Influence of L-Asparaginase on Antibody Production and Growth of Tumors in Allogeneic Mice

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SUMMARY

The effect of L-asparaginase on immunosuppression in mice was studied with procedures measuring the ability to produce circulating antibodies against sheep erythrocytes and allogeneic tumor cells in the primary immune response in C57BL/Na X A/Jax F1 mice was markedly suppressed at a dose of 25 i.u. of L-asparaginase/mouse, and the degree of the immunosuppression was just parallel to the dose of enzyme administered. On the contrary, the enzyme failed to suppress the secondary immune response to sheep erythrocytes. The production of cytotoxic antibody in mice bearing allogeneic tumors was also depressed by the enzyme treatment; however, the duration of the immunosuppression was temporary. Successful tumor allograft was observed with enzyme treatment in the systems of C57BL/Na versus L1210 and CBA/Na versus EL4. The minimum effective dose for allogeneic tumor take was 1000 i.u. of L-asparaginase/mouse for a system of C57BL/Na versus L1210 and 200 i.u. for a system of CBA/Na versus EL4. Tumor allograft in a system of C57BL/Na versus 6C3HED-OG or -RG was regressed at a dose of 800 to 1000 i.u. of L-asparaginase. Histological findings revealed that the cells associated with immune reactions were scarce in the tumor-grafted area and ascites of the enzyme-treated mice, as compared to those of the 0.9% NaCl solution-treated mice. These results indicate that L-asparaginase acts as one of the potent immunosuppressive agents. The mechanisms of immunosuppression by the enzyme are discussed.

INTRODUCTION

Many investigators have reported that L-asparaginase inhibits tumor growth in the mouse (4—7, 17), rat (18), dog (27), and human (13, 25) through the inhibition or deletion of tumor-specific metabolism relating to L-asparagine. Since L-asparagine is synthesized by asparagine synthetase from L-aspartic acid, L-glutamine, ATP, and Mg2+ in mammalian cells (19, 28), the deprivation of exogenous L-asparagine does not affect the metabolism of normal mammalian cells.

With increasing evidence of the significant role played by immunity in the control of cancer, it becomes very desirable for the antineoplastic agent to satisfy the prerequisite that it should be devoid of immunosuppression and yet effective against cancer. In all senses, it has been considered that L-asparaginase might belong to a new class of therapeutic agents for cancers just compatible with the above prerequisite. However, several investigators have reported that this enzyme inhibited DNA and RNA synthesis in regenerating rat liver (2, 3) and PHA1-induced lymphocyte blastogenesis (23, 26) and that the patient treated with the enzyme tended to suffer from such toxicities as liver damage, hypocholesteremia, coagulopathy, hypoproteinemia, etc. (10, 12). These reports imply that the enzyme itself might have an action directed against the metabolism of certain normal cells through the deprivation of exogenous L-asparagine.

The present experiment is undertaken to investigate whether or not L-asparaginase acts as an immunosuppressive agent and, furthermore, to clarify the mode of action of the enzyme on certain normal cells with the use of a system of tumor-host drug relationship.

MATERIALS AND METHODS

Mice. Mice of strains CBA/Na (H-2d), C57BL/Na (H-2b), C57BL/Na × A/Jax F1, and BDF1 were used. CBA/Na, C57BL/Na, and A/Jax were maintained by single line in the breeding laboratory of Nagoya University School of Medicine. BDF1 mice were supplied from Sanyo Seiyaku Co., Ltd., Tokyo, Japan.

Tumors. The following ascitic leukemias were used: EL4 (histocompatibility H-2d, L-asparaginase-resistant, and L-asparagine-independent tumor) (M. Miura, unpublished observation); L1210 (histocompatibility H-2d, L-asparaginase-resistant and L-asparagine-independent tumor) (M. Miura, unpublished observation); 6C3HED-RG1 (histocompatibility H-2k, L-asparaginase-resistant and L-asparagine-independent tumor) (7); and 6C3HED-OG (histocompatibility H-2k, L-asparaginase-sensitive and L-asparagine-dependent tumor) (21, 33). EL4, L1210, 6C3HED-OG, and 6C3HED-RG1 were maintained through serial transfers in C57BL/Na, BDF1, CBA/Na, and CBA/Na mice, respectively.

Titration of Anti-SE Hemolysin. SE (Kyokuto Sango Co., Ltd., Tokyo, Japan) were washed 3 times with 0.9% NaCl solution and then adjusted to a 2% suspension. Mice were immunized i.p. with 0.3 ml of this suspension and bled on Day 3 or 4 after immunization. Sera obtained were inactivated for 30 min at 56°, titrated for hemolysin, serially diluted (0.3 ml), and then incubated with SE (0.1 ml of a 2% suspension) and

1 The abbreviations used are: PHA, phytohemagglutinin; SE, sheep erythrocytes.

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guinea pig serum (0.1 ml of a 10% guinea pig serum). After incubation for 2 hr at 37°, the determination of hemolysin titers was made. Titers referred to the highest dilution of the serum showing complete hemolysis and were expressed as \( \log_2 S^{-1} \) of the dilutions. If a titer lay between \( n \) and \( n + 1 \) (\( n \), an integral number induced from \( \log_2 S^{-1} \) of the dilutions), it was expressed as \( n + 0.5 \). For this test, lyophilized commercial guinea pig serum (Kyokuto Sangyo Co., Ltd.) was used as a source of complement.

**Cytotoxic Test.** The test procedure was based on the method of Gorer and O’Gorman (10). Briefly, equal volumes (0.05 ml) of tumor cells (5 \( \times 10^6/ml \)), serum dilution, and pooled guinea pig serum diluted 1:3 as a source of complement were incubated for 45 min at 37°. The pooled guinea pig serum was previously absorbed in the cold with mouse ascitic tumor cells, 6C3HED-OG or EL4, in a ratio of 9 parts serum to 1 part packed, washed tumor cells to remove natural heteroantibody.) Viability of cells (percentage) was determined microscopically after the addition of freshly prepared 0.2% trypan blue solution. Titers referred to the dilution of the serum in which the proportion of dead cells (stained) was nearest at 50% and were expressed as \( \log_2 2^{-1} \) of the dilutions. If a titer lay between \( n \) and \( n + 1 \), the same calculation as that for the hemolysin titer was made.

**Tumor Allograft Procedure.** The allograft procedure consisted of the s.c. injection of 1 \( \times 10^7 \) tumor cells (0.2 ml) into the shorn back of each mouse. Tumor growth was followed by measurement of the longest and the shortest diameters, and size was expressed as a mean of both tumor diameters.

**L-Asparaginase.** *Escherichia coli* L-asparaginase (KW 0-20-1, Lot No. 101-116; specific activity 288 to 300 i.u./mg of protein), obtained from Kyowa Hakko Co., Ltd., Tokyo, Japan, was highly purified and devoid of contaminants of bacterial origin. The enzyme was dissolved with 0.9% NaCl solution before use. Mice were given this enzyme i.p., either simultaneously with, before, or after tumor inoculation. The enzyme was dissolved with 0.9% NaCl solution before use. Mice were given this enzyme i.p., either simultaneously with, before, or after tumor inoculation.

**Effect of L-Asparaginase on Primary Immune Response to SE in Mice.** As shown in Table 1, C57BL/Na mice treated with more than 200 i.u. of L-asparaginase/mouse produced anti-SE with a titer of less than 2 (range, <2), while 0.9% NaCl solution-treated control mice produced much higher anti-SE, with a mean titer of 6.6 (range, 5 to 8).

The immunosuppressive dose of the enzyme was determined with C57BL/Na X A/Jax F1 mice immunized with SE. As indicated in Experiment 2, inhibition of anti-SE production was obtained at a dose of 25 i.u. of L-asparaginase/mouse. The degree of suppression of anti-SE production was just parallel to the dose of enzyme administered.

**Effect of L-Asparaginase on the Secondary Immune Response to SE in Mice.** C57BL/Na mice previously immunized with SE were used. As shown in Table 2, 200 or 1000 i.u. of L-asparaginase in either single or divided doses caused no inhibition of anti-SE production on Day 3 after rechallenge with SE.

**RESULTS**

**Effect of L-Asparaginase on Primary Immune Response to SE in Mice.** As shown in Chart 1A, the EL4 tumor growth in the CBA/Na mice treated with the enzyme was somewhat slower initially than that in 0.9% NaCl solution-treated ones. However, in the enzyme-treated group, 50.0% tumor take (4 out of 8 mice) was observed and, in the surviving mice, the survival of tumor graft was prolonged compared with that in 0.9% NaCl solution-treated mice. Chart 1B showed the L1210 tumor growth curve in C57BL/Na mice. In either the enzyme-treated or 0.9% NaCl solution-treated group, tumor take was not observed in any of the mice treated with the enzyme.

**Effect of L-Asparaginase on the Tumor Growth in Allogeneic Mice (Table 3).** L-Asparaginase-resistant 6C3HED-RGI, L1210, and EL4 tumors were grafted to histoincompatible mice, C57BL/Na, and CBA/Na, respectively. Allogeneic tumor take was observed in C57BL/Na mice grafted with L1210 and CBA/Na mice grafted with EL4 when mice were treated with more than 200 i.u. of L-asparaginase.

With treatment of 200 i.u. in a single dose, 600 i.u. in 3 divided doses, and 800 i.u. in 4 divided doses, the tumor take was observed at 16.6, 50.0 and 50.0% of CBA/Na mice given transplants of EL4, respectively. In the system of L1210 versus C57BL/Na, more than 1000 i.u. of L-asparaginase in either single or divided doses were necessary for the successful tumor allograft. Eight hundred i.u. of L-asparaginase treatment in 4 divided doses caused no tumor take in the system of 6C3HED-RGI versus C57BL/Na. In C57BL/Na mice inoculated with 6C3HED-OG tumor cells, no tumor take was obtained with 1000 i.u. of L-asparaginase in 5 divided doses.

**Effect of L-Asparaginase on EL4 Tumor Growth in the Allogeneic C57BL/Na Mice Preimmunized with the Same Tumor.** A total of 32 mice preimmunized with EL4 cells (1 \( \times 10^7 \)) were rechallenged with the s.c. inoculation of the same tumor cells (1 \( \times 10^7 \)). These mice were given L-asparaginase i.p. at various dosages from 200 to 1000 i.u./mouse simultaneously with the tumor inoculation. As shown in Table 4, no tumor take was observed in any of the mice treated with the enzyme.

**Comparison of the Allogeneic Tumor Growth between L-Asparaginase-treated and 0.9% NaCl Solution-treated Mice.** As shown in Chart 1A, the EL4 tumor growth in the CBA/Na mice treated with the enzyme was somewhat slower initially than that in 0.9% NaCl solution-treated ones. However, in the enzyme-treated group, 50.0% tumor take (4 out of 8 mice) was observed and, in the surviving mice, the survival of tumor graft was prolonged compared with that in 0.9% NaCl solution-treated mice. Chart 1B showed the 6C3HED-OG tumor growth curve in C57BL/Na mice. In either the enzyme-treated or 0.9% NaCl solution-treated group, tumor take was not achieved. In the enzyme-treated ones, no tumor growth was seen during the observation period.

**Effect of L-Asparaginase on the Capability of Production of Cytotoxic Antibody in Mice.** In the systems of EL4 versus CBA/Na and L1210 versus C57BL/Na, none of the mice treated with the enzyme produced detectable cytotoxic antibody on Day 12 after tumor inoculation, while 0.9% NaCl solution-treated control mice produced the antibody with mean titers of 3.1 (range, 1.5 to 5.0) and 3.9 (range, 2.5 to 4.5), respectively.

However, on Day 18 after tumor inoculation, a mean anti-SE titer of 3.1 (range, 1.5 to 5.0) and 3.9 (range, 2.5 to 4.5) for L1210-bearing C57BL/Na mice, respectively.
Table 1

Effect of L-asparaginase on primary immune response to SE

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Strain of mice</th>
<th>No. of mice</th>
<th>Treatment</th>
<th>Titer of anti-SEa</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C57BL/Na</td>
<td>7</td>
<td>0.9% NaCl solution (0.2 ml i.p. × 5)b</td>
<td>5, 6, 6, 7, 7, 8 (6.6)</td>
</tr>
<tr>
<td>9</td>
<td>L-Asparaginase (200 i.u. i.p. × 5)b</td>
<td>&lt;2, &lt;2, &lt;2, &lt;2, &lt;2, &lt;2 (2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>L-Asparaginase (200 i.u. i.p. × 1)c</td>
<td>&lt;2, &lt;2, &lt;2, &lt;2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>C57BL/Na × A/J F1</td>
<td>5</td>
<td>0.9% NaCl solution (0.2 ml i.p. × 1)c</td>
<td>6, 7, 7, 8 (6.8)</td>
</tr>
<tr>
<td>5</td>
<td>L-Asparaginase (5 i.u. i.p. × 1)c</td>
<td>6, 7, 7, 8 (7.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>L-Asparaginase (25 i.u. i.p. × 1)c</td>
<td>3, 4, 5, 5, 5 (4.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>L-Asparaginase (50 i.u. i.p. × 1)c</td>
<td>2, 3, 4, 4 (3.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>L-Asparaginase (100 i.u. i.p. × 1)c</td>
<td>0, 1, 2, 4 (2.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>L-Asparaginase (200 i.u. i.p. × 1)c</td>
<td>0, 0, 0, 1, 2 (0.6)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Antisera tested were obtained on Day 4 after the immunization of a 2% SE suspension. Titers were expressed as log10 5 of dilutions. Values in parentheses, mean.
b L-Asparaginase was administered i.p. 5 times before and after the immunization of SE suspension (on Days —2, —1, 0, +1, and +2).
c L-Asparaginase was given concurrently with the immunization of SE suspension.

Table 2

Effect of L-asparaginase on secondary immune response to SE in C57BL/Na mice

<table>
<thead>
<tr>
<th>No. of mice</th>
<th>Treatment</th>
<th>Titer of anti-SEa</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>L-Asparaginase (200 i.u. i.p. × 5)b</td>
<td>8, 8, 8, 9, 10 (8.6)</td>
</tr>
<tr>
<td>5</td>
<td>L-Asparaginase (1000 i.u. i.p. × 1)c</td>
<td>8, 8, 8, 9 (8.2)</td>
</tr>
<tr>
<td>5</td>
<td>L-Asparaginase (200 i.u. i.p. × 1)c</td>
<td>7, 7, 8, 9, 10 (8.2)</td>
</tr>
<tr>
<td>5</td>
<td>0.9% NaCl solution (0.2 ml i.p. × 5)b</td>
<td>8, 8, 8, 9, 9 (8.4)</td>
</tr>
</tbody>
</table>

a Antisera tested were obtained on Day 3 after the secondary immunization of 0.3 ml of a 2% SE suspension. Titers were expressed as log10 5 of dilutions. Values in parentheses, mean.
b L-Asparaginase was administered i.p. 5 times before and after the immunization of SE suspension (on Days —2, —1, 0, +1, and +2).
c L-Asparaginase was given concurrently with the secondary immunization of SE suspension.

treatment with the enzyme inhibited the production of detectable antibody in C57BL/Na mice bearing 6C3HED-OG on Days 8, 12, and 18 after tumor inoculation.

Comparison of Histopathological Findings on Immune Reactions between L-Asparaginase-treated and 0.9% NaCl Solution-treated Mice. Histopathological examination was carried out to find the difference of the host reactions against the allogeneic tumor (EL4) between L-Asparaginase-treated and 0.9% NaCl solution-treated mice. As shown in Figs. 1 to 8, extensive tumor development in the inoculated s.c. area, invasion of tumor to adjacent tissues (muscle), and ascites consisting of tumor cells were detected in the mice treated with the enzyme. On the contrary, in the 0.9% NaCl solution-treated ones, necrosis of tumor cells and infiltration

Table 3

Effect of L-asparaginase on tumor growth in allogeneic mice

<table>
<thead>
<tr>
<th>Tumor grafted</th>
<th>Recipient mice</th>
<th>L-Asparaginase treated</th>
<th>0.9% NaCl solution treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>6C3HED-RGI</td>
<td>C57BL/Na (H-2b)</td>
<td>0/11 (200 i.u. i.p. × 4)b</td>
<td>0/10</td>
</tr>
<tr>
<td>6C3HED-OG</td>
<td>C57BL/Na (H-2b)</td>
<td>0/6 (200 i.u. i.p. × 5)b</td>
<td>0/10</td>
</tr>
<tr>
<td>L1210</td>
<td>C57BL/Na (H-2b)</td>
<td>0/6 (200 i.u. i.p. × 4)b</td>
<td>0/6</td>
</tr>
<tr>
<td>EL4</td>
<td>CBA/Na (H-2b)</td>
<td>4/8 (200 i.u. i.p. × 4)b</td>
<td>0/16</td>
</tr>
<tr>
<td>1 × 10^7 cells, s.c.</td>
<td>3/6 (200 i.u. i.p. × 3)c</td>
<td>1/6 (200 i.u. i.p. × 1)d</td>
<td>0/4 (100 i.u. i.p. × 1)</td>
</tr>
<tr>
<td>1 × 10^7 cells, s.c.</td>
<td>0/4 (50 i.u. i.p. × 1)c</td>
<td>0/4 (50 i.u. i.p. × 1)c</td>
<td>0/4 (50 i.u. i.p. × 1)c</td>
</tr>
</tbody>
</table>

a L-Asparaginase given on Days —4, —3, —2, and —1.
b L-Asparaginase given on Days —4, —3, —2, —1, 0, +1, and +2.
c L-Asparaginase given concurrently with tumor graft.
d L-Asparaginase given on Days —1, +3, and +7.
Treatment No. of mice with progressive tumor growth/No. of mice tested

<table>
<thead>
<tr>
<th>Treatment</th>
<th>0/3</th>
<th>0/2</th>
<th>0/3</th>
<th>0/2</th>
<th>0/6</th>
<th>0/6</th>
<th>0/6</th>
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<tbody>
<tr>
<td>0.9% NaCl solution (0.2 ml i.p.)</td>
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<td>L-Asparaginase (200 i.u. i.p. X 1)</td>
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<tr>
<td>L-Asparaginase (200 i.u. i.p. X 1)</td>
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<tr>
<td>L-Asparaginase (200 i.u. i.p. X 4)</td>
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<tr>
<td>L-Asparaginase (200 i.u. s.c. X 4)</td>
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</tr>
<tr>
<td>L-Asparaginase (1000 i.u. i.p. X 1)</td>
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<tr>
<td>L-Asparaginase (1000 i.u. s.c. X 1)</td>
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</tbody>
</table>

a L-Asparaginase was given concurrently with the secondary homograft of EL4.

b L-Asparaginase was administered i.p. or s.c. 4 times (on Days -4, -3, -2, and -1) before the secondary homograft of EL4.

Table 4
Influence of L-asparaginase on the growth of EL4 tumor in EL4-preimmunized (1 x 10^6 cells, s.c.) CBA/Na mice

The experiment revealed that L-asparaginase possessed immunosuppressive action. A dosage of L-asparaginase was more than 25 i.u./mouse for the suppression of anti-SE production and more than 200 i.u. for the allogeneic tumor take. On the contrary, a single injection of 4 i.u. of L-asparaginase/mouse brought about a strong inhibition of the growth of sensitive tumors, 6C3HED-OG (33) and EARADI (4) in vivo. Thus, the ratio of tumor-effective dose to immunosuppressive dose is approximately 1:6 for the suppression of anti-SE production and 1:50 for the successful tumor allograft. Therefore, it can be concluded that there is a great difference between immunosuppressive dose and tumor effective dose of L-asparaginase.

Considering the fact that L-asparaginase could inhibit the primary immune response to SE but could not inhibit the secondary one to SE, the immunocompetent cells might be required for exogenous L-asparagine in the primary immune process but not in the secondary one. In other words, these cells might have insufficient asparagine synthetase activity for the production of endogenous L-asparagine necessary for antibody synthesis or the cell proliferation in the primary immune process but sufficient enzyme activity for it in the secondary immune process. The report of Horowitz et al. (15) that asparagine synthetase activity was low in nontreated mouse spleen and lymph node cells may support the above speculation.

As demonstrated in Chart 2, the ability to produce cytotoxic antibody was partially recovered in surviving C57BL/Na mice bearing L1210 tumor and CBA/Na mice bearing EL4 tumor on Day 18 after the cessation of the enzyme treatment. It might therefore be possible to say that the inhibitory effect of the enzyme on cytotoxic antibody production was temporary in these mice.

Cytotoxic antibody could not be detected on Days 8, 12, and 18 after the cessation of the enzyme treatment in C57BL/Na mice bearing 6C3HED-OG tumor. The difference of the immune response of C57BL/Na bearing 6C3HED-OG tumor from that of the other 2 systems, C57BL/Na versus L1210 and CBA/Na versus EL4, in the enzyme-treated condition can be understood, in part, by the following explanation: the L-asparaginase-sensitive tumor, 6C3HED-OG, was killed by the massive dose of the enzyme and, consequently, the intact tumor cells necessary for immunization could not remain in the process of immunization, while L-asparaginase-resistant L1210 and EL4 tumors could grow until establishment of immunization and enough intact tumor cells could be supplied as an antigen for immunization. There are, of course, many factors affecting antibody production, such as antigenicity or antigen content of tumor and host immunological capability, and so on.

The following mechanisms can be considered concerning the inhibitory action of L-asparaginase on antibody formation.

Inhibition of Immunoglobulin Biosynthesis. The known metabolic pathways of L-asparagine are its utilization for protein synthesis and its conversion to aspartate by hydrolysis or to a ketosuccinamic acid by transamination (20). Thus, it is clear that L-asparagine is one of the important amino acids for
protein synthesis. A fall of serum albumin level and various coagulation factors in the sera of the patients treated with the enzyme might be due to the block of biosynthesis of these substances under the lack of exogenous L-asparagine (11).

The suppression of antibody production by L-asparaginase is partially understandable because of the block of immunoglobulin biosynthesis under such a condition. It remains unsolved, however, as to whether or not the lack of exogenous L-asparagine induced by the enzyme is solely responsible for these phenomena. Campbell et al. (8) reported that L-asparaginase from E. coli had small but definite glutaminase activity. Miller et al. (22) confirmed the presence of glutaminase activity in the enzyme and found that the sera of leukemic patients during the enzyme treatment contained neither L-asparaginase nor L-glutamine. The glutaminase activity of our particular samples was about 4% of the L-asparaginase activity. This glutaminase activity might serve as one of the immunosuppressive factors because glutamine is also one of the important amino acids for synthesizing of protein and nucleic acids.

**Suppression of the Proliferation of the Immunocompetent Cells.** It has been demonstrated (T. Uetani and M. Miura, unpublished observations) that mice immunized with SE by the enzyme treatment produced a smaller number of antibody-forming cells and spleen-nucleated cells than those immunized by the 0.9% NaCl solution treatment. Tsutsui (32) and other investigators (1, 9, 14, 29, 31) reported that the transformed lymphocyte induced by PHA in vitro had morphological and functional characteristics similar to those of the immunoblast or lymphogonia found in lymph node, spleen, or peripheral blood in the animals stimulated by an antigen. Miura et al. (23) clearly demonstrated that PHA-transformed lymphocyte was L-asparaginase sensitive and, if the enzyme were put into lymphocyte culture with PHA, blastic transformation was completely suppressed with the inhibition of DNA and RNA synthesis. These reports support a hypothesis that L-asparaginase inhibits the proliferation of immunocompetent cells primarily stimulated by an antigen and, consequently, brings about immunosuppression.

The results on the augmented growth of the tumor in allogeneic mice treated with the enzyme suggest that the enzyme inhibits not only the production of circulating antibody but also cellular immunological reactivity. The histological examination also provided clear evidence on the suppression of cellular immunological reactions by the enzyme treatment.

It might be concluded, therefore, that immunosuppressive action of L-asparaginase results from inhibition of the proliferation of immunocompetent cells in addition to the block of immunoglobulin biosynthesis under the lack of exogenous L-asparagine.

**ADDENDUM**

Since this paper was contributed on July 23, 1969, 3 papers concerning the immunosuppressive action of L-asparaginase have been presented by other investigators (16, 24, 30).

**REFERENCES**

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**Chart 2. Effect of L-asparaginase on the production of cytotoxic antibody in mice bearing allogeneic tumors.** Numbers on the curves of cytotoxic antibody titers indicate the number of mice tested. **Vertical bars** and range of cytotoxic antibody titers in each group; L-Asp., L-asparaginase; Saline, 0.9% NaCl solution.
L-Asparaginase Effect on Antibodies and Tumor Growth


Influence of l-Asparaginase on Antibody Production and Growth of Tumors in Allogeneic Mice

Moriji Miura, Kohei Kawashima, Tadaaki Uetani, et al.

Cancer Res 1971;31:114-121.

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