Effects of L-Asparaginase on Lymphocyte Surface and Blastogenesis

Isaiah J. Fidler and Paul C. Montgomery
School of Dental Medicine and Center for Oral Health Research, University of Pennsylvania, Philadelphia, Pennsylvania 19104

SUMMARY

L-Asparaginase is immunosuppressive, inhibiting both the cellular and humoral responses, as well as the concanavalin A- and/or phytohemagglutinin-induced blastogenesis of lymphocytes. However, its mechanism of action is presently unclear. This study dealt with the enzyme effects on the lymphocyte surface leading to interference with the binding of concanavalin A. The mitogen was labeled with $^{125}$I and added to lymphocyte cultures prior to or following incubation with L-asparaginase.

The data permitted the following conclusions. L-Asparaginase treatment prior to introduction of mitogen reduced the capacity of the lymphocyte to bind concanavalin A. Enzymatic treatment of lymphocytes 90 min after the introduction of mitogen did not influence this binding. Addition of L-asparaginase, L-glutamine, and aspartic acid did not reverse the inhibitory effects of the enzyme. The decrease in mitogen binding (20%) led to a 95% decrease in subsequent DNA synthesis. Mitogen binding to receptors on lymphocytes precedes their subsequent stimulation. It appears that the decrease of mitogen binding by L-asparaginase results from an alteration on the lymphocyte surface. Any such alteration by the enzyme could account for its immunosuppressive activity.

INTRODUCTION

L-Asparaginase is a chemotherapeutic agent which has been used to treat various neoplasms in man and animals (for a review, see Ref. 12). Initially, it was believed that the enzyme might act only on malignant cells which are asparagine dependent. However, extended clinical investigations have indicated that L-asparaginase may have toxic effects on normal tissue. Embryotoxic effects of L-asparaginase (1) as well as inhibition of the early mitosis of regenerating liver in rats have been reported (7).

L-Asparaginase is immunosuppressive, inhibiting graft-versus-host reaction (18), skin graft rejection (10, 13, 17, 24), and antibody production (8, 11, 28, 31), as well as immune reactivity to an allografted tumor (30). The enzyme has been reported to enhance experimental metastasis, an effect lasting for 48 hr (14, 15). Moreover, the enzyme suppresses the PHA-induced blastogenesis of human lymphocytes (3, 4, 21, 36).

Several hypotheses have been suggested to account for the immunosuppressive effects of L-asparaginase. The most favored explanation has been that the depletion of L-asparagine from the culture media brings about this inhibitory effect (26). Since all preparations of L-asparaginase contain L-glutaminase activity, a glutamine depletion has been suggested as another possible cause of immunosuppression (10, 26). However, addition of L-asparagine or L-glutamine to the culture media does not reverse L-asparaginase-induced inhibition (32). It would appear that the inhibitory mechanisms are in fact more complex than depletion phenomena.

Recently, it has been reported that L-asparaginase inhibits the synthesis of membrane glycoproteins as well as bringing about cleavage of these subunits from the cell surface. Rapid cell death in sensitive leukemic cells might well be attributed to such cell membrane rupture (9). Since immunoglobulins are glycoproteins and function as antigen receptor sites on certain lymphocytes, it is possible that some of the immunosuppressive effects of the enzyme could be accounted for by its action on cell surface glycoproteins.

Similarly, L-asparaginase might interfere with binding of mitogens. Therefore, the present studies were undertaken to determine the relationship between the inhibition by L-asparaginase of initial binding of Con A to the lymphoid cell surface and subsequent blastogenesis (27, 34). This relationship was examined in an attempt to clarify and define a possible mechanism for the immunosuppressive effects of L-asparaginase.

MATERIALS AND METHODS

Animals. Fischer rats were used throughout these experiments.

Con A. Con A (3 times crystallized) was obtained as lyophilized powder from Miles Yeda, Ltd., Israel.

$^{125}$I-Labeled Con A. Con A was dissolved in phosphate-buffered saline, pH 7.4. The concentration was determined spectrophotometrically at 280 nm with $E_{1^\%}^{1^\text{cm}} = 12.9$ (27). Iodination was carried out by modifying the method of Hunter and Greenwood (20). Eight hundred $\mu$Ci of a 1-mg/ml solution of Con A in phosphate-buffered saline were added to 1000 $\mu$Ci $^{125}$I (NEZ-033H, sodium iodide in 0.1 M NaOH, reductant free, New England Nuclear, Boston, Mass.). The iodination was accomplished by the addition of 100 $\mu$Ci of

The abbreviations used are: PHA, phytohemagglutinin; Con A, concanavalin A; TCA, trichloroacetic acid; HBSS, Hank's balanced salt solution; MEM, Eagle's minimal essential medium; UdR-$^{13}$H, 5-iodo-2'-deoxyuridine-$^{13}$H.
chloramine-T (2.4 mg/ml, H2O). After 5 min, the reaction was terminated by the addition of 200 μl of sodium bisulfite (2.4 mg/ml, H2O). The labeled Con A was separated from other reaction products by chromatography on Sephadex G-25, dialyzed exhaustively, and concentrated by vacuum dialysis.

**L-Asparaginase.** The enzyme used was *Escherichia coli* L-asparaginase supplied by Merck, Sharp and Dohme Research Laboratories, West Point, Pa. One lot number (C-8378) was used for all experiments. L-Asparaginase solutions were prepared so that 0.1 ml added to cultures gave a final concentration of 0.1, 1.0, and 10 i.u./ml culture.

**125I-labeled L-Asparaginase.** L-Asparaginase, 10,000 i.u., was dissolved in 1.0 ml of water and dialyzed against 0.01 M phosphate buffer, pH 7.4. The iodination was carried out with 500 μCl 125I, and the protocol was identical to that described earlier for the Con A. Labeled asparaginase was separated from the other reaction products by Sephadex G-25 chromatography. Unbound iodine was removed by exhaustive dialysis, and concentration was carried out by negative pressure dialysis. Both labeled and unlabeled L-asparaginase preparations were characterized by isoelectric focusing.

**125I Counting.** A Packard Model 5219 automatic γ counter was used to determine the 125I radioactivity. All samples were counted 3 times for 5 min.

**Isoelectric Focusing Analysis.** Aliquots (10 to 50 μl containing 200 to 800 μg) of each sample were applied to thin-layer, 4 to 5% polyacrylamide gels containing ampholine carrier ampholytes (pH 3 to 10, LKB). The details of the thin-layer method have been described previously (5, 24). Once focusing had been achieved, pH gradients were measured, and the gels were soaked in 10% TCA. Protein bands were stained with 0.1% Coomassie blue, the gels were dried, and contact autoradiographs were made to visualize iodinated samples (23, 37).

**Preparation of Lymphoid Cultures.** Peripheral blood lymphocyte cultures were prepared according to the method of Wilson (38). Blood from rats weighing 200 to 300 g was collected by cardiac puncture with heparinized syringes. Following sedimentation with dextran-sodium citrate, the leukocyte-rich plasma was collected and washed twice with HBSS. Cultures were set up in 13-× 100-mm glass culture tubes and contained 106 lymphocytes/ml of MEM supplemented with sodium pyruvate, vitamin solution, L-glutamine, and 5% fetal calf serum. The cultures were incubated at 37° in 5% CO2 and air.

**Lymph Node Cells.** In some experiments, lymph node cells were used. Rats were exsanguinated as described previously. Their peripheral and mesenteric lymph nodes were removed aseptically, minced, and passed through a 70 mesh stainless steel sieve into cold HBSS. After passage through a glass wool column, the cells were washed twice and then suspended in MEM supplemented as described earlier. Cultures containing 106 lymphocytes/ml were set up in tubes and incubated at 37° in 5% CO2 and air.

**Con A-125I**

**Lymphocyte Binding Studies.** A study of the initial binding of Con A-125I to lymphoid cell surface was performed with a given number of cells suspended in 1.0 ml of supplemented MEM. Binding was routinely carried out at 37° for 90 min, and the lymphocytes were then washed 3 times with HBSS. The amount of 125I radioactivity in the final pellet was determined.

**Inhibition of Mitogen Binding with L-Asparaginase.** For study of the L-asparaginase interference with mitogen binding to lymphocyte surface, the following studies were performed. Lymphocyte cultures (peripheral and/or lymph node) were set up as described previously. These cultures were divided into 3 experimental groups: (a) Cultures receiving 10 i.u. L-asparaginase; (b) cultures receiving Con A-125I (specific activity, 0.02 to 0.05 μCi/μg); and (c) no additives. All cultures were incubated for 90 min and then treated as follows: (a) Con A-125I; (b) 10 i.u. L-asparaginase; and (c) Con A-125I. The cultures were incubated for another 90 min, centrifuged, and washed 3 times with HBSS; the final cell pellet was monitored for radioactivity in a γ-counter. Each culture was counted 3 times for 5 min. The amount of radioactive activity in control cultures (no enzyme treatment) was compared to that in cultures treated before or after addition of radioactive mitogen.

**Initial Mitogen Binding Correlated to Subsequent DNA Synthesis of Cultured Lymphocytes.** For correlation of the inhibitory effects of L-asparaginase on initial binding of mitogen and subsequent DNA synthesis (blastogenesis), the following study was performed. Lymphocyte cultures were divided into 2 groups: Group A was incubated with 10 i.u./ml/106 lymphocytes (as described previously), and Group B served as untreated control. Following 60 min incubation with the enzyme, all cultures were centrifuged at 1000 rpm for 10 min. Supernatants containing the enzyme or control media were discarded, and the cultures were refed with media containing 1.0 μg Con A-125I per ml (specific activity, 0.02 μCi/μg). After 1 hr all cultures were again centrifuged, supernatants were discarded, and the cellular pellets were resuspended in fresh media and incubated at 37° in 5% CO2 for 4 days. On the 4th day, 0.5 μCi UdR-125I (New England Nuclear) per ml (specific activity, 102 mCi/μM) was added to 15 tubes in each group while the other 15 tubes received only Con A-125I and served as controls. After 4 hr the cultures were centrifuged at 2000 rpm for 10 min and washed 3 times with HBSS; the final cellular pellet was monitored for 125I radioactivity. The 125I counts in cultures receiving only Con A-125I were subtracted from the total counts representing both Con A-125I and UdR-125I, thus determining the net incorporation of UdR-125I. UdR-125I, an analog of thymidine, is specifically incorporated into the DNA of the cell. It allows for easy sample preparation and easy counting of radioactivity as described by Surianu et al. (35).

**Lymphocyte Binding Assay.** For determination of possible binding of L-asparaginase to the lymphocyte surface, the following experiment was performed. Peripheral and nodal lymphocyte cultures were prepared as described previously. All cultures were divided into 3 experimental groups treated sequentially as follows. Group A was incubated for 60 min with 20 i.u. of L-asparaginase-125I (specific activity, 0.05 μCi/i.u.). After 60 min the cultures were centrifuged at 2000 rpm for 8 min, and the supernatant was removed and added to cultures of Group B. These were now incubated for 60 min. Following incubation the process was repeated, and the
Isaiah J. Fidler and Paul C. Montgomery

supernatant from Group B was now added to cultures of Group C. After 1 hr more, Group C cultures were centrifuged, and the supernatant was collected for radioactive monitoring. All cultures were washed 3 times with HBSS, and the final cellular pellets were monitored for $^{125}$I radioactivity to detect whether L-asparaginase-$^{125}$I was cell bound or free in the culture media.

**Viability.** Cellular viability was determined by direct cell counts and trypan blue exclusion test.

**Statistical Analysis.** Statistical analysis was carried out by the Student $t$ test.

**RESULTS**

**Isoelectric Analysis of L-Asparaginase.** Fig. 1A shows the isoelectric spectra obtained with 3 batches of L-asparaginase. Discrete differences in the binding pattern are apparent. Suggestions that the multiplicity of banding was due to sieving effects of the gel and thus attributable to multimeric forms of the enzyme were not substantiated on further experimentation. Fig. 1B shows the isoelectric spectra of an L-asparaginase preparation loaded anodally (Track a) or divided and loaded from both the anode and cathode (Track b). The banding spectra are indistinguishable, indicating that sieving effects are probably not responsible for the banding complexity. In addition molecules as large as M.W. 360,000 have been shown to enter and focus without sieving in this gel system (P. C. Montgomery and B. P. Rosner. The Secretory Antibody Response. Antibody Induced by Dinitrophenylated Type III Pneumococcus, submitted for publication). Further evidence that molecular sieving is not responsible for the banding complexity comes from studies with another gel system that allows focusing of molecules with molecular weights of 900,000 (P. Montgomery, unpublished observations). In lieu of the unexplained banding differences in enzyme preparations, a single enzyme batch was used throughout these investigations.

**Iodination of Con A.** $^{125}$I-labeled Con A eluted as a single peak appearing at the void volume of the Sephadex G-25 column. The specific activity of the preparation used in these studies ranged from 0.02 to 0.1 μCi/μg of protein.

**Iodination of L-Asparaginase.** Chart 1 shows the Sephadex G-25 elution profile of the radioactivity after L-asparaginase was iodinated. L-Asparaginase elutes at the void volume of the column. The radioactivity appearing in Fractions II and III is completely dialyzable and elutes in a manner identical to that of the unreacted isotope. In addition, no protein is present in these fractions. Fractions II and III have been attributed to unreacted isotope present after the iodination is complete. The specific activity of L-asparaginase-$^{125}$I was 0.005 μCi/i.u.

The biochemical integrity of iodinated L-asparaginase was studied by isoelectric focusing. Fig. 2 shows the spectrum of the labeled enzyme visualized by protein staining (Track a) and the spectrum of labeled enzyme visualized by contact autoradiography (Track b), which shows the distribution of radioactivity in the various bands. The spectrum of the labeled L-asparaginase is identical to that of the unlabeled enzyme. Further proof that the biochemical integrity of the iodinated molecule is maintained comes from data of retention of biological activity as demonstrated in studies of host immunosuppression and will be published at a later date. However, it is not known at present whether any of the individual 4 components shown by isoelectric focusing analysis possess any differences in enzymatic activity.

**The Effect of L-Asparaginase on Binding of Con A-$^{125}$I to Lymphocyte Surface.** The effect of L-asparaginase on the initial binding of $^{125}$I-labeled Con A to lymphocytes was studied (Tables 1 and 2). In both experiments the results were similar and demonstrated the following: (a) L-asparaginase-treated lymphocytes had significantly lower counts (Con A-$^{125}$I) than control cultures ($p < 0.001$); (b) lymphocytes treated with L-asparaginase 90 min after introduction of the mitogen had significantly higher counts than those treated before mitogenic stimulation ($p < 0.001$);
L-Asparaginase and Lymphocyte Blastogenesis

and (c) significant differences were not discernible between control lymphocytes and those treated with L-asparaginase after the binding of Con A-125I to their surface took place.

**Dose-Response Curve: Inhibition of Mitogen Binding by L-Asparaginase with or without Supplementation with Amino Acids.** The inhibitory effects of increasing concentrations of L-asparaginase on Con A-125I binding to rat lymphocytes were studied. In addition, we have attempted to reverse this inhibition by supplementing the cultures with the amino acids L-asparagine and L-glutamine, the chief substrates of the enzyme; and aspartic acid, the precursor of the substrate of the enzyme (Table 3). Following 90 min incubation with the enzyme, the cultures were centrifuged and washed twice with HBSS, and the amino acids were added. Fifteen min later, Con A-125I was introduced to the cultures. The data demonstrated that the interference with binding of Con A-125I to the lymphocyte surface by L-asparaginase is dose related. In control cultures that were not treated with the enzyme, Con A-125I adsorption represented a mean cpm of 18,500, while cultures treated with L-asparaginase, 10 i.u./ml, exhibited about 11,300 cpm (about 39% reduction; p < 0.005). In cultures treated with 1 i.u./ml, the bound Con A-125I represented 14,800 cpm (21% reduction; p < 0.005). Treatment with L-asparaginase, 0.1 i.u./ml, had no inhibitory effects. The concentrations of L-asparaginase that were effective in reducing binding of Con A-125I were similar to those that bring about inhibition of PHA-induced blastogenesis (3, 4, 21, 26, 36). In our study the inhibitory effect of L-asparaginase on mitogen binding could not be reversed with supplementation of media with L-asparagine, L-glutamine, and aspartic acid. No statistically significant differences were discernible between the bound Con A-125I in cultures supplemented with up to 100-fold concentration of amino acids and their nonsupplemented controls. This finding closely agrees with previous work by Shons et al. (32), demonstrating the inability to reverse L-asparaginase inhibition of blastogenesis in human mixed lymphocyte cultures by the addition of L-asparagine, L-glutamine, and aspartic acid.

**The Inhibitory Effect of L-Asparaginase on Initial Mitogen Binding Correlated to Subsequent DNA Synthesis.** Lymphocytes that interact with antigens presumably do so because they possess specific receptors at their surface. In our experiments we have demonstrated that pretreatment of lymphoid cells by L-asparaginase interferes with the initial binding of the mitogen. The following study was carried out to show that this decrease in Con A-125I binding was related to the inhibition of blastogenesis as measured by cellular DNA synthesis. Lymph node and peripheral lymphocytes were incubated for 60 min with or without L-asparaginase (10 i.u.). Then cells were centrifuged and washed free of the enzyme. Following that, all cultures were stimulated with 1.0 μg of Con A-125I for 60 min. Again, cultures were centrifuged and...
The effects of L-asparaginase on the binding of Con A-125I to surface of rat lymphocytes

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Radioactivity (cpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Control Con A-125I</td>
<td>2440 ± 325d</td>
</tr>
<tr>
<td>B. Con A-125I, then L-asparaginase</td>
<td>2262 ± 570d</td>
</tr>
<tr>
<td>C. L-Asparaginase, then Con A-125I</td>
<td>1597 ± 142d</td>
</tr>
</tbody>
</table>

The effects of L-asparaginase on the binding of Con A-125I to surface of rat lymphocytes

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Radioactivity (cpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Control Con A-125I</td>
<td>6265 ± 800d</td>
</tr>
<tr>
<td>B. Con A-125I, then L-asparaginase</td>
<td>5229 ± 700d</td>
</tr>
<tr>
<td>C. L-Asparaginase, then Con A-125I</td>
<td>3995 ± 590d</td>
</tr>
</tbody>
</table>

The effects of L-asparaginase concentration and amino acids on the binding of Con A-125I to surface of rat lymphocytes

<table>
<thead>
<tr>
<th>Lymphocyte culture group</th>
<th>Radioactivity (cpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>With amino acids</td>
</tr>
<tr>
<td></td>
<td>(cpm)</td>
</tr>
<tr>
<td>0</td>
<td>18,450 ± 610</td>
</tr>
<tr>
<td>0.1</td>
<td>18,700 ± 1,210</td>
</tr>
<tr>
<td>1.0</td>
<td>14,650 ± 640</td>
</tr>
<tr>
<td>10.0</td>
<td>11,150 ± 580</td>
</tr>
</tbody>
</table>

The absorption of L-asparaginase-125I to the Surface of Lymphoid Cells. We investigated the possibility that L-asparaginase might bind competitively to lymphoid receptor sites, thus reducing the binding of mitogen. It was hypothesized that if L-asparaginase binds to the cell surface a significant amount of the enzyme would be removed from the culture media and be directly associated with the lymphoid cells (Table 5). However, our data demonstrated the opposite. L-Asparaginase-125I does not bind to the lymphocyte surface.

DISCUSSION

The inhibitory effects of L-asparaginase on lymphocyte blastogenesis are well documented (3, 4, 21, 26, 32, 36), but...
the mechanism of action has yet to be defined conclusively. Our data demonstrate that L-asparaginase reduced the binding of Con A to the lymphoid cell surface and decreased lymphocyte blastogenesis as measured by DNA synthesis. It is not known whether lymphocytes require an exogenous source of L-asparaginase, a nonessential amino acid, to undergo blastogenesis. In our experiments, the media contained 2 μmoles of L-glutamine per ml and no nonessential amino acids. Fetal calf serum probably contains small quantities of L-asparaginase (the level of L-asparaginase in normal human plasma is 40 nmoles/ml). One i.u. of L-asparaginase activity is defined as the quantity of enzyme that releases 1 μmole of ammonia from L-asparaginase in 1 hr. Therefore, for complete catalysis of all the available exogenous asparagine in our media, about 0.05 i.u. L-asparaginase per ml would have been sufficient. Since our data and those of others demonstrated that the minimum level of enzyme necessary for a biological effect is at least 1 i.u./ml, it appears that the inhibitory effects are not simply due to degradation of exogenous asparagine.

The inhibitory effects of the enzyme on mitogen binding and subsequent blastogenesis were not due to L-asparaginase lymphocytotoxicity, as described previously (26, 29).

In our experiments the exposure time of lymphoid cells to L-asparaginase was limited to 60 to 90 min. This was in contrast to other investigations in which the enzyme was added to the cultures and allowed to remain throughout the experiment (3 to 5 days). Our studies demonstrated that L-asparaginase did not bind to the lymphocyte surface. Thus the only interaction between the enzyme and lymphoid cells was limited to the incubation period. In addition we were unable to reverse the enzymatic interference with mitogen binding to the lymphocyte with L-asparaginase, L-aspartic acid, or L-glutamine. Some cultures received up to 300-fold of the normal amounts of L-asparaginase following incubation and removal of L-asparaginase, yet when Con A was added 15 min later its binding did not return to normal levels. If the decrease in mitogen binding, inhibition of blastogenesis, or indeed generalized immunosuppression was due to a simple depletion of intra- or extracellular L-asparaginase or L-glutamine, then the addition of high concentrations of these amino acids should have reversed the effects of the enzyme. From the above, it would seem that the mechanism of L-asparaginase-immunosuppressive effects is probably not due simply to a depletion of free asparagine.

L-Asparaginase sequentially inhibits protein, RNA, and DNA synthesis. Specifically, it has been shown to inhibit the synthesis of glycoproteins (9). Membrane glycoproteins are known to turn over rapidly. The absence of replacement glycoproteins could conceivably lead to membrane lysis and cell death. Furthermore, L-asparaginase is capable of cleaving formed membrane glycoproteins, probably at the carbohydrate amino acid linkage which involves the amide group of asparagine and N-acetylglucosamine residue (33). The possibility of cell surface alteration by the enzyme is of great interest in the light of recent findings, demonstrating that lymphocytes which interact with antigens, do so because they have specific receptors at their surface. These surface receptors are thought to represent the type of glycoproteins that are synthesized by individual cells (19). Moreover, the antigen binding receptors are probably immunoglobulins (6, 22).

L-Asparaginase suppresses lymphocyte blastogenesis when added to cultures prior to or simultaneously with mitogen or antigen. It becomes less inhibitory when added at various times after mitogen stimulation. Once lymphocytes are induced to undergo blastogenesis, the event cannot be inhibited by treatment with the enzyme. Lymphocytes, cultured with PHA and L-asparaginase and then washed free of both after 48 to 72 hr, did not undergo blastogenesis unless PHA was added again (26). This suggested that L-asparaginase might interfere with PHA attachment to the lymphocyte surface.

The receptors for Con A of pig lymphocyte plasma membranes are known to be glycoproteins occupying exposed positions on the cell surface (2). Different lymphoid cell populations from the same animal react differentially to stimulation with Con A or PHA. This is not due to quantitative variability in binding of Con A to their surfaces (34) but rather to the fact that activation of lymphocytes by mitogen is highly specific and probably due to a particular linkage between the mitogen, surface receptors, and a cellular activation mechanism (34).

The interaction of antigen or mitogen with immunocompetent cell receptors and the subsequent triggering of the cellular activation mechanism are the initial steps in the immune response. Obviously, any interference with this preliminary step could lead to immunosuppression. We have demonstrated that pretreatment of rat lymphoid cells with L-asparaginase brings about a significant reduction in binding of Con A to their surface which leads to inhibition of subsequent DNA synthesis. This inhibition of Con A binding is not unique to the mitogen system alone. In a previous publication (16) we reported that treatment with L-asparaginase of lymphocytes sensitized in vivo to dinitrophenylated bovine γ-globulin brought about a similar decrease in absorption of iodinated dinitrophenyl ligand to their surface.

The immunosuppressive activity of L-asparaginase appears to be due to surface modifications of immunocompetent cells. These modifications could be attributed to a direct disruption of cell surface antigen or mitogen receptor sites either by cleavage of these surface glycoproteins and/or inhibition of new glycoprotein synthesis causing binding site depletion. Alternatively, the enzyme could act indirectly on some other cell surface component causing a dispersal or rearrangement of binding sites in a manner postulated for certain proteolytic enzymes (25). In any event, the immunosuppressive mechanism of L-asparaginase is indeed a complex phenomenon. Additional in vivo and in vitro studies are underway to clarify the mechanism further.

ACKNOWLEDGMENTS

We thank Dr. Pete Wang for his preparation of L-asparaginase-125I and Con A-125I. We are especially indebted to Miss Ellen Ferguson for her excellent technical assistance.

REFERENCES

2. Allan, D., Anger, J., and Crompton, M. J. Glycoprotein Receptors for Concanavalin A Isolated from Pig Lymphocyte Plasma
Effects of L-Asparaginase on Lymphocyte Surface and Blastogenesis

Isaiah J. Fidler and Paul C. Montgomery


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/32/11/2400

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.