ABSTRACT

Twenty-four h after tumor transplantation increases of free glutamine in plasma, liver, and kidney occurred simultaneously with the exponential phase of tumor growth. Kidney and muscle glutamine synthetase also increased in the first 2 days following tumor transplantation, while kidney and liver glutaminases decreased. The levels of free glutamine in plasma and tissues, and the activities of glutamine synthetase and glutaminase, tended to approach normal values in the last days of life of the tumor-transplanted animals. Eleven days after transplantation, liver glutamine synthetase activity diminished. The results are discussed in terms of a glutamate/glutamine intercellular cycle which could augment the circulating glutamine, the main source of nitrogen for tumor cells.

INTRODUCTION

Glutamine is described as the main source of energy and nitrogen for rapidly dividing cells such as enterocytes (1), reticulocytes (2), thymocytes (3), and tumor cells (4, 5). In a previous report, the host to tumor net flux of glutamine was confirmed in vivo, in mice inoculated with Ehrlich ascites tumor cells (6). Glutamine can be produced by the muscle (7), the brain, the kidney, the liver, and adipose tissues (8). Furthermore, there is hepatic glutamine release in response to metabolic acidosis (9). Wu et al. report a decrease in glutamine synthetase (EC 6.3.1.2) in the liver of tumor-bearing rats (10). The work of Bhatcharjee et al. (11) confirms changes in the levels of liver glutamine synthetase following Ehrlich ascites tumor transplantation in mice. The fact that the kidney and the liver also contain phosphate-dependent glutaminases (EC 3.5.1.2), which give rise to a glutamate/glutamine energy consuming cycle (12), called for a systematic study of the contributions made by the host tissues to circulating glutamine during tumor development. The results reported here suggest that, in addition to the contribution from skeletal muscle, an intercellular glutamate/glutamine cycle might be operating in the liver and in the kidney, to produce a net increase in the plasma glutamine needed for tumor growth.

MATERIALS AND METHODS

Ehrlich Ascites Cell. A hyperploid Lettré strain, kindly supplied by Dr. T. Galeotti (Università Cattolica Sacro Cuore, Rome, Italy), was maintained in 2-month-old, female albino Swiss mice OF1 (SPF Ico) purchased from Paliab (Barcelona, Spain). The animals received standard Paliab food with the following composition: carbohydrates 49.8%, lipids 5.0%, proteins 23.5%, minerals 5.7%, cellulose 4.0%, water 12%, and tap water ad libitum. They were kept at temperatures of 22-24°C by Dr. T. Galeotti (Università Cattolica Sacro Cuore, Rome, Italy), was provided by Dr. T. Galeotti (Università Cattolica Sacro Cuore, Rome, Italy).

Enzyme Assays. Four series of 21 mice each were inoculated with 5 × 10^6 tumor cells from four different tumor-inoculated animals. The assays were carried out 1, 2, 4, 7, 11, 14, and 16 days after the tumor transplantation. Nine nontransplanted animals were used as controls.

RESULTS

Table 1 shows the levels of free glutamine in the plasma, ascitic liquid, liver, and kidney of tumor-bearing mice. Twenty-four h after tumor transplantation, the levels of plasma glutamine were significantly higher than those of the controls until the 11th day. The life-span of inoculated mice was 16 ± 1 days. Similar patterns were observed for liver and kidney free-glutamine content after transplantation. The maximum values of free glutamine levels in plasma, liver, and kidney coincided with the logarithmic phase of tumor growth. The concentration of glutamine was always higher in plasma than in ascitic liquid, because glutamine is rapidly consumed by tumor cells (6).

Two different catalytic activities are reported in vitro for the glutamine synthetase enzyme: the synthetase activity and the γ-glutamyl transfer reaction. The transferase activity in vitro is at least 18–20-fold higher than that of synthetase. Fig. 1 shows the glutamine synthetase level measured as transferase activity, in mice hind-quarter tissues after inoculation with Ehrlich ascites tumor cells. Skeletal muscle did not show any glutaminase activity. Transferase activity was assayed because of its

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GLUTAMINE CONTRIBUTION BY THE HOST TO TUMOR

Table 1 Free glutamine levels in plasma, ascitic liquid, and kidney of tumor-bearing mice during tumor growth period

Daily food intake in tumor-bearing mice was determined in three different series of six animals, and expressed as percentage of the daily food intake of controls. Values of plasma and ascitic liquid, means ± SE of 12 infected animals, determined by reversed-phase high-performance liquid chromatography using precolumn derivatization with Dansyl chloride. Values of glutamine contents in liver and kidney, means ± SE of six infected mice, determined by enzymatic analyses.

<table>
<thead>
<tr>
<th>Days after tumor transplantation</th>
<th>% Daily food intake</th>
<th>Plasma (nmol/ml)</th>
<th>Ascitic liquid (nmol/ml)</th>
<th>Liver (µmol/g fresh tissue)</th>
<th>Kidney (µmol/g fresh tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
<td>222 ± 8</td>
<td>ND</td>
<td>2.84 ± 0.27</td>
<td>0.47 ± 0.10</td>
</tr>
<tr>
<td>1</td>
<td>69 ± 2*</td>
<td>306 ± 14</td>
<td>ND</td>
<td>4.90 ± 0.66*</td>
<td>0.93 ± 0.01</td>
</tr>
<tr>
<td>2</td>
<td>78 ± 5*</td>
<td>305 ± 12*</td>
<td>ND</td>
<td>3.98 ± 0.23*</td>
<td>1.05 ± 0.11*</td>
</tr>
<tr>
<td>4</td>
<td>105 ± 6</td>
<td>254 ± 11*</td>
<td>7.5 ± 0.4</td>
<td>3.14 ± 0.31</td>
<td>1.51 ± 0.25*</td>
</tr>
<tr>
<td>7</td>
<td>100 ± 9</td>
<td>259 ± 9*</td>
<td>12.5 ± 1.8</td>
<td>2.81 ± 0.37</td>
<td>1.34 ± 0.07*</td>
</tr>
<tr>
<td>11</td>
<td>77 ± 7</td>
<td>254 ± 8*</td>
<td>67.5 ± 5.3</td>
<td>2.85 ± 0.31</td>
<td>0.67 ± 0.12</td>
</tr>
<tr>
<td>14</td>
<td>87 ± 8</td>
<td>197 ± 11</td>
<td>50.9 ± 5.2</td>
<td>1.58 ± 0.11*</td>
<td>0.42 ± 0.06</td>
</tr>
<tr>
<td>16</td>
<td>91 ± 3</td>
<td>200 ± 6</td>
<td>60.3 ± 5.9</td>
<td>1.57 ± 0.17*</td>
<td>0.48 ± 0.13</td>
</tr>
</tbody>
</table>

* ND, ascitic liquid was not detectable.

\* P < 0.0005 versus control.
\* P < 0.001 versus control.
\* P < 0.01 versus control.
\* P < 0.005 versus control.
\* P < 0.0025 versus control.
\* P < 0.05 versus control.

greater sensitivity and because the glutamine synthetase levels in muscle are very low. Relatively small, but significant increases were observed 24 h after inoculation. Figs. 2 and 3 display the glutamine synthetase and phosphate-dependent glutaminase levels of both kidney and liver. The results are expressed per gram of fresh tissue. Similar patterns were obtained when the results related to mg of protein extract. Identical variations of the enzyme levels in liver and kidney were found when the γ-glutamyl transfer reaction was assayed (results not shown). A decrease in liver glutamine synthetase level was observed 11 days after inoculation. These results agree well with the early report of Wu et al. (10) which shows that the free glutamine concentrations in the tissues of rat bearing Walker carcinoma 256 significantly decrease, and that glutamine synthetase activities also decline in the livers of tumor-bearing animals, in which tumor weight amounted to 8–17% of the total body weight. However, in the present work, a decrease of the glutaminase level to less than 50% of the controls was observed 24 h after tumor transplantation.

In the kidney, following tumor inoculation, simultaneous but opposite changes for both enzymes were observed; glutamine synthetase level increased to a maximum at the fourth day while the glutaminase level decreased. The values of both enzymes were close to normal at the end of animal life-span. The changes in the in vitro activities of both enzymes are really changes in their concentrations. This was demonstrated by mixing kidney extracts from the controls with extracts taken from mice 4 days after inoculation. The combined activities of the two homogenate mixtures were equal to their sums, which rules out the possibility that an activator of glutamine synthetase, or an inhibitor of glutaminase, might be present in the extracts of the inoculated mice. The levels of brain glutamine synthetase and glutaminase were unchanged at the 4th day after inoculation.
Because a decrease of 30%, in the food intake, was observed in the first 2 days after inoculation, the levels of glutamine synthetase and glutaminase were determined in starved animals. After 48 h of starvation, no changes were observed in the levels of kidney and muscle glutamine synthetase and liver glutaminase. However, a significant decrease of liver glutamine synthetase (17%), and a slight increase of kidney phosphate-dependent glutaminase (9%), were observed (results not shown). These results are contrary to those observed in liver and kidney, and suggest that the changes of glutamine synthetase and glutaminase in the first days after inoculation were not an adaptive response to decreased feeding. The levels of glutamine synthetase and glutaminase in livers and kidneys of mice injected with cell-free sterile asctic liquid remained unchanged.

DISCUSSION

One of the most important systemic effects produced by tumors in the host is the induction of a negative nitrogen balance (19). Glutamine is postulated as the major nitrogen carrier from the host to the tumor (20). Previous studies report that the concentrations of glutamine in plasma are always higher than in ascitic liquid (6). This indicates a net flux of glutamine from the host to the tumor, and so there must be a continually replenished source of circulating glutamine somewhere in the host body. The most obvious source of circulating glutamine is skeletal muscle where it is an end product of amino acid catabolism (7). The increase of glutamine synthetase observed in muscles following tumor transplantation suggests that the presence of the tumor elicits an increase in glutamine release by the muscle in the first days after inoculation when the specific growth rate reaches a peak (21).

Both glutamine synthetase and glutaminase are simultaneously active in the liver, but the regulation of their activities in vivo is poorly understood (8). Moreover, the metabolic conditions determine whether there is an overall hepatic uptake or release of glutamine (22, 23).

Several authors describe the operation in liver and kidney of a glutamate/glutamine intercellular cycle (24, 25). Crabtree and Newsholme describe the substrate cycles as regulatory devices to enhance the sensitivity to regulatory modulators (26). This hypothesis makes the important prediction that the cycling rates vary from one metabolic state to another.

In this study, no significant changes in the levels of hepatic glutamine synthetase were observed until the 11th day after inoculation. However glutaminase sharply decreased in the first day following tumor transplantation, an indication that the glutamine production capability of the whole liver was increased. The rise in liver glutamine content observed during the first 2 days following transplantation supports this statement. The results reported here for the kidneys of tumor-bearing animals indicate that shortly after transplantation, one branch of the cycle was increased, while the other was diminished. Consequently, a net increase occurred in both tissue free-glutamine and circulating glutamine. Nevertheless, in the last days of life, glutamine synthetase decreased and glutaminase increased to control values. Squires and Brosnan (27) report that renal synthesis of glutamine decreased in rats treated with NH4Cl solution in drinking water. In similar conditions, Tong et al. (28) demonstrate that kidney glutaminase levels raised due to an increase in the rate of synthesis. Thus, the ammonia detected in Ehrlich ascites tumor-bearing animals (6) could explain the rise of glutaminase levels found in the kidney in the last days of life. The results presented here suggest that the tumor elicits a specific response in the host nitrogen metabolism so that the whole organism is mobilized to augment circulating glutamine; the prime source of nitrogen for the tumor cell.

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Contribution by Host Tissues to Circulating Glutamine in Mice Inoculated with Ehrlich Ascites Tumor Cells

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