Immunosuppressive Effects of L-Asparaginase

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SUMMARY

The immunological effects of L-asparaginase were studied in man. L-Asparaginase suppressed the development of delayed hypersensitivity to primary antigens in cancer patients receiving therapeutic doses of the drug. The development of delayed hypersensitivity skin reactions was completely or partially suppressed in 5 out of 8 patients immunized with 2 mg keyhole limpet hemocyanin and in 5 out of 6 patients immunized with a synthetic amino acid copolymer of glutamic acid, lysine, alanine, and tyrosine. L-asparaginase did not change established delayed hypersensitivity skin reactivity.

Production of antibodies to keyhole limpet hemocyanin was delayed and suppressed by L-asparaginase and 3 out of 8 patients did not produce any antibody to this antigen. Antibody production was delayed and antibody titers were lower compared to controls in the other 5. Production of antibodies to the synthetic amino acid copolymer of glutamic acid, lysine, alanine, and tyrosine was delayed by treatment.

Lymphocytes of patients receiving L-asparaginase did not respond to phytohemagglutinin or specific antigens when cultured in media containing their own on-treatment serum, but responded at normal level when washed and cultured in media containing normal serum or their own pretreatment serum.

Phytohemagglutinin-induced blastogenic responses of lymphocytes from hematologically normal subjects were suppressed by the addition of serum from patients on L-asparaginase treatment.

INTRODUCTION

L-Asparaginase is a new antitumor agent with activity in acute leukemia (7, 11, 31). The antitumor activity is apparently related to the depletion of L-asparagine, which is indispensable to L-asparagine-dependent tumors (1, 3, 13). This enzyme was found to inhibit the in vitro blastogenic responses to human peripheral blood lymphocytes stimulated with PHA2 or specific antigens to which the donors of lymphocytes were sensitive (2, 22, 23). The inhibition of lymphocyte blastogenesis was not due to cytotoxicity, but rather to specific depletion of exogenous and endogenous L-asparaginase. L-Asparaginase also suppressed the production of hemolytic plaque-forming cells and hemagglutinating antibody in mice immunized with sheep red blood cells (24, 26).

The immunosuppressive effects in mice were due to inhibition of the proliferation of committed immunologically competent cells.

The present report describes the immunosuppressive effects of L-asparaginase in man. L-Asparaginase was found to inhibit the primary immune response, in terms of both the humoral antibody production and the development of delayed hypersensitivity. In addition, serum of patients receiving L-asparaginase strongly inhibited the in vitro blastogenic responses of the lymphocytes of hematologically normal subjects.

MATERIALS AND METHODS

Subjects and Therapy. Fourteen patients of both sexes, ages 5 to 74 years, who received therapeutic doses of *Escherichia coli* L-asparaginase (Merck, Sharp and Dohme Research Laboratories, West Point, Pa.) were studied. The dose of L-asparaginase ranged from 12,000 to 400,000 i.u./sq m body surface area i.v./day for 3 to 38 days. Eight patients had AML, 2 had ALL, and 4 had nonlymphoid solid tumors (2 breast carcinomas, 1 osteogenic sarcoma, and 1 nonfunctioning adrenocortical carcinoma).

No other antitumor drugs were given with the L-asparaginase. Five of the leukemia patients received L-asparaginase as initial treatment and 5 received the drug after other chemotherapeutic agents had failed to control their disease. The 4 solid tumor patients received L-asparaginase as initial treatment for their metastatic disease. Sites of metastasis were limited to 1 organ. They were hematologically normal and in good general condition. All but 1 of the leukemia patients received L-asparaginase treatment alone.

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patients were in good clinical condition. That patient had fever and was on combination antibacterial-antibiotic therapy.

Healthy volunteers and untreated patients with primary or metastatic solid tumors, ages 25 to 50 years, served as controls for the primary immune response in untreated subjects (5). Specifically, this group contained 14 healthy volunteers ages 25 to 50 years, 9 patients who had prior surgical removal of a primary melanoma and were free of disease when studied, and 12 patients with metastatic malignant melanoma, not on chemotherapy and in good clinical condition. The response of these 3 groups to KLH were similar.

Primary Immunization. KLH was prepared according to the method of Campbell et al. (4). For primary immunization, 2 mg KLH in 0.1 ml solution were injected s.c. in the deltoid region. GLAT, a synthetic polymer of glutamic acid, lysine, alanine, and tyrosine (M.W. 35,000) (19) was provided through the courtesy of Dr. Paul H. Maurer. One hundred µg GLAT in 0.1 ml solution were injected intracutaneously in the forearm. Nine patients were actively immunized. Four patients received 1 antigen within 4 hr of the start of therapy. The other five received 1 antigen within 4 hr after the start of treatment (Day 1) and the other antigen 2 days later (Day 3) in the contralateral arm. The immune response was followed over the next 3 to 4 weeks. Thus, 5 patients were immunized with both KLH and GLAT, 3 with KLH alone, and 1 with GLAT alone.

Measurement of Immunity. As a test of the development of new delayed hypersensitivity, the patients were skin tested intradermally 3 weeks after immunization with 100 µg GLAT and/or 100 µg KLH in 0.1 ml 0.85% NaCl solution. As a test of established delayed hypersensitivity, skin tests were done with the antigens candidin, dermaphtophytin, and dermophytophytin “O,” (Hollister-Stier Laboratories, Dallas, Texas. Original extracts were diluted to 1:10 in 0.85% NaCl solution.) and streptokinase-streptodornase (Varidase) (Lederle Laboratories, Pearl River, N. Y.). These skin tests were done before therapy and the patients were skin tested again 3 weeks after therapy with 1 of the established delayed hypersensitivity antigens to which they were initially positive. This was done to differentiate true immunosuppression from the development of generalized immunological deficiency. The skin test sites were examined 24 and 48 hr after injection. Induration was measured in millimeters in 2 diameters at right angles and the result was expressed as the average of these measurements. Skin tests were regarded as positive if the longer diameter at 24 and 48 hr was at least 3.5 mm, as unquestionably positive if the longer diameter was between 1 and 3 mm, and as negative if no induration was present at all. All previously unimmunized subjects have negative skin reactions to KLH and GLAT.

Antibody to KLH and GLAT were determined by passive hemagglutination with serum specimens collected weekly. Tanned red cell hemagglutination was performed according to the method of Stavitsky (29).

Leukocyte cultures designed to measure lymphocyte blastogenic responses were done by previously described methods (5). Venous blood was defibrinated with glass beads, dextran was sedimented, and the leukocyte-rich plasma was cultured. Lymphocytes (10^6) in 1 ml serum and 2 ml spinner modified Eagle’s minimum essential medium were cultured in 13- x 100-mm glass round-bottom screw-cap tubes. Cultures were stimulated with 0.05 ml PHA-M, 0.1 ml SLO, (Difco Laboratories, Detroit, Mich.), 0.1 ml Varidase, 0.1 ml vaccinia solution (Wyeth Laboratories, Inc., Marietta, Pa.), 0.1 ml KLH (10 or 100 µg/ml), and 0.1 ml GLAT (10 or 100 µg/ml). When allogenic serum was used in culture, leukocytes were centrifuged and washed twice with medium containing 10% fetal bovine serum before culture with the appropriate test serum. The culture tubes were incubated upright at 37° in 5% carbon dioxide in air for 5 days. Three hr before harvesting, 2 µCi thymidine-3H (Schwarz Bio Research, Orangeburg, N. Y.) (specific activity, 1.9 Ci/mmmole) were added to each tube. The cells then were washed twice with cold 0.85% NaCl solution. The acid-insoluble material was precipitated twice with cold 5% trichloroacetic acid, dissolved in 0.5 ml Hyamine (Packard Instrument Co., Inc., Downer’s Grove, Ill.) at 60° for 15 min, taken up in 0.5% PPO (Packard) and 0.01% dimethyl-POPOP (Packard) in toluene, brought to a volume of 12 ml with toluene, and counted in a Packard Tri-Carb Model 3375 liquid scintillation counter. Radioisotope incorporation was expressed as cpn/10^6 lymphocytes. The counts of the unstimulated controls were subtracted from the stimulated culture to estimate net incorporation.

Serum electrophoresis was performed with cellulose acetate and immunoglobulin levels were determined by a radial immunodiffusion method (6).

RESULTS

Effect of L-Asparaginase on Primary Immune Response to KLH and GLAT. Nine patients underwent a total of 14 immunizations with KLH and/or GLAT immediately (Day 1) or 2 days (Day 3) after initiation of L-asparaginase treatment (Table 1). Three out of 8 patients immunized with KLH did not develop delayed hypersensitivity skin reactions when tested with 100 µg KLH 3 weeks after immunization, 2 developed questionably positive skin reactions, and 3 developed clearly positive reactions. All control subjects developed positive delayed hypersensitivity skin reactions. Established delayed hypersensitivity skin reactions were tested at the same time. All but 1 showed positive reactions to at least 1 of these antigens. In selected subjects in this and other (5) studies, biopsies of even small (less than 5-mm diameter) reactions interpreted clinically as delayed hypersensitivity have shown typical perivascular small round cell infiltration.

Antibody production to KLH was suppressed in every patient studied. Three out of 8 patients did not produce any hemagglutinating antibody to KLH during the 21-day study period. Compared to normal controls, antibody titers among these patients were lower and appeared later. Seven days after immunization, only 1 out of 8 patients had developed antibody, whereas all control subjects already had antibody at this time. In this 1 patient, antibody was no longer detectable on Day 21.
Table 1

Effect of L-asparaginase on primary immune response in man

<table>
<thead>
<tr>
<th>Drug</th>
<th>Subject</th>
<th>Age</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>Total L-asparaginase (i.u./sq m)</th>
<th>Period drug given (days)</th>
<th>Immunization (Day)(^a)</th>
<th>Skin test (mm)b</th>
<th>Antibody titer</th>
<th>Absolute lymphocyte count</th>
<th>Survival after study finished (mo.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KLH  (2 mg s.c.)</td>
<td>I. D.</td>
<td>47</td>
<td>F</td>
<td>Breast carcinoma</td>
<td>525,000</td>
<td>14</td>
<td>1</td>
<td>0</td>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>J. H.</td>
<td>52</td>
<td>F</td>
<td>Adrenal carcinoma</td>
<td>700,000</td>
<td>7</td>
<td>1</td>
<td>10</td>
<td>11.5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>V. B.</td>
<td>37</td>
<td>M</td>
<td>Osteogenic sarcoma</td>
<td>1,555,000</td>
<td>21</td>
<td>1</td>
<td>12</td>
<td>12</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>B. L.</td>
<td>15</td>
<td>M</td>
<td>AML</td>
<td>350,000</td>
<td>7</td>
<td>1</td>
<td>0</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>B. S.</td>
<td>37</td>
<td>F</td>
<td>AML</td>
<td>700,000</td>
<td>14</td>
<td>1</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>C. H.</td>
<td>23</td>
<td>F</td>
<td>AML</td>
<td>700,000</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>J. F.</td>
<td>26</td>
<td>F</td>
<td>AML</td>
<td>600,000</td>
<td>21</td>
<td>3</td>
<td>3</td>
<td>9</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>N. S.</td>
<td>5</td>
<td>M</td>
<td>AML</td>
<td>210,000</td>
<td>18</td>
<td>1</td>
<td>5</td>
<td>5</td>
<td>0</td>
<td>0</td>
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<td>Patient average</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4.1</td>
<td>8.1</td>
<td>0</td>
<td>0.50</td>
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<td>Controls</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>10.1</td>
<td>0</td>
<td>4.75</td>
</tr>
<tr>
<td>GLAT  (100 mg) intra-cutaneously</td>
<td>J. H.</td>
<td>52</td>
<td>F</td>
<td>Adrenal carcinoma</td>
<td>700,000</td>
<td>7</td>
<td>3</td>
<td>0</td>
<td>11.5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>V. B.</td>
<td>37</td>
<td>M</td>
<td>Osteogenic sarcoma</td>
<td>1,555,000</td>
<td>21</td>
<td>3</td>
<td>9</td>
<td>12</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>C. H.</td>
<td>23</td>
<td>F</td>
<td>AML</td>
<td>700,000</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>J. F.</td>
<td>26</td>
<td>F</td>
<td>AML</td>
<td>600,000</td>
<td>21</td>
<td>1</td>
<td>3</td>
<td>9</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>H. R.</td>
<td>34</td>
<td>M</td>
<td>AML</td>
<td>700,000</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>11</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>N. S.</td>
<td>5</td>
<td>M</td>
<td>AML</td>
<td>210,000</td>
<td>18</td>
<td>3</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Patient average</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<td>2.7</td>
<td>9.4</td>
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</tr>
<tr>
<td>Controls</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>6.6</td>
<td>0</td>
<td>0.44</td>
</tr>
</tbody>
</table>

\(a\)Day relative to initiation of L-asparaginase treatment.
\(b\)Skin test was done Day 21. Mean of 2 diameters at the right angle.

Six patients were immunized with 100 μg GLAT intra-cutaneously. Two out of 6 did not develop delayed hypersensitivity skin reactions when tested with 100 μg GLAT 3 weeks after immunization. Three developed minimal delayed hypersensitivity to GLAT and only 1 developed normal delayed sensitivity to this antigen. It is of interest that both patients who failed to develop any delayed hypersensitivity to GLAT were immunized on Day 3 of treatment. Overall, 3 out of 9 patients immunized on Day 1 showed a normal primary delayed hypersensitivity response, while only 1 out of 5 immunized on Day 3 showed such a response.

Three of the 6 patients did not develop hemagglutinating antibody to GLAT during the period of study. Production of antibody to GLAT in patients receiving L-asparaginase appeared to be delayed. Seven days after immunization none of 6 patients produced antibody, although it was produced by 22.2% of controls. Fourteen days after immunization none of the 6 patients produced antibody, while it was detected in 55.6% of controls. By Day 21 approximately 50% of both patient and control groups had antibody.

Absolute peripheral blood lymphocyte counts were within normal limits in all except 1 of these patients (N. S.), on both the day of immunization and the day of skin testing. In addition, 28 days of L-asparaginase treatment did not cause lymphopenia or monocytopenia in 4 hematologically normal nonlymphoid solid tumor patients (Table 2).

Serum proteins were measured in 7 of the patients (Table 3). A marked drop of total protein was observed. Mean total protein was 6.4 g/100 ml before L-asparaginase treatment and 5.1 g/100 ml during and shortly after L-asparaginase treatment. The decrease in serum protein was due mainly to a fall in albumin concentration; γ-globulin was not influenced. Mean value of γ-globulin was 1.1 g/100 ml before and 1.1 g/100 ml during treatment. Mean value of serum albumin was 3.4 g/100 ml before and 2.5 g/100 ml during treatment.

Immunoglobulin levels were measured in these patients twice a week while they were receiving L-asparaginase. There was no decrease in the levels of either IgG, IgA, or IgM during and shortly after L-asparaginase treatment. There was no significant difference in immunoglobulin levels between...
Effect of L-asparaginase on total leukocyte and lymphocyte counts

<table>
<thead>
<tr>
<th>Day</th>
<th>Leukocytes/cu mm</th>
<th>Lymphocytes/cu mm</th>
<th>Neutrophils/cu mm</th>
<th>Monocytes/cu mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>6375a (4000–8500)</td>
<td>1095 (610–2210)</td>
<td>4960 (3180–5865)</td>
<td>190 (80–305)</td>
</tr>
<tr>
<td>7</td>
<td>5600 (4300–7300)</td>
<td>1033 (511–1701)</td>
<td>4320 (3285–6426)</td>
<td>211 (146–450)</td>
</tr>
<tr>
<td>14</td>
<td>5325 (3700–6100)</td>
<td>1195 (778–1708)</td>
<td>3805 (2627–4814)</td>
<td>272 (0–488)</td>
</tr>
<tr>
<td>21</td>
<td>4972 (4090–5900)</td>
<td>937 (462–1475)</td>
<td>3560 (2509–4446)</td>
<td>219 (57–354)</td>
</tr>
<tr>
<td>28</td>
<td>5725 (3900–7900)</td>
<td>1123 (660–2133)</td>
<td>3920 (2691–5056)</td>
<td>185 (45–312)</td>
</tr>
</tbody>
</table>

aMean value of counts from 4 hematologically normal solid tumor patients receiving L-asparaginase.

Table 3

Effect of L-asparaginase on serum proteins (g/100 ml)

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Total protein (g/100 ml)</th>
<th>Albumin (g/100 ml)</th>
<th>γ-globulin (g/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-treatment</td>
<td>During treatment</td>
<td>Pre-treatment</td>
</tr>
<tr>
<td>1</td>
<td>7.1</td>
<td>5.8</td>
<td>4.4</td>
</tr>
<tr>
<td>2</td>
<td>7.0</td>
<td>5.0</td>
<td>3.8</td>
</tr>
<tr>
<td>3</td>
<td>6.7</td>
<td>5.2</td>
<td>3.4</td>
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<tr>
<td>4</td>
<td>6.6</td>
<td>4.5</td>
<td>3.3</td>
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<td>5</td>
<td>6.3</td>
<td>4.9</td>
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<td>5.8</td>
<td>5.6</td>
<td>2.8</td>
</tr>
<tr>
<td>7</td>
<td>5.6</td>
<td>4.4</td>
<td>2.7</td>
</tr>
<tr>
<td>Average</td>
<td>6.4</td>
<td>5.1</td>
<td>3.4</td>
</tr>
</tbody>
</table>

Effect of L-Asparaginase on Established Delayed Hypersensitivity. The effect of L-asparaginase on established delayed hypersensitivity was studied in 6 patients (2 solid tumors, 3 AML, and 1 ALL) receiving therapeutic doses of L-asparaginase (Table 4). Two days prior to initiation of L-asparaginase treatment, skin tests were performed on 1 forearm with candidin, dermatophytin, dermatophytin “O,” and Varidase. Each patient showed positive delayed hypersensitivity reaction to at least 2 of these antigens. Out of 22 tests, 18, or 81.8%, were positive at 24 and/or 48 hr after injection. The mean diameter of induration was 9.7 mm. Two days after initiation of daily L-asparaginase, 2 or 3 antigens which elicited positive reactions were injected on the opposite forearm. These skin tests were repeated at weekly interval on alternating forearms as long as the treatment continued. All of the 26 skin tests done in this

Table 4

Effect of L-asparaginase on established delayed hypersensitivity skin test

<table>
<thead>
<tr>
<th>No. of patient</th>
<th>No. of antigen</th>
<th>No. of skin test</th>
<th>No. of positive tests</th>
<th>Positive skin test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pretreatment</td>
<td>6</td>
<td>4</td>
<td>22</td>
<td>18</td>
</tr>
<tr>
<td>During treatment</td>
<td>6</td>
<td>4</td>
<td>26</td>
<td>26</td>
</tr>
</tbody>
</table>

Candidin, dermatophytin, dermatophytin “O,” and vaccinia were used as antigens. 

Mean value of positive skin tests.
manner elicited positive delayed hypersensitivity reactions at 24 and/or 48 hr after injection. The mean diameter of these positive skin tests was 9.2 mm. These results indicated that L-asparaginase had no effect on established delayed hypersensitivity.

Effect of Serum of Patients Receiving L-Asparaginase on Lymphocyte Blastogenesis. Peripheral blood lymphocytes were obtained from 2 healthy donors and 6 untreated hematologically normal nonlymphoid solid tumor patients. They were washed twice with Eagle's minimum essential medium containing 5% fetal bovine serum and cultured in media and various ratios of their own serum and the serum of patients receiving L-asparaginase. The sera from 3 patients undergoing daily L-asparaginase treatment were studied. One patient with breast carcinoma received 12,500 i.u./sq m L-asparaginase daily and 2 with acute leukemia received 25,000 i.u./sq m daily. These patients had no side effects of treatment at the time of study. Sera were obtained 30 min after the first injections and after 2 to 28 days of treatment, just before the daily injection.

All sera obtained in this manner inhibited the PHA-induced blastogenic responses of the cells of the 8 hematologically normal subjects (Chart 1). Washed PHA-stimulated lymphocytes incorporated 35,000 cpn/10^6 lymphocytes when cultured in 1 ml autochthonous serum. However, the same lymphocytes incorporated 0 cpn when cultured in 1 ml patients' serum. The inhibitory effect of patients' serum was dose dependent. Thymidine-^3^H uptake of PHA-stimulated normal lymphocytes was suppressed 69.9% by only 0.05 ml patients' serum. Pretreatment patients' serum was not inhibitory.

Blastogenesis of Lymphocytes from Patients Receiving L-Asparaginase. Lymphocytes from 3 nonlymphoid solid tumor patients were cultured before initiation of L-asparaginase and thereafter every week until the drug was discontinued. Before drug treatment, lymphocytes of each patient responded vigorously to PHA and specific antigens. Mean thymidine-^3^H uptake of PHA-stimulated lymphocytes was 52,300 cpn/10^6 lymphocytes. Mean thymidine-^3^H uptake of SLO-stimulated lymphocytes was 6,000 cpn. On Day 7 of L-asparaginase treatment, mean thymidine-^3^H uptake of PHA-stimulated lymphocytes was only 690 cpn/10^6 lymphocytes and mean thymidine-^3^H uptake of SLO-stimulated lymphocytes was 90 cpn/10^6 lymphocytes when the cells were cultured in 1 ml autochthonous serum. When the lymphocytes of these treated patients were washed and cultured in media containing 1 ml normal serum, including their own pretreatment serum, they responded to PHA or specific antigen stimulation at almost the normal level (Chart 2).

Chart 1. Inhibition of lymphocyte blastogenesis by sera of patients receiving L-asparaginase. Blastogenic response of normal lymphocytes to 0.05 ml PHA showed marked inhibition when as little as 10% of the serum in the culture (0.10 ml) was derived from a patient on L-asparaginase. The blastogenic response is plotted in cpn on the ordinate and the percentage of the serum in the culture (1-ml total) derived from the patient is plotted on the abscissa. The remainder of the serum in the culture was normal serum.

Chart 2. Lymphocyte blastogenesis in patients receiving L-asparaginase. Diminished blastogenic responses of the lymphocytes of patients on L-asparaginase were restored to normal levels when the cells were washed and cultured in normal serum. PHA dose, 0.05 ml. Antigens included SLO, streptokinase-streptodornase, or vaccinia. o, use of patients' pretreatment serum instead of normal serum.

In 1 patient who developed delayed hypersensitivity skin reactions to KLH and GLAT in spite of treatment, the lymphocytes responded to these antigens in vitro only when washed and cultured in media containing normal serum.
DISCUSSION

KLH is a strong primary antigen in man, producing both antibody and delayed hypersensitivity skin reactions in all immunized subjects (5, 30). Both 100 μg and 5 mg produce the same degree of immunity in man (5). Five out of 8 patients immunized with 2 mg KLH followed by therapeutic doses of L-asparaginase failed completely or partially to develop delayed hypersensitivity skin reactions when skin tested 3 weeks after immunization.

GLAT is also a good primary antigen in man (19). About 90% of the normal control subjects immunized with 100 μg GLAT developed delayed hypersensitivity skin reactions. Five of 6 patients immunized with 100 μg GLAT followed by therapeutic doses of L-asparaginase failed completely or partially to develop delayed hypersensitivity skin reactions when tested 3 weeks after immunization. Thus, considering the fact that L-asparaginase did not suppress established delayed hypersensitivity, it is likely that L-asparaginase was responsible for the complete or partial suppression of the development of new delayed hypersensitivity.

L-Asparaginase had no effect on established delayed hypersensitivity skin reactions. There are several possible explanations for the discrepancy. First, there is some evidence that L-asparaginase enters the extra vascular spaces poorly (12). Thus, it may be excluded from sites of delayed reactions. Second, protein synthesis and blastogenesis may not be necessary for the cellular events of delayed sensitivity. Thus, it would proceed even in the presence of effective concentrations of enzyme.

Primary antibody responses have been reported to be normal both in patients with acute leukemia (15, 27) and in patients with nonlymphoid solid tumors (9, 17, 28). They may be moderately diminished in patients with very widespread metastases (18). Essentially all control subjects immunized with KLH produced humoral antibody by 7 days after immunization. In contrast, 3 of 8 patients immunized with KLH followed by daily therapeutic doses of L-asparaginase did not produce antibody to KLH during the 21-day study period. Among 5 who did produce antibody, only 1 had a detectable titer at 7 days after immunization. Antibody titers of these patients were also lower than controls. The data suggest that L-asparaginase was the cause of this immunosuppressive effect. GLAT normally produced a weak primary antibody in healthy normal subjects. A delay in the appearance of antibody was the major effect of L-asparaginase treatment on the response to this antigen.

Immunoglobulin levels have been reported to be normal in AML and only IgA has been reported to be slightly diminished in ALL (20, 21). During L-asparaginase treatment, immunoglobulin levels remained essentially normal while the total protein levels were markedly decreased. The decrease was mainly due to decreased albumin.

Plasma levels of L-asparaginase can reach more than 10 i.u./ml after a single dose of 25,000 i.u./sq m i.v. (13). Since the half-life of L-asparaginase in plasma is 12 to 30 hr, the plasma level of the drug often reached more than 20 i.u./ml after 7 daily injections (12). Thus, sera from patients receiving L-asparaginase contain high concentrations of the enzyme. L-asparaginase is known to inhibit PHA-induced lymphocyte blastogenic responses completely when added to lymphocyte cultures at a concentration of 1 i.u./ml (22, 23). Therefore, it is evident that sera of patients receiving L-asparaginase should contain enough enzyme to inhibit PHA-induced blastogenesis strongly.

High blood ammonia levels, hyperglycemia, hyperlipemia, hypofibrinogenemia, hyperbilirubinemia, and elevated serum amylose levels have been reported to occur during L-asparaginase treatment (8, 32). None of 3 patients whose sera was inhibitory in these experiments showed the above side effects. Moreover, serum obtained 20 min after the first injection was already markedly inhibitory. Since L-asparaginase is known to be nontoxic to normal lymphocytes (2, 25) and did not decrease the number of circulating lymphocytes in the present study, the normal responses of washed patients' lymphocytes in normal serum are readily understood.

Impaired host defense mechanisms have been observed in patients with several types of malignant disease (10). Delayed hypersensitivity is normal in patients with untreated acute leukemia. It is normal in patients with solid tumors in good condition and impaired in patients with metastatic solid tumor in poor condition (14, 16).

These reports suggest that some of our study patients might have had impaired cellular immunity before treatment. However, the fact that all but 1 patient immunized with KLH and/or GLAT showed positive established delayed hypersensitivity to at least 1 of a battery of established delayed hypersensitivity antigens, and the fact that all but 1 patient had normal levels of circulating lymphocytes at both the time of immunization and the time of skin testing, suggest that these patients had normal cellular immunity. The normal blastogenic reactions of their lymphocytes when cultured in normal serum also indicates their immunocompetence.

It seems then that L-asparaginase can impair the primary immune response and the function of lymphocytes circulating in its presence. It had no effect on the expression of delayed hypersensitivity to a battery of antigens. Suppression of the immune response was partial rather than complete in most of the patients studied.

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