafter, cells were rinsed twice with 70% and 95% ethanol, respectively. They were allowed to dry and solubilized in 200 μL of 0.2 N NaOH/0.2% sodium dodecyl sulfate solution. Radioactivity and protein content were measured by the same procedures described for glutamine transport measurements.

Thymidine Transport Measurements

Cells were seeded at the same densities as described in the 3H-thymidine and 3H-leucine incorporation experiments. After 24 hours, the culture medium was removed and changed to fresh medium containing the appropriate glutamine concentrations. Thymidine transport was determined by the methods described previously for the glutamine transport experiments. Briefly, after rinsing the cells twice with CholKRP, a substrate mixture (0.25 mL) containing 3H-thymidine (5 μCi/mL) as well as 1 μmol/L unlabeled thymidine was added to the cells for 30 seconds at 37 C. Transport was terminated by washing three times with ice-cold buffer. Measurements of radioactivity and protein content were performed by the methods described above. Saturable, or carrier-mediated sodium-independent, thymidine transport was determined by subtracting the nonsaturable component, defined as that portion of uptake not sensitive to inhibition by excess (1 mm) unlabeled thymidine, from total sodium-independent uptake measured in the absence of excess unlabeled thymidine. The sodium-dependent thy-

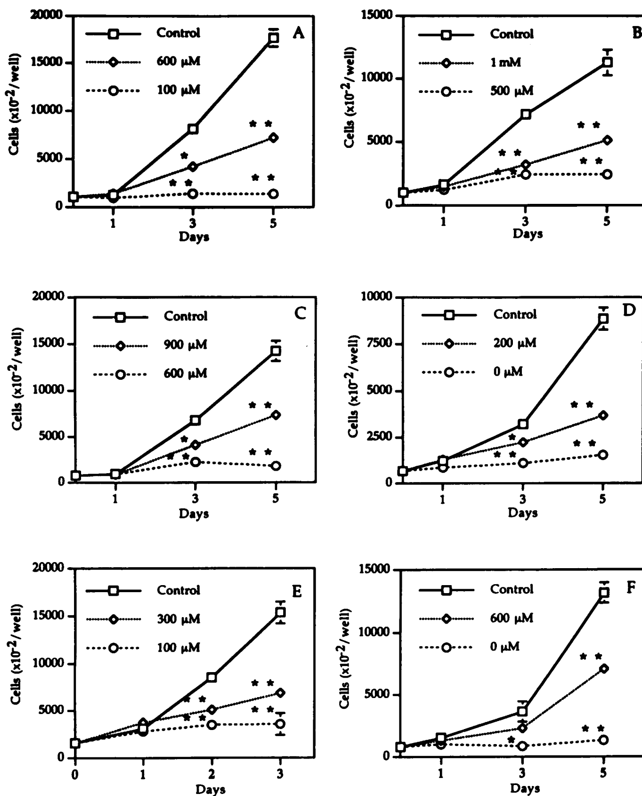

Figure 1. Effects of glutamine deprivation on cell growth in (A) HT29, (B) KM12C, (C) HBL100, (D) T47D, (E) SK-Hep, and (F) HepG2. Data are presented as the mean cell number ± standard deviation for triplicate determinations. Where not shown, error bars are within the symbol; $^*p < 0.05$ and $^{**}p < 0.01$ *vs.* control cells.

midine uptake was determined by subtracting the uptake value in CholKRP from that in NaKRP. Transport velocities were expressed in units of picomoles per milligram of protein per minute.

All experiments were performed at least twice. Data (mean ± standard deviation) were analyzed and compared with Student's t test or one-way analysis of variance. A p value < 0.05 was considered statistically significant.

RESULTS

Effect of Glutamine Concentration on Cell Growth

The effect of glutamine concentration on cell growth was determined in each cell line (Fig. 1). Cell proliferation rates were dependent on glutamine concentrations in every cell line, but the degree of dependence on glutamine for growth varied considerably. For example, in HT29 cells, growth was attenuated significantly in 600 μmol/L and 100 μmol/L glutamine compared with control (2 mm glutamine) beginning 3 days after glutamine deprivation (Fig. 1A). The T47D and HepG2 cells could survive and even grow slowly in the absence (0 mm) of glutamine (Figs. 1D, 1F). In contrast, KM12C and HBL100 cells showed slow cell growth, even in 500 μmol/L and 600 μmol/L glutamine, respectively (Figs. 1B, 1C), and could not survive in 0 mm glutamine (data not shown). The glutamine concentrations in which cells could grow very slowly for each cell line were 100 μmol/L (HT29), 500 μmol/L (KM12C), 600 μmol/L (HBL100), 0 μmol/L (T47D), 100 μmol/L (SK-Hep), and 0 μmol/L (HepG2), and those in which they could grow about half as fast as control were 600 μmol/L, 1 mm, 900 μmol/L, 200 μmol/L, 300 μmol/L, and 600 μmol/L, respectively.

Kinetics of Sodium-Dependent Glutamine Transport

Glutamine uptake by these 6 cell lines was linear for at least 2 minutes, and the sodium-dependent component was shown to account for more than 90% of total glutamine uptake. Therefore, 1 minute was chosen for the measurement of initial rate sodium-dependent glutamine transport velocities and was determined at concentrations between 10 μmol/L and 5 mm in both sodium and choline buffers. Figure 2 illustrates the Eadie-Hofstee plot of the kinetic data obtained from the sodium-dependent component. As reported previously by our group,[2] HepG2 cells exhibited a biphasic kinetic plot with a transport affinity (Km) value of 263 ± 68 μmol/L. The other five cell lines showed single-affinity transport systems with transporter affinities (Km) ranging from 152 μmol/L to 565 μmol/L and with maximum transport velocities (Vmax) ranging from 7395 to 21,048 pmol/mg protein/minute.

Characterization of Tumor Cell Glutamine Transporters

To characterize the sodium-dependent glutamine transport systems, the uptake of 10 μmol/L L-glutamine was measured in the absence and presence of 5-mm concentrations of individual unlabeled amino acids (Fig. 3). Osmotic effects of inhibitors were compensated for by the addition of sucrose to control assays. Sodium-dependent glutamine transport has been characterized previously in the two hepatoma cell lines (SK-Hep and HepG2) and is mediated primarily by System ASC.[2] Nearly identical profiles of amino acid inhibition were observed in the other cell lines studied. Sodium-dependent glutamine transport was inhibited significantly by glutamine, alanine, serine, and asparagine ($p < 0.001$), but was unaffected by MeAIB, glutamate, or arginine. Mild inhibition was observed in the presence of histi-

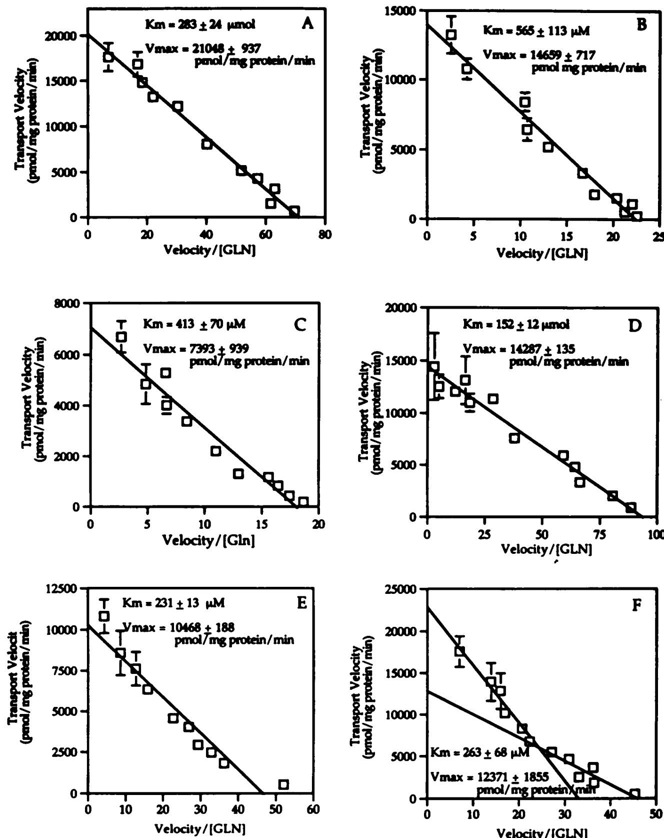

Figure 2. Eadie–Hofstee plot of saturable sodium (Na+)-dependent glutamine transport in (A) HT29, (B) KM12C, (C) HBL100, (D) T47D, (E) SK-Hep, and (F) HepG2. Transport velocity is plotted as a function of velocity/[Gln]. Data points are mean ± standard deviation for triplicate determinations. Where not shown, the error bar is contained within the symbol.

dine. These data indicate that sodium-dependent glutamine transport in these six cell lines is mediated by carriers with characteristics indicative of System ASC.

DNA Synthesis

Figure 4 shows the effect of glutamine deficiency on 3H-thymidine incorporation rates in HT29, KM12C, T47D, and SK-Hep, and the data are expressed as percent of control values. Glutamine deficiency resulted in remarkable decreases in 3H-thymidine incorporation in these four cell lines. For HT29, as shown in Figure 4A, 3H-thymidine incorporation decreased in the 600 μmol/L and 100 μmol/L groups significantly after 1 day (control, 100 ± 0; 600 μmol/L, 71 ± 6% ($p < 0.01$); 100 μmol/L, 37 ± 4% [$p < 0.01$]) and 3 days (control, 100 ± 5; 600 μmol/L, 53 ± 8% ($p < 0.01$); 100 μmol/L, 25 ± 4% [$p < 0.01$]). For KM12C, 3H-thymidine incorporation decreased in the 500-μmol/Lm group significantly after 3 days (control, 100 ± 18%; 500 μmol/L, 54 ± 7% [$p < 0.01$]). For T47D, DNA synthesis decreased in both the 200 μmol/L and 0 μmol/L groups after 1 day (control, 100 ± 4%; 200 μmol/L, 78 ± 4% ($p < 0.05$); 0 mm, 34 ± 8% [$p < 0.01$]), 3 days (control, 100 ± 13%; 200 μmol/L, 47 ± 11% ($p < 0.01$); 0 mm, 36 ± 5% [$p < 0.01$]), and 5 days (control, 100 ± 3%; 200 μmol/L, 69 ± 13% ($p < 0.05$); 0 mm, 58 ± 5% [$p < 0.01$]). For SK-Hep, DNA synthetic rates decreased in the 100-μmol/L group after 1 day (control, 100 ± 3%; 100 μmol/L, 31 ± 3% [$p < 0.01$]), 3 days (control, 100 ± 5%; 100 μmol/L, 32 ± 4% [$p < 0.01$]), and 5 days (control, 100 ± 16%; 100 μmol/L, 58 ± 4% [$p < 0.01$]).

Protein Synthetic Rates

Rates of 3H-leucine incorporation into acid-insoluble material were measured in all cell lines (Fig. 5), and the

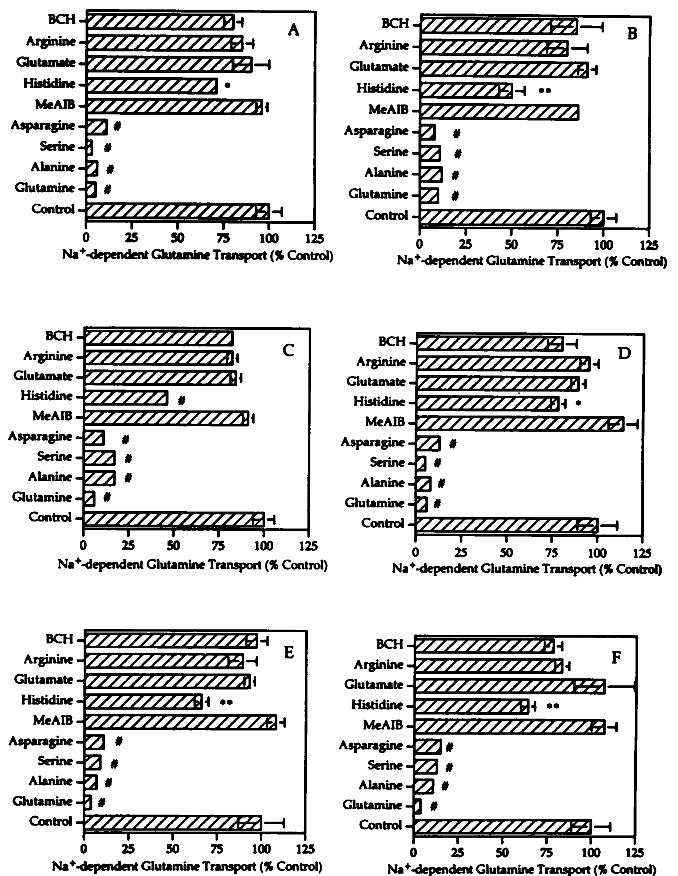

Figure 3. Amino acid inhibition profile of sodium (Na+)-dependent glutamine transport in (A) HT29, (B) KM12C, (C) HBL100, (D) T47D, (E) SK-Hep, and (F) HepG2. Data are expressed as percentage of control rates (absence of unlabeled amino acid) of glutamine uptake, which were 957 ± 70% (HT29), 177 ± 14% (KM12C), 202 ± 10% (HBL100), 1186 ± 121% (T47D), 887 ± 87% (SK-Hep), and 635 ± 26 pmol·mg-1 protein·min-1 (HepG2). Data are presented as the mean ± standard deviation for quadruplicate determinations. The abbreviations used are: BCH, 2-aminobicyclo-(2,2,1)-heptane-2-carboxylic acid; MeAIB, 2-(methylamino) isobutyric acid; *$p < 0.05$; **$p < 0.01$; #$p < 0.01$ *vs.* control.

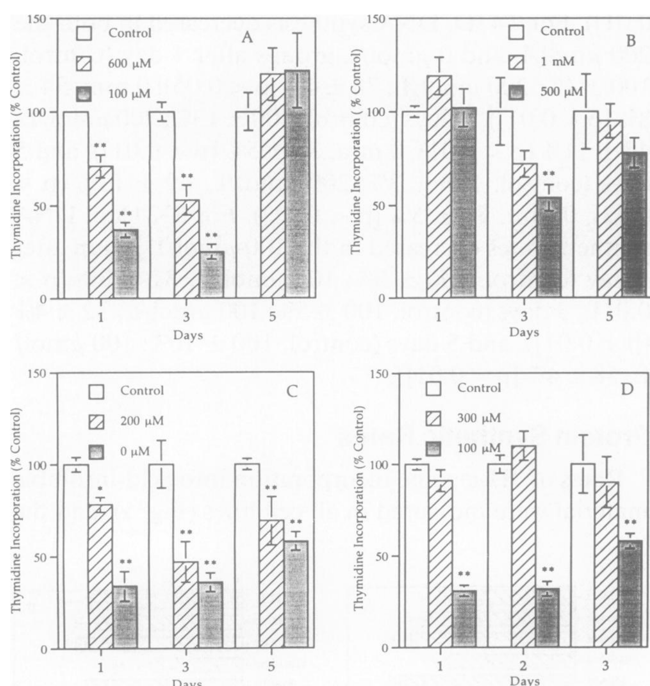

Figure 4. Effect of glutamine deprivation on 3H-thymidine incorporation into DNA after 1, 3, and 5 days (1, 2, and 3 days for SK-Hep) in (A) HT29, (B) KM12C, (C) T47D, and (D) SK-Hep. Data are expressed as percentage of control values and are the mean ± standard deviation for quadruplicate determinations; $^*p < 0.05$; $^{**}p < 0.01$ *vs.* control.

data are expressed as the percent of control values. Glutamine deficiency resulted in significant decreases in protein synthesis rates in each cell line, particularly in the lower glutamine concentration groups. The incorporation of 3H-leucine was significantly decreased after 3 days for HT29 (control, 100 ± 14%; 100 μmol/L, 78 ± 2% [$p < 0.05$]), KM12C (control, 100 ± 8%; 1 mmol/L, 73 ± 9% ($p < 0.01$); 500 μmol/L, 58 ± 5% [$p < 0.01$]), and SK-Hep (control, 100 ± 10%; 100 μmol/L, 76 ± 10% [$p < 0.05$]), but there was no difference in rates between the three glutamine groups on days 1 and 5, indicating the transient nature of the response in these three cell lines. Protein synthesis was significantly decreased for HBL100 and HepG2 after 3 days (control, 100 ± 11%; 600 μmol/L, 52 ± 5% ($p < 0.01$), control, 100 ± 5%; 0 μmol/L, 70 ± 10% ($p < 0.01$), respectively) and after 5 days (control, 100 ± 5%; 900 μmol/L, 72 ± 10% ($p < 0.01$); 600 μmol/L, 70 ± 9% ($p < 0.01$), control, 100 ± 8%; 0 μmol/L, 77 ± 2% ($p < 0.01$), respectively). For T47D, 3H-leucine incorporation was decreased in 0 μmol/L group after 1 day (control, 100 ± 2%; 0 μmol/L, 77 ± 7% [$p < 0.01$]), 3 days (control, 100 ± 5%; 0 μmol/L, 49 ± 10% [$p < 0.01$]), and 5 days (control, 100 ± 9%; 0 μmol/L, 60 ± 7% [$p < 0.01$]). Collectively, the results show that severe glutamine deprivation results in either transient (HT29, KM12C, and SK-Hep) or sustained (HBL100, T47D, and HepG2) depression of protein synthetic rates in tumor cells. The transient or sustained nature of the effect, however, appears to bear no relationship to the degree of glutamine dependence for growth among the cell lines.

Thymidine Transport

Thymidine transport was linear for at least 1 minute, and the sodium-independent component was shown to account for more than 85% of total thymidine uptake. Therefore, 30 seconds was chosen for the measurement of the initial-rate sodium-independent transport velocity. Figure 6 depicts the effect of glutamine deprivation on thymidine transport in HT29, KM12C, T47D, and SK-Hep. Glutamine deprivation resulted in significant

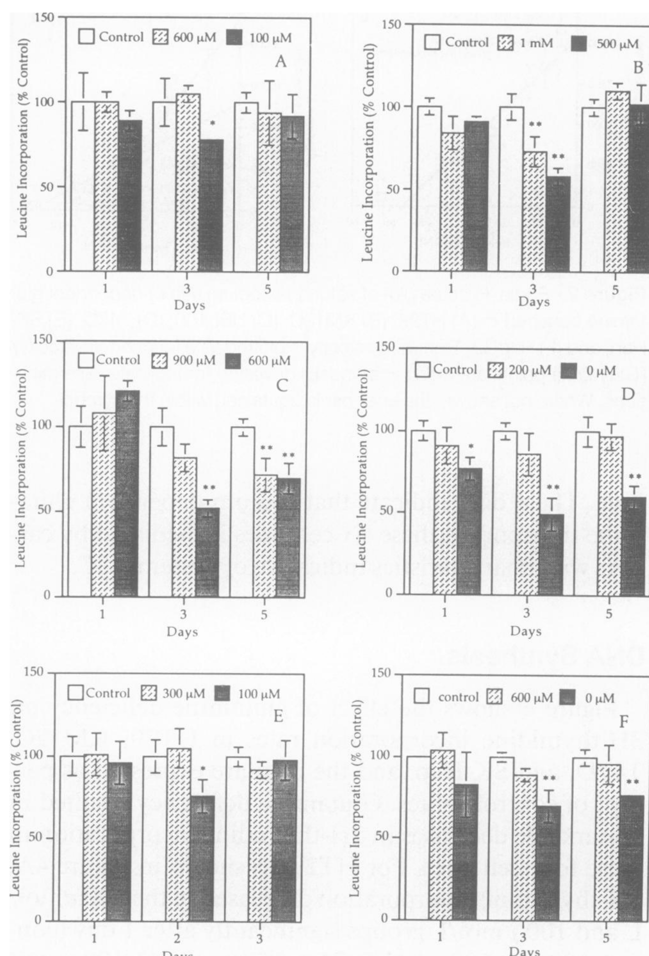

Figure 5. Effect of glutamine deprivation on 3H-leucine incorporation into protein after 1, 3, and 5 days (1, 2, and 3 days for SK-Hep) in (A) HT29, (B) KM12C, (C) HBL100, (D) T47D, (E) SK-Hep, and (F) HepG2. Data are expressed as percentage of control values and are the mean ± standard deviation for quadruplicate determinations; $^*p < 0.05$; $^{**}p < 0.01$ *vs.* control.

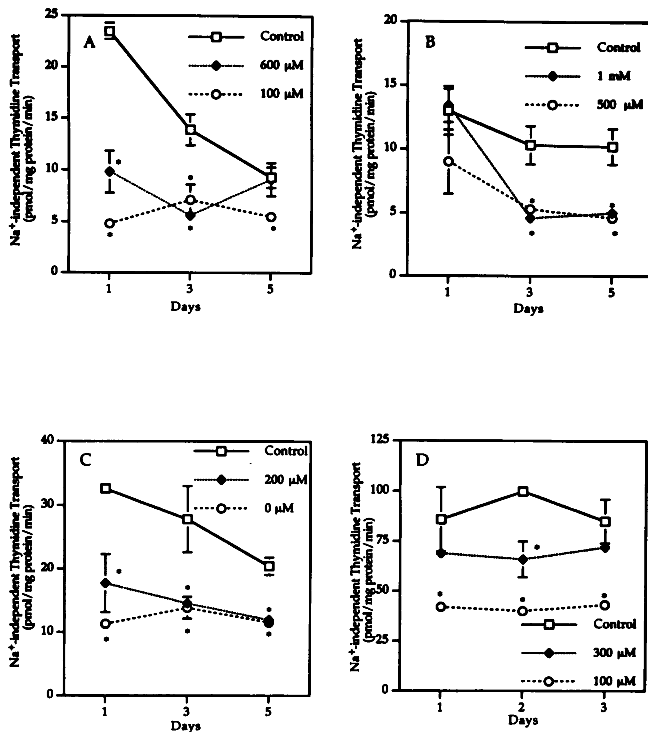

Figure 6. Effect of glutamine deprivation on sodium (Na±)-independent thymidine transport after 1, 3, and 5 days (1, 2, and 3 days for SK-Hep) in (A) HT29, (B) KM12C, (C) T47D, and (D) SK-Hep. Data are presented as the mean ± standard deviation for quadruplicated determinations; *p < 0.01 *vs.* control.

decreases in carrier-mediated thymidine transport velocities in these cell lines at all time points for HT29, T47D, and SK-Hep and after 3 and 5 days for KM12C. With the exception of day 5 for the two colon cancer cell lines (HT29 and KM12C), these data are consistent with the corresponding depressed rates of thymidine incorporation into DNA in response to glutamine deprivation (Fig. 4).

DISCUSSION

One major nutritional difference between normal and cancer cells is the latter's greatly increased propensity for the use of the amino acid glutamine.[1] Tumors exhibit increased glutamine uptake and consumption manyfold that of normal tissues, eventually outstripping the body's ability to provide this conditionally essential nutrient and resulting in net negative nitrogen balance and cachexia in the host.[1] As shown in the present study, cancer cells transport glutamine into the cytoplasm *via* System ASC2. Given the nearly identical substrate profiles (Fig. 3), yet disparate affinities for glutamine (Kms = 150 − 565 μmol/L, [Fig. 2]), the System ASC described here probably represents a family of closely related integral membrane transport proteins. The affinities of the carriers in all of the cells studied are below the normal circulating glutamine concentration (600 μmol/L); therefore, at least half of the transporters are normally bound, ensuring adequate glutamine uptake except under conditions of severe deprivation. Interestingly, the KM12C colon cancer had the highest Km (Fig. 2) and was the most sensitive to glutamine deprivation (Fig. 1), consistent with the diminished ability of its carrier to efficiently transport glutamine at reduced concentrations. Similarly, hepatomas have been shown to exhibit increased transporter activity in response to glutamine deprivation, presumably in an effort to acquire what little glutamine is available in the plasma with maximal efficiency.

The glutamine concentrations used in this study were chosen to mimic those levels commonly used in cell culture studies (2–4 mm) as well as those concentrations normally observed in the plasma (approximately 600 μmol/L) or within a poorly vascularized center of a tumor (0–300 μmol/L). In all malignant cells, the rate of cell growth was proportional to glutamine availability. At physiologic concentrations, the rate of cell proliferation was variable, indicating that cell cycle kinetics are regulated, in part, by the extracellular glutamine concentration. In low glutamine media, growth of all cells ceased as shown by no net change in cell number as a function of time. When excess glutamine was again provided, rates of cell growth increased (data not shown). Turowski et al.[8] showed that even higher ambient glutamine concentrations were able to further stimulate cell growth of cancer cells. The caveat of their model, however, is that such concentrations (as high as 10 mm) are nonphysiologic and are never achieved *in vivo,* even in those patients receiving glutamine-supplemented nutrition whose blood levels may reach 0.9 mm.

The mechanism by which glutamine regulates the growth of these malignant cells is related to its influence on DNA replication and, to a lesser extent, protein synthesis. As shown in Figures 4 and 5, leucine incorporation and thymidine incorporation were both proportional to glutamine availability. Although it has been established that glutamine may serve as a positive regulator of protein synthesis in enterocytes,[9] skeletal muscle[10] and liver,[11] and of DNA synthesis in other cell types,[12] the specific signals that control these biosynthetic pathways as they relate to glutamine availability are unclear. As both are energy-dependent processes, it is possible that low glutamine levels result in depressed cellular ATP content, as has been shown in human leukemia cells.[13] However, in those studies, restoration of cellular ATP levels *via* the addition of exogenous adenine failed to restore the proliferation rates of the cells, indicating that glutamine exerts additional effects on cellular growth exclusive of its effects on energy pools. The observation

that some cells "recover" protein synthetic rates comparable to those of control cells between 3 and 5 days of glutamine deprivation (Fig. 5), whereas cell proliferation rates remain depressed (Fig. 1), provides further evidence that differences in ATP levels between the groups cannot fully account for glutamine's effects on growth. One possible explanation for the glutamine deprivation-dependent inhibition of cell proliferation may involve its effects on nucleotide pools available for DNA biosynthesis, similar to that observed in activated lymphocytes,[14] and this possibility remains to be explored. Nonetheless, the basis for the differential effects of individual amino acids on gene expression remains poorly understood and, as such, is a fruitful area of research.[15] Based on the results presented in this report, it could be argued that glutamine deprivation (to different "threshold" levels in individual tumor cells) may exert negative specific effects on the expression of genes necessary for cellular proliferation, whereas those for cellular homeostasis remain unaffected. This hypothesis is not unreasonable as glutamine has been shown to selectively induce the expression of "heat-shock" proteins[16] and proteins involved in early embryogenesis[17] in the absence of effects on cellular energy. Additionally, glutamine has been shown to effectively replace growth factors (*i.e.*, serum) in the induction of DNA synthesis in quiescent 3T3-fibroblasts.[12]

The "antiproliferative" effects of glutamine deprivation on tumor cells is further manifested by the observation that thymidine transport also paralleled the availability of glutamine (Fig. 6). One might postulate that in the absence of, or upon reductions in levels of a key substrate (glutamine), tumor cells respond by reducing metabolic activities associated with growth, such that only vital biochemical pathways continue to function. The upregulation of other amino acid transporters may reflect a reprioritization of cellular metabolism and occur in an effort to support synthetic reactions essential for homeostasis as cells remain in G0. For example, the recovery of protein synthetic rates in the HT29 cells after 5 days of glutamine deprivation (Fig. 5) may correspond to the previously reported induction of enterocytic differentiation in this cell line after growth in glutamine-free media.[18]

Given these known effects of glutamine on cell growth and gene expression, the experiments presented here have important clinical implications with regard to tumor metabolism and nutrition. Based on the data in Figure 1, it may be inferred that the relative impoverishment of glutamine levels within a poorly vascularized tumor results in reduced cell proliferation. This explanation is probably somewhat simplistic because the availability of other substrates (*e.g.*, oxygen, nucleosides, glucose) also is likely to modulate growth. Based on the data from the growth curve and Figure 1, it is clear that some cancers are more sensitive to glutamine deprivation than others. The basis for this differential sensitivity is unclear, but may involve factors such as the ability to induce expression of glutamine synthetase, which appears to occur more readily in relatively glutamine-independent cells.[13] Of possible significance in this regard is the observation that transformed cells adapted to survive in the absence of glutamine are more tumorigenic than their glutamine-dependent counterparts.[19] The avidity with which tumor cells extract and use glutamine also has caused some concern in the initiation and development of prospective clinical studies of the use of glutamine-supplemented nutritional regimens in patients with cancer.[20] Animal studies using glutamine-enriched solutions suggest that supplemental glutamine, whereas not affecting tumor growth, does slightly increase the aneuploid–diploid ratio in the tumor.[21] In similar animal studies, it was shown that a glutamine-supplemented diet exerts protective effects on host tissues and a sensitizing effect on the tumor toward methotrexate cytotoxicity.[22,23] Nonetheless, the basis for the formulation of nutritional regimens targeted to benefit the patient with cancer must inevitably involve the exploitation of differences in transformed and nontransformed cellular glutamine requirements and metabolism.

In summary, the studies presented here are an important first step in elucidating the basis for the avid requirement of tumor cells for glutamine and in understanding cancer cell responses to glutamine levels likely encountered *in vivo.* Future studies will need to address the difference between "proliferative" and metabolic effects of glutamine in malignant and nonmalignant cells. For example, the identification of putative "glutamine-dependent proliferation genes" that are no longer expressed (or whose products are inactivated below certain ambient glutamine concentrations must be established. Design of rational nutritional regimens to benefit the host with cancer can only be achieved after these fundamental differences are elucidated.

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