Immunosuppression by L-asparaginase
by Joe Kay Moody

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY in Microbiology
Montana State University

Abstract:
The immunosuppressive effects produced by E. coli E-asparaginase and the cell and/or cell types affected were investigated.

L-asparaginase was shown to suppress the immune responses to SRBC, LPS, SSS III and to prolong allograft survival. The suppressed response to SRBC was reconstituted with normal and SRBC primed peritoneal macrophages. Bone marrow was not able to reconstitute the LPS response in an irradiated, enzyme-treated mouse, but did reconstitute the response in an irradiated recipient which did not receive enzyme. This implied the presence of a radio-resistant cell in the recipient, conceivably the macrophage, which interacted with the donor bone marrow. Intravenous enzyme treatment caused atrophy of the liver, spleen, lymph nodes, and the thymus while IP treatment affected only the thymus.

Intraperitoneal enzyme suppression of the LPS response reduced the plaque forming cells per spleen and per million spleen cells while IV suppression reduced only the plaque forming cells per spleen. Intraperitoneal enzyme treatment conceivably affected the macrophage processing and/ or presentation of antigen, therefore reducing the plaque forming cell per spleen and per million cells.

Intravenous treatment eliminated a non-antibody producing cell ("T" cell) and the macrophage, thereby reducing the total number of cells in the spleen which artificially elevated the antibody producing cells ("B" cells) per million spleen cells. Enzyme induced prolongation of allograft survival also implicated the "T" cell and possibly the macrophage. Immunoglobulin levels were not affected by enzyme which reinforced the idea of a resistant "B" cell.

It was concluded that the macrophage and "T" cells were affected by L-asparaginase in the experiments described, and that "B" cells were not the primary target of the enzyme.

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ABSTRACT

The immunosuppressive effects produced by E. coli L-asparaginase and the cell and/or cell types affected were investigated.

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Intraperitoneal enzyme suppression of the LPS response reduced the plaque forming cells per spleen and per million spleen cells while IV suppression reduced only the plaque forming cells per spleen. Intraperitoneal enzyme treatment conceivably affected the macrophage processing and/or presentation of antigen, therefore reducing the plaque forming cell per spleen and per million cells.

Intravenous treatment eliminated a non-antibody producing cell ("T" cell) and the macrophage, thereby reducing the total number of cells in the spleen which artificially elevated the antibody producing cells ("B" cells) per million spleen cells. Enzyme induced prolongation of allograft survival also implicated the "T" cell and possibly the macrophage. Immunoglobulin levels were not affected by enzyme which reinforced the idea of a resistant "B" cell.

It was concluded that the macrophage and "T" cells were affected by L-asparaginase in the experiments described, and that "B" cells were not the primary target of the enzyme.

INTRODUCTION

In 1953, Kidd (23,24) observed that certain murine leukemias were inhibited by a component of guinea pig serum, later identified as the enzyme, L-asparaginase. In recent years the enzyme was shown to be a potent producer of immunosuppression by virtue of its cytostatic or cytocidal effects on lymphocytes (17,18,45).

Most animals and bacteria produce a form of L-asparaginase that causes conversion of asparagine to aspartic acid and ammonia. Only a few forms of the enzyme have been shown to produce the anti-leukemic and/or the immunosuppressive effects.

Initially, small quantities of the active enzyme were obtained from the members of the Superfamily Cavioidae, of which the guinea pig is a member, but bacteria have been the main source of L-asparaginase. Escherichia coli became the main source of L-asparaginase in the early sixties. The enzyme from E. coli has the same molecular weight (138,000) and the same anti-leukemic and immunosuppressive properties as the guinea pig enzyme.

The L-asparaginase from certain strains of E. coli was purified into two distinct forms; E.C. I and E.C. II.

The E.C. I fraction contained very little anti-leukemic or immunosuppressive activity while the E.C. II fraction had maximum activity. These two fractions were separated by varying the concentrations of ammonium sulfate in the protein precipitation steps during purification.

In 1966, Schwartz, et al. (39) showed that the E.C. I fraction was constitutive in E. coli and had a low avidity for its substrate; the E.C. II fraction was inducible and possessed a high substrate avidity.

The proposed mechanisms of anti-leukemic action of E. coli L-asparaginase were provided by the studies of Broome and Schwartz (9), Broome (11, 11a), and Sobin (41). These studies showed that resistant leukemic cells had an increased ability to synthesize asparagine and did not require exogenous amino acid for protein synthesis. Asparagine synthetase, an enzyme facilitating the transfer of an amino group from glutamine to aspartic acid yielding asparagine, was deficient or absent in sensitive cells, while resistant cells maintained relatively high levels. All normal cells possessed synthetase activity and low levels could act as an indicator for sensitivity to L-asparaginase (11,41).

Immunosuppression by L-asparaginase was also believed to be caused by depletion of exogenous asparagine

(18,45). Friedman and Chakrabarty (18) supported this premise by their ability to reverse the immunosuppressive effects by supplementation of exogenous asparagine. Weksler and Weksler (45) showed the same results by using an interesting dialysis system that removed the exogenous asparagine by L-asparaginase without allowing the latter to contact the cells.

Asparaginase was shown to lower the amount of asparagine in the blood for 42 to 72 hours post-treatment. These blood concentrations of asparagine were much less than required for protein synthesis in vitro (12). Other tissues displayed a reduction in asparagine levels, but three hours after treatment there was a gradual increase in asparagine levels in normal tissues. This increase was not seen in the blood or in asparaginase sensitive lymphoma cells. An increase in normal tissues despite low levels of serum amino acid at three hours suggested that synthesis of asparagine was taking place within the resistant cells. Within 48 hours after treatment with asparaginase, normal mitotic indices were obtained in normal tissues but not in sensitive lymphoma cells. Thus, lymphoma cells appeared to be irreversibly damaged by the action of L-asparaginase. Based on

these observations, Becker and Broome (5) postulated that asparaginase interfered with some early stage in cell proliferation.

A controversy yet to be resolved was initiated by the work of Benezra, et al. (6). They alluded to the possibility that asparaginase was not responsible for tumor remissions or immunosuppression. Their data showed, at least in an in vitro system, that glutaminase caused the suppressive effects and that depletion of asparagine did not play a critical role in immunosuppression. This exception can only be explained by differences in experimental protocol and enzyme preparations since many preparations of L-asparaginase causing anti-leukemic and immunosuppressive effects were reported free of glutaminase activity.

In the middle to late sixties, reports appeared which suggested that L-asparaginase from E. coli inhibited lymphocytic blastogenesis (3,16,26,35). Using human lymphocytes, Astaldi, et al. (3) found that upon phytohaemagglutinin (PHA) stimulation, those lymphocytes previously treated with enzyme failed to attain the control levels of mitosis. Thus, human peripheral lymphocytes apparently required L-asparagine for their PHA induced transformation and were not able to synthesize this amino acid in sufficient

quantities when exposed to enzyme. Weksler and Weksler (45) also found that asparaginase markedly inhibited PHA induced lymphocytic transformation and did not interfere with cell viability as measured with trypan blue exclusion.

In a related study, Weiner, et al. (44) showed that protein synthesis in lymphocytes was reduced to 40% of the controls in the absence of L-asparagine. In addition, cells depleted of asparagine with enzyme or cells grown in a medium lacking the amino acid, displayed decreased mitotic indices and reduced levels of protein synthesis.

In 1969, Schwartz (40) suggested the possible relationships between the inhibition of lymphocyte blastogenesis and the immunosuppressive effects caused by L-asparaginase. Using sheep erythrocytes (SRBC) as an antigen and the Jerne Plaque Assay (21) for enumeration of IgM producing cells, Schwartz found that immunosuppression was established over a wide range of enzyme doses, 25 to 1000 International Units (I.U.). Berenbaum (7) predicted a maximum effect would occur two days after contact with the antigen, the time corresponding to a period of maximum cell proliferation after antigenation. Interestingly, the effect was maximum from 48 hours before to 4 hours after asparaginase administration. The effect of the enzyme was temporary and after an interim

of 6 days without treatment, the animals would respond normally to antigens. The importance of timing was also confirmed by Chakrabarty and Friedman (15).

Berenbaum (7) investigated the effectiveness of various enzyme preparations from different sources. The effectiveness of the L-asparaginase varied tremendously with the source from which it was derived. The in vivo half-life in mice differed from 19 hours with guinea pig asparaginase to 30 minutes with yeast enzyme. Berenbaum (7) also showed that yeast asparaginase with ineffective in producing immunosuppression while the guinea pig enzyme was very effective.

Since the pioneering work of Schwartz (40) and Berenbaum (7), numerous studies have confirmed the suppressive effects of the enzyme on the response to SRBC (12,15, 18,19,29,30,31,35,40). These reports demonstrated how both serum titers and antibody producing cells in the spleen were reduced by administration of L-asparaginase. In these studies, enzyme doses were varied from 10 to 1000 I.U., and both enzyme and antigen were given by the intraperitoneal (IP) route.

More recent investigations have included the use of a thymus independent antigen, endotoxin (LPS), to investigate

the effect of L-asparaginase on bone marrow derived or thymus derived lymphocytes. Friedman (18) demonstrated that IP administration of asparaginase suppressed the response to 100 µgm of LPS administered by the same route.

Kahn and Hill (22) found the response to bovine serum albumen was suppressed by L-asparaginase extending the immunosuppressive effects to a pure protein antigen.

L-asparaginase was found to prolong the survival of allografts (18,25,30,38,42,45). Graft survival was shown to depend on a critical timing of enzyme administration. Since both reduction of the humoral response and homograft survival depended on early contact with the enzyme, the early "processing" phase of the immune response to antigens seemed to be affected. The second set graft rejection was delayed by the presence of asparaginase (18), but Nelson, et al. (30) failed to suppress the secondary response to SRBC. The latter is in contrast to the observations by Friedman and Chakrabarty (18) who were able to obtain suppression of the secondary response to SRBC. Friedman and Chakrabarty (18) showed a greater reduction in the numbers of 7S plaque forming cells than in the 19S producing cells when the secondary response was impaired. This suggested a blockage of the 19S to 7S transition characteristic of a secondary response. On

the other hand, suppression of a primary response to SRBC with L-asparaginase depleted the numbers of both 7S and 19S antibody producing cells.

Since L-asparaginase produced an impaired antibody response to a variety of antigens, it would not be unexpected to find lowered immunoglobulin levels. In 1970, Burgio, et al. (13) measured the gamma globulin levels in children with acute lymphocytic leukemia and rheumatoid arthritis who were under asparaginase treatment. They found the gamma globulin levels slightly increased. The reason for this increase is presently unexplainable.

The cellular site of action of L-asparaginase has recently received attention by several groups of investigators (17,45). In 1971, Friedman (17), using a bone marrow-thymus reconstitution system, showed a cell within the bone marrow to be a target for asparaginase. Thus, Friedman was able to reconstitute the immune capacity of lethally irradiated mice with thymus cells from enzyme treated mice and bone marrow from non-enzyme treated mice.

In 1971, Weksler and Weksler (45) investigated many aspects of L-asparaginase induced immunosuppression. Lymphocytopenia with spleen, thymus, and lymph node atrophy was produced by L-asparaginase treatment. Adrenalectomized and

enzyme-treated mice displayed reduced responses to antigens which indicated that L-asparaginase induced immunosuppression did not require adrenal steriods.

Hobik (20) was able to suppress the graft-versus-host (GvH) reaction by treatment of spleen cells with asparaginase. Weksler and Weksler (45) were also able to suppress the GvH reaction by incubation of donor spleen cells with enzyme in vivo but not in vitro.

Rodney and Good (37) showed that PHA stimulated lymphocytic transformation was dependent upon a population of thymus derived lymphocytes. In view of Weksler and Weksler's (45) observations on PHA stimulated blastogenic inhibition, marked thymic atrophy, and homograft prolongation, he postulated the thymus derived lymphocyte was the affected cell.

Weksler and Weksler (45) also noted an abnormal migration or "homing" of asparaginase treated lymphocytes. Labeled lymphocytes from nodes were incubated in vitro with L-asparaginase and injected into syngenic recipients. The enzyme treated lymphocytes migrated primarily to the liver instead of the spleen and lymph nodes.

The purpose of this work was to investigate the cellular and humoral aspects of L-asparaginase induced

immunosuppression. Major emphasis was placed on the mechanisms underlying the suppressive effect caused by the enzyme and the various cells and/or cell types affected by L-asparaginase.

Recent concepts of cells or cell types affected will be reviewed and discussed. Investigations were carried out to further clarify the total scheme of events involved in L-asparaginase induced immunosuppression.

MATERIALS AND METHODS

I. Mice

Inbred Balb/c ($H\text{-}2^d$) mice obtained from the National Institutes of Health (N.I.H.) were used throughout the study. CBA/J ($H\text{-}2^k$) mice (Roscoe B. Jackson Memorial Laboratories, Bar Harbor, Maine) were used as donors of skin grafts, and A/Jax ($H\text{-}2^a$) mice were used in some initial weight loss studies. All mice were fed autoclaved Purina 50-10-c fortified mouse chow and acidified-chlorinated water (Appendix I). All cages were cleaned weekly and filled with sterilized corn cob bedding. Litters were weaned at 4 weeks of age and all mice used in the study, with the exception of 4 to 6 month old macrophage donors, were between 4 and 12 weeks of age.

II. L-asparaginase

Lyophylized preparations of *E. coli* L-asparaginase ("Lyovac") were obtained from Merck, Sharp, and Dohme (West Point, Pennsylvania). Each bottle contained 50,000 International Units (I.U.) of the enzyme plus 200 mg of mannitol. The enzyme was weighed out immediately before

use and rehydrated in 0.25 ml of physiological (0.85%) saline for injection. After the vials were opened, the powdered enzyme was kept at -70°C. until use and was found to maintain its immunosuppressive activity for 40 to 60 days.

The enzyme dosages varied from 50 I.U. to 1000 I.U. per dose, depending on the experimental design.

III. Antigens

Sheep erythrocytes (SRBC) in Alsevers (Colorado Serum Company, Denver, Colorado) were washed three times in saline and resuspended in saline to the desired concentration.

Endotoxin (LPS) (00111:B4,Lot558185) was purchased from Difco Laboratories (Detroit, Michigan) in 100 mg quantities. For injection, the LPS was resuspended in saline to a concentration of 40 µgm per ml and a volume of 0.25 ml was administered. For coating SRBC, the LPS was suspended in 0.01 M phosphate buffer (Appendix I) pH 7.4 and boiled for 2 1/2 hours. A 1:10 dilution was made in phosphate buffered saline (PBS) (Appendix I) and 10 ml of diluted, boiled LPS was added to 0.25 ml of packed, thrice-washed, SRBC. This mixture was incubated at 37°C. for 30 minutes, washed three

times in PBS, and resuspended in Duttons Balanced Salts Solution (DBSS) at a 15% concentration for plaquing.

Pneumococcal Polysaccharide Type III (SSS III), kindly supplied by Dr. P. J. Baker, NIH, Bethesda, Maryland, was rehydrated in saline to a concentration of 1 mg per ml. A volume of 0.8 ml (0.8 mg) of SSS III was added to 1 ml of 0.1% chromium chloride ($CrCl^3$). Sheep erythrocytes were washed twice in saline and added to the mixture of SSS III and $CrCl^3$ and allowed to stand at room temperature for 5 minutes. The cells were washed three times in saline and resuspended to a concentration of 20% in saline. This 20% suspension of SSS III coated SRBC was used for plaquing.

IV. Hemagglutination (HS) and Hemolysin (HL) Titrations

For HA titrations, blood was collected from the retro-orbital plexus and allowed to clot at room temperature for one hour. The clot was centrifuged at 3000 rpm for 10 minutes to separate the serum from the cells. The serum samples for the HA and HL titrations were frozen at 5°C. until used. Twofold serial dilutions of the serum were made in saline and the volume, after dilution, was 0.2 ml. Two tenths ml of a 1% suspension of SRBC and 0.1 ml of saline were added making the final volume 0.5 ml. This was

incubated at 37°C. for one hour and held at 5°C. overnight. The tubes were centrifuged for three minutes at 3000 rpm and the reciprocal of the last serial dilution showing agglutination was considered the HA titer of the serum.

Hemolysin (HA) titrations were performed by the same procedure as the HA titrations with two exceptions. One tenth of a ml of 10% guinea pig complement (Colorado Serum Company, Denver, Colorado) was added instead of the 0.1 ml of saline. The tubes were incubated for one hour at 37°C. and read immediately. The reciprocal of the last serial dilution showing complete hemolysis was considered the titer of the serum.

V. Jerne Plaque Technique

A modification of the Jerne Slide Technique, as described by Mishell and Dutton (28), was used to plaque against SRBC. The spleen cell suspensions were not adjusted to 10 million cells per ml before plaquing. Instead, the spleen cells were suspended in 4 ml of DBSS and were plaqued undiluted and as a 1:5 and 1:25 dilution. The number of IgM producing cells was counted from slides incubated with guinea pig complement. The IgG plaques were developed by the addition of rabbit anti-mouse IgG antiserum to the guinea pig

complement. The facilitated plaque counts included both direct and indirect plaques.

The number of cells in the undiluted preparation was counted on a hemocytometer. After incubation with complement, the plaque forming cells were counted and the number of plaque forming cells per million spleen cells and per spleen was calculated.

The procedure for plaquing with endotoxin (LPS) is the same with the SRBC above except the erythrocytes must be coated with the endotoxin before being placed in the overlay agar (See Materials and Methods III). Since mice respond primarily with an IgM response to endotoxin, facilitation with IgG antiserum was not necessary.

The preparation of SRBC for plaquing against SSS III was mentioned earlier (See Materials and Methods III).

Mouse spleen cells for plaquing were suspended in Medium 199 (Microbiological Associates), pH 7.15. Coated SRBC were suspended in Hanks Balanced Salts Solution (HBSS) (Microbiological Associates), pH 7.15. The agarose overlay tubes were prepared with HBSS. Actual preparation for and pouring slides was identical to the SRBC method of Mishell and Dutton (39). The slides were incubated for one hour at 37°C. After incubation, the facilitating antiserum, rabbit

anti-μ antiserum, was applied. This serum was previously titrated to determine the optimum concentration, as too concentrated a serum would inhibit plaque formation. The slides were incubated another hour and diluted guinea pig complement (1:10 in HBSS) was added to the slides. A further two hour incubation at 37°C. was carried out and the slides were scored for plaque forming cells. IgM anti-SSS III plaques were facilitated with a pre-titrated anti-IgM serum.

VI. Skin Grafting

The procedure of Billingham and Silvers was used (8). Inbred CBA/J ($H\text{-}2^k$) mice were used as trunk skin donors and Balb/c ($H\text{-}2^d$) mice served as graft recipients. Mice were anesthetized with sodium pentabarbital (Appendix II) by the method of Pilgrim and DeOme (34).

Trunk skin was removed from mice that had been dehaired with "Nair" (Calcium thioglycolate) the day prior to use. This skin was scraped to remove the piniculus carnosa. The skin was then cut with a cork bore to a diameter of one cm and placed in sterile HBSS, pH 7.15. Mice to be grafted were dehaired with "Nair" one day prior to use. They were anesthetized (Appendix II) and a graft bed approximately one

cm in diameter was prepared immediately above the rib cage. The bed was cut to expose the piniculus carnosa. The graft was held in place by first layering a piece of lawn, impregnated with vaseline and sterilized, over the new graft. A sterile gauze compress was applied and covered with 4 or 5 wraps of one inch gauze. The entire area was covered with three wraps of one inch wide plaster cast.

Six days later, the cast was removed and the progress of graft rejection was observed for two or three weeks. Grafts were considered rejected when 100% of the graft became necrotic.

VII. Macrophage Stimulation and Harvesting

Balb/c mice 4 to 6 months of age were used as macrophage donors. Older mice yielded larger numbers of macrophages. These mice were given three ml of fluid thioglyocolate medium IP. Four days later, the mice were sacrificed and the macrophages harvested by flushing the peritoneal cavity with 5 ml of Medium 199, pH 7.15. The cells were centrifuged at 3000 rpm for 10 minutes and resuspended in 4 ml of Medium 199, pH 7.15. The peritoneal exudate cells (PEC) were incubated at 37°C. for two hours in Falcon plastic petri dishes. The non-adherent cells were removed by

pouring off the cells with the supernate, and the adhering macrophages were collected in sterile Medium 199 by scraping the petri dish with a sterile rubber spatula. Cell numbers were determined by the use of the hemocytometer and their viability was evaluated by the use of trypan blue exclusion (See below).

VIII. Cell Viability Determination (Trypan Blue Exclusion)

A 0.5% solution of trypan blue in distilled water was prepared. The cells (0.05 ml) to be tested were mixed with 0.1 ml of the dye and 0.85 ml of saline and allowed to stand at room temperature for 10 minutes. After 10 minutes, the cells were counted in a hemocytometer and only those cells not excluding the dye were considered viable.

IX. Histologic Techniques

All tissues were removed from the mice as soon as possible after death. They were placed in 10% buffered formalin (Appendix I) and allowed to remain for two to three weeks before embedding. The tissues were embedded using the standard procedures and stained with Harris' Hemotoxylin and Eosin according to the procedure of Weesner (43).

X. Immunoglobulin Determinations

Relative levels of the classic immunoglobulins were determined by a modified Masseyelf and Zisswiller technique (27). The sera to be tested were collected from the retro-orbital plexus and frozen at 5°C. until use. One cm discs were cut with a cork bore from a 1.5 mm thick 1% agarose sheet prepared with 0.05 M HCl-phosphate buffer pH 8.4 (Appendix I). To the discs were added 0.03 ml of diluted Meloy (Meloy, Fallschurch, Virginia) goat antiserum specific for one of the mouse immunoglobulins. The discs were incubated in a moist chamber for 12 to 18 hours. At the end of this time interval, a two mm well was placed in the center of each disc. Into the center well were placed 5 lamda of each serial dilution of mouse serum. The discs were allowed to incubate at room temperature for 48 hours and the diameter of the precipitation rings were measured. Ring diameters were compared to a set of standards prepared using serum of normal mice. The results of the treated mice were reported as a relative variation from the normal levels.

XI. Irradiation

Mice were given 675 or 900 rad whole body gamma irradiation using a ^{60}Co source, delivering 97 rad/min. onto a plexiglass cage (H and H Plastics, Lincoln, Nebraska). The cage was placed in a 17.5 x 17.5 cm field on top of a turntable which was rotated at two rpm and placed 60 cm from the source to the center of the mouse. Maximum backscattering was achieved by placing a 5 inch masonite block between the turntable and the plexiglass cage containing the 12 mice.

XII. Reconstitution Methods

Mice were sacrificed and the two femurs were harvested and cleaned of any muscle tissue. The bones were placed in Medium 199, pH 7.15, and chilled in an ice bath. The ends of the femurs were cut off, and the marrow aspirated from the lumen. The extracted marrow was pipetted in a capillary pipette until a single cell suspension was obtained. Cell numbers were determined and the appropriate dilutions were made in Medium 199, pH 7.15 or HBSS for injection.

Thymuses were removed from young mice and mashed through a number 100 mesh sterile stainless steel screen,

using Medium 199 or HBSS as the suspending medium. After a uniform cell suspension was obtained, the cells were centrifuged at 3000 rpm for 10 minutes and resuspended in sterile medium. The cells were counted, checked for viability by dye exclusion, and diluted to the desired concentration of viable cells for IV injection.

Macrophage stimulation and harvesting were described earlier (Materials and Methods VII). After the viable cell numbers were determined, a dilution yielding 25 to 30 million cells per ml was made. Animals were reconstituted IP with 12.5 to 15 million cells suspended in 0.5 ml of Medium 199, pH 7.15.

XIII. Calculation of "K" Values

The "K" value allows one to assign a significant level of organ weight gain or loss between treated and control groups relative to body weight,

$$\text{where } K = \frac{\frac{\text{Treated Organ Weight}}{\text{Treated Body Weight}}}{\frac{\text{Normal Organ Weight}}{\text{Normal Body Weight}}}.$$

Any "K" value greater than 1.3 indicates a significant organ weight increase, and any number lower than 0.7 indicates a significant organ weight decrease.

XIV. Blood Cell Staining

All blood cells were stained with Wrights Stain by the procedure of Bauer, et al. (4).

RESULTS

I. Effect of L-asparaginase Administration on Adult Mice

Balb/c and A/Jax mice served as recipients of 3 - 1000 I.U. doses of L-asparaginase administered IV in 0.25 ml of saline on each of three consecutive days. After the initial three doses, enzyme was given IV every other day for two weeks. The results of total body weight loss in Balb/c and A/Jax mice are shown in Table I.

Balb/c mice, treated with enzyme, displayed a profound body weight loss for the first 5 days of treatment. Continued treatment did not cause any further weight loss. A/Jax mice also displayed a severe weight loss after treatment with 1000 I.U. and death occurred within 5 to 10 days. A/Jax can better tolerate 100 I.U., however, a 40% mortality was observed by 10 days. With a discontinuation of treatment, mortality continued to 80% by day 15. The remaining 20% recovered without any further treatment. Table I illustrates the strain variations in body weight and mortality after L-asparaginase treatment.

Organ weights are also affected by enzyme treatment. Two-month old Balb/c mice were given 1000 I.U. of enzyme IV

on three consecutive days and sacrificed on the fourth day. Table II lists the total body weights, the spleen, thymus, and liver weights and the "K" values of treated and control mice.

Balb/c mice displayed marked reduction of spleen, thymus, and liver weights after IV treatment with L-asparaginase. Lymph nodes also displayed signs of atrophy after mice were treated IV with enzyme. Three to 4 weeks after cessation of enzyme treatment, the organ and body weights had recovered.

The effect on the antibody response of 3000 I.U. of enzyme administered IV was measured by hemolysin (HL) titers, hemagglutination (HA) titers and plaque forming cells in Balb/c mice.

Two-month old mice were injected with 1000 I.U. of L-asparaginase IV on each of three consecutive days. On the second day of enzyme treatment, the mice received 1×10^8 SRBC IV in 0.25 ml of saline. Five days later, the mice were bled from the retro-orbital plexus, sacrificed, and their spleens removed. The results of the HA-HL titrations and plaque forming cell response are reported in Table III.

L-asparaginase was effective, when given IV, in suppressing the humoral response to SRBC given IV to Balb/c

TABLE I

Body Weight in Balb/c and A/Jax Mice
Following Enzyme Treatment

No. & Strain of mice	Treatment route	Average wgt. (gm) at day 0	Average Weight (gm) Days after Initial Enzyme Treatment 1	2	3	5	10	14
10 (Balb/c)	Saline (IV)	20.5	21.5	22.8	22.34	23.52	24.1	25.6
10 (Balb/c)	Asp-Ase[1] (IV)	21.2	21.4	18.06	17.25	15.92	15.5	15.6
10 (A/Jax)	Saline (IV)	18.35	18.4	19.15	19.3	19.92	27.1	--
10 (A/Jax)	Asp-Ase[1] (IV)	19.23	19.2	17.33	15.1	12.1 (5 mice)	All Dead	--
5 (A/Jax)	Saline (IV)	19.30	19.23	19.91	20.64	21.47	25.2	--
5 (A/Jax)	Asp-Ase[2] (IV)	19.75	19.8	18.23	16.62	14.03	12.3 (3 mice)	--

[1]Asp-Ase = 1000 I.U. administered IV

[2]Asp-Ase = 100 I.U. administered IV

TABLE II

Body and Organ Weights, and "K" Values[1] for Balb/c Mice

No. of mice	Treatment	Average[2] Body wgt. (gm)	Ave. Organ wgts. in mg ("K" Value) Spleen	Thymus	Liver
10	Saline (IV)	23.12	148	71	1573
10	Asp-Ase (IV)	17.93	60 (0.53)	18 (0.315)	700 (0.0572)

[1]"K"-Value - See Materials and Methods XIII

[2]Average Body Weight (gm) - Average body weight on day of sacrifice

TABLE III

Hemolysin, Hemagglutination and Plaque Forming Cell Responses in Balb/c Mice

No. of mice	Treatment route	Antigen[1] route	Ave. Spleen weight (mg)	Direct Plaque Forming Cells HA[2]	HL[3]	per 10^6	per Spleen
15	Saline (IV)	IV	137	960	620	152	4.1×10^4
20	Asp-Ase (IV)	IV	52	35	20	18	6.2×10^2
8	Δ-Asp-Ase (IP)[4]	IV	128	--	--	137	3.75×10^4

[1]Antigen = 1×10^8 SRBC IV in 0.25 ml Saline

[2-3]HA-HL = Hemagglutination and Hemolysin titers are reported as the reciprocal of the last serial dilution showing agglutinated RBC or complete lysis respectively.

[4]Δ-Asp-Ase = Heat denatured enzyme (30 minutes at 65°C.)

mice. Heat denaturing the enzyme at 65°C. for 30 minutes negated its suppressive effects. Broome (10) showed that heat denaturation destroyed its enzymatic activity. Due to its aggregated state after heating, it had to be given IP.

II. Pathologic Effects of L-asparaginase

Two-month old Balb/c mice were given 1000 I.U. of enzyme IV on three consecutive days and sacrificed on day 4. The spleens, livers, axillary lymph nodes, and thymuses were removed and processed for histologic examination (See Materials and Methods IX). Plates I-IV are photomicrographs showing the thymus, spleen, lymph node, and liver section from control and enzyme treated Balb/c mice.