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L-Asparaginase : a review of the literature

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L-Asparaginase - A Review of the Literature

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One of the most attractive and optimistic areas of current cancer research is that concerning the metabolic and nutritional differences between neoplastic and normal cells. The treatment of malignant tumors with the enzyme L-asparaginase may represent the first example of a mode of therapy directed at a specific nutritional difference between certain neoplastic and normal cells. This review is an attempt to describe, in more or less chronologic order, the original research dealing with the discovery of L-asparaginase as a tumor-inhibiting agent and to trace its development as a cancer chemotherapeutic drug.

In 1953 Kidd observed the regression of two types of transplanted lymphomas following multiple injections given intraperitoneally of normal guinea pig serum into mice carrying these tumors, whereas the lymphomas of untreated control mice grew rapidly, killing their hosts within thirty days.[1] The latter was true of the growth of similar tumors in other mice given repeated injections of horse serum or rabbit serum. In similar experiments Kidd showed that the cells of a transplanted lymphosarcoma of rats were temporarily kept from proliferating by multiple intraperitoneal injections of guinea pig serum, while the cells of two transplanted mammary carcinomas of mice, and those of fibrosarcoma, grew unimpeded in hosts that were similarly treated. These findings, which were thusfar unique, provided an example of a naturally occurring substance, viz., the guinea pig serum, that brought about regression of a single type of cancer cells in living animals without doing obvious harm.

Kidd went on to show that the active serum constituent was probably a protein, and suggested that it might be one of the components of complement. It was shown microscopically that the cells of subsequent lymphomas died rapidly and were resorbed following injections of relatively large amounts of guinea pig serum intraperitoneally into mice carrying them. No microscopic changes referable to the guinea pig serum were seen in the normal tissues or organs of mice receiving it. Mouse lymphoma cells, suspended artificially in a physiologic saline solution, regularly remained viable following incubation in vitro in mixtures with guinea pig serum. From this classic study, Kidd concluded that the regression of the growths was brought about in vivo through some interaction in which the host and the unknown active constituent of the guinea pig serum both participated.

Within a few years several investigators became interested in this apparently unique feature of the guinea pig serum, and began varied experiments in an effort to further elucidate the principles involved.[2-8] Jameson and his associates studied the inhibition of a fibrosarcoma, that was transplanted into a rat, by repeated intraperitoneal injections of normal guinea pig serum.[2] They felt that gamma globulin was somehow implicated, and that this inhibition was enhanced by a single injection of heterologous gamma globulin given at the time of implantation. To them the gamma globulin appeared to act in one of several ways. By itself, in the dose used, it had no effect on tumor growth, although larger doses had a stimulating effect on the growth. When it was given in

conjunction with guinea pig serum, their results were equivocal-- in some instances it suppressed the growth of the tumor entirely, and yet in other instances it had no added effect on the inhibitory action of the guinea pig serum. They came to the rather vague conclusion that the course taken by the tumors was probably dependent on an unknown tumor-host relationship.

Yet other investigators felt that the properdin system, well known to form a natural bactericidal system in conjunction with complement and magnesium ions, was somehow involved. Herbut and his group[3] had felt at one point in their investigations that the tumor-inhibiting activity of the guinea pig serum might be due to this naturally occurring protein called properdin. Subsequent experiments, however, convinced them that the tumor-inhibiting principle was in all probability not properdin, but that the properdin system in animals bearing transplantable tumors was somehow altered.

Meanwhile, Jameson and associates continued to study the action of the guinea pig serum and found it to be effective in inhibiting the ascites form of the Murphy-Sturm lymphosarcoma in Wistar rats.[4] Their preliminary studies suggested, too, that the properdin system was possibly involved in part. The C'_3 fraction of hemolytic complement, however, did not appear to them to be involved in the activity. Of greater import was the fact that their experiments showed that the serum had to be present at the same time as the tumor cells in order to be effective, it being ineffective in inhibiting the tumor after growth had started.

Herbut continued to investigate the possible role of the

properdin system in transplantable cancer.[5] At this time he concluded that 1) the operating mechanism for the regression of tumor size was not through the properdin system, and 2) that properdin levels of sera from C3H mice varied inversely with growth of tumor 6C3HED, but that this fluctuation was probably an indicator of tumor growth rather than its determinant.

While continuing to try to identify and isolate the tumor-inhibiting principle, although having given up the idea that the properdin system was responsible, Herbut and Kraemer[6] became interested in determining first if any of the organs of the guinea pig contained the tumor-inhibiting principle thus serving as a source of this material in serum, and second, if they did, whether similar organs of other animals might also contain this same substance. Saline extracts of livers from several species of animals were tested for tumor-inhibiting activity against Gardner lymphosarcoma 6C3HED carried subcutaneously by C3H mice. Complete regression of growth was obtained with the extract of guinea pig livers; marked, but incomplete, retardation of growth was induced by extracts of sheep, hog and rabbit livers; slight and inconsistent retardation with extracts of horse and bovine livers; and none with extracts from two human livers.

Intrigued by these findings, Herbut and his fellow investigators[7] tested the ammonium sulfate globulin fraction of physiologic saline extracts of livers from several species of animals for their tumor-inhibiting activity against the Gardner lymphosarcoma. Complete regression of tumor or marked retardation of growth was obtained with the globulin fraction of

guinea pig, hog, rabbit, horse, cow, sheep, and veal livers. Slight retardation of tumor growth was obtained with the globulin fraction of lamb liver, and that from normal human livers from three groups of patients — newborn to eighteen months old, nine to twelve years old, and a 26 year old, respectively — and from one patient with chronic lymphocytic leukemia. Preparations of livers from patients with bronchial asthma, pneumonia, acute stem cell and myeloid leukemias, and carcinoma of the kidney and ovary brought about no retardation of growth. These authors concluded by saying that, in general, the tumor-inhibiting activity of the fractions paralleled their globulin content, but whether the tumor-inhibiting principle was globulin itself or something else merely associated with this protein remained to be proved.

It remained only until 1961 for the active constituent of the guinea pig serum to be elucidated. Broome[8-9] obtained his evidence in two ways: first, by a study of the induction of resistance to guinea pig serum by 6C3HED cells grown in tissue culture; and secondly, by a direct comparison of the L-asparaginase activity in various preparations of guinea pig serum with their tumor-inhibitory properties. His experiments, ingeniously performed, provided strong evidence that the inhibitory action of guinea pig serum on 6C3HED cells was due to its L-asparaginase activity, and he concluded that it was probable the anti-lymphoma action on other sensitive cell strains was by a similar mechanism, and indicated that a distinct form of L-asparagine metabolism was possessed by this group of tumors.

Other investigators still remained somewhat skeptical.

Beyse and associates[10] felt that the fact that suppression had been obtained only with tumors which had a long history of transplantation, or with tumors carried in heterogeneous stocks, made it difficult to evaluate the relevance of the finding to primary cancer. They felt that since the tumors that were known to be sensitive were in some degree incompatible with their present hosts, it had been difficult to assess the activity of the serum factor free of any accompanying immune reactions attributable to histo-incompatibility. Thus, they tested the sensitivity to guinea pig serum of eighteen new leukemias that arose from their inbred mouse colonies. All the tests were carried out during early transplant generations in mice from their own colonies, maintained under strict conditions of inbreeding. They felt there was little necessity to consider the intervention of isoantigenic disparity in the instances they reported. Of the 18 leukemias tested, ten were found to be suppressed by guinea pig serum. These leukemias included both those which were spontaneously occurring and those that had been radiation-induced. They concluded that a number of new leukemias were found to be highly sensitive to suppression by guinea pig serum, under conditions where isoimmunity was certainly not a contributory influence. They even stated that the possibility that some tumors in man may be responsive to similar treatment should be considered.

Up until that time, no animal closely related to the guinea pig had been tested in regard to the suppressive properties of its sera towards transplantable leukemias. Therefore, Beyse and his group sought a different line of investi-

gation.[11] They obtained several rodents of the same suborder Hystricomorpha in order to test the activity of their sera against transplantable leukemias. In view of Broome's contention[8-9] that this inhibition was mediated by the L-asparaginase content of the guinea pig serum, they also determined the activity of this enzyme in each serum sample. They found that serum from all members of the super-family Cavioidea, with the exception of the capybara, showed both protection against the leukemia graft and L-asparaginase activity. It was concluded that the fact serum from three distinct genera, other than the guinea pig, possessed both L-asparaginase and the property of inhibiting an experimental leukemia strengthened Broome's conclusion that these two characteristics are causally related. From further experiments with serial dilutions of serum, they obtained evidence that the same quantitative relationship existed between leukemia inhibition and L-asparaginase activity, irrespective of the source of the serum.

To further support Broome's proposal, Mashburn and Wriston described experiments on the effectiveness of partially purified L-asparaginase of guinea pig serum in inhibiting tumor growth.[12] These authors showed that there is a direct relationship between L-asparaginase activity and tumor-inhibitory activity, regardless of the specific activity of the L-asparaginase used for testing. Several L-asparaginase samples of intermediate specific activity were tested for anti-lymphoma activity. These preparations showed an initial inhibitory effect on tumor growth which was not sustained throughout their four day injection period. When these samples were

again assayed for L-asparaginase activity, it was noted that there had been a complete loss of the enzyme's activity. The conclusion was arrived at that this observation supported the view that L-asparaginase of guinea pig serum is responsible for the anti-lymphoma activity.

To add further confirmation, Boyse and workers[13] described one of the sensitive leukemias, EARAD1, induced by X-radiation, which they had mentioned in a previous article[10], in a (C37BL/6xA)F_1 hybrid, and how they used it in further tests of the relationship between the L-asparaginase activity and the leukemia-inhibitory activity of guinea pig serum. Guinea pig serum was fractionated by procedures based on different properties of serum proteins -- 1) solubility, 2) molecular size, and 3) ion exchange. These procedures, applied singly or in series of two or three steps, gave preparations in which L-asparaginase activity was directly and quantitatively correlated with the leukemia-inhibitory activity of the original guinea pig serum pool.

By this time numerous biochemical investigators had detected aspafaginase activity in several micro-organisms, including E. coli and B. coagulans. Mashburn and Wriston[14] reported results of experiments which indicated that the L-asparaginase from E. coli also inhibited the growth of the Gardner lymphosarcoma in mice as did the guinea pig serum, but that the corresponding enzyme from B. coagulans was without effect. In addition, they compared the effects of E. coli asparaginase and guinea pig serum on two additional mouse tumors, and found that the guinea pig serum-sensitive tumor was also inhibited by E. coli asparaginase, whereas

the tumor that did not respond to guinea pig serum was unaffected by the bacterial enzyme.

Herbut and Seld, continuing from their earlier experiments[6-7], undertook to isolate the anti-tumor principle from both the serum and the liver of the guinea pig, and in doing so, to isolate asparaginase in pure form.[15] By a variety of separation techniques, a 1400-fold purification of the guinea pig serum L-asparaginase and a 50-fold purification of the guinea pig liver asparaginase were effected. They found that while asparaginase from both sources produced tumor inhibition, two to three times as many units of guinea pig liver asparaginase were required to attain the same degree of inhibition as with the guinea pig serum asparaginase preparations.

Broome had not remained idle since his fortuitous discovery in 1961. He obtained a purified L-asparaginase from yeast and tested it on the growth of guinea pig serum-sensitive lymphoma 6C3HED in C3H mice, but found that no inhibition of the tumor resulted.[16] He explained this by the extremely rapid rate of clearance of the enzyme in the mouse. Whereas guinea pig serum L-asparaginase persisted in the blood in considerable amounts for three or more days following IV injection, the yeast enzyme was almost completely cleared in less than one hour. His ultracentrifuge studies suggested that the yeast L-asparaginase was in the form of molecular aggregates, of a kind likely to be taken up with great avidity by the reticuloendothelial system.

Patterson <u>et al</u>[17] were apparently the first to concretely propose a mechanism of action of the L-asparaginase in its tumor-inhibiting activity. These authors studied the in <u>vitro</u>

growth response of the Jensen sarcoma and its nutritional variants (JA-1 and JA-2) to media containing normal guinea pig serum and partially purified asparaginase and media devoid of asparagine. A comparison of the morphology of cells grown in the presence of guinea pig serum and media devoid of asparagine showed a remarkable similarity. Further, the growth characteristics of cultures exposed to guinea pig serum and partially purified asparaginase were essentially the same. These results suggested the active component of guinea pig serum was asparaginase which catalyzed the destruction of extracellular asparagine.

Sebin and Kidd, however, detracted from this finding by showing that certain lines of lymphoma cells require L-asparagine and others don't.[18]

Yellin and Wriston were quick to point up the fact, though, that tumors susceptible to asparaginase indeed do require asparagine in order to divide normally in tissue culture, and that no other enzyme which has a tumor-inhibitory effect had been so closely linked to a specific metabolic requirement of the tumor cell.[19] These investigators obtained a highly purified preparation of L-asparaginase from guinea pig serum and suggested that their evidence was highly conclusive that the enzyme was responsible for the tumor-inhibiting principle of guinea pig serum. They cited the fact that the importance of demonstrating correlations between the growth of tumors on the one hand and unique or highly modified metabolic pathways on the other was widely recognized and served as an impetus for much current cancer research. No other enzyme except L-asparaginase up to that time had shown such a

striking effect in attempts to treat cancer. They went on to say that there are no known metabolic pathways which require asparagine, and if one assumes that asparaginase inhibits the tumor by depriving it of asparagine as Patterson et al suggested[17], the central problem of the role of asparagine still remains. It is not known whether asparaginase acts indirectly by way of ribonuclease, for example, or limits the availability of asparagine for a novel biosynthetic pathway in the tumors.

To add another small problem in the investigations of L-asparaginase, Roberts and co-workers isolated two L-asparaginase components from E. coli.[20] The early emerging component on column chromatography was capable of causing complete regression of the Gardner 6C3HED lymphosarcoma and was stable on prolonged incubation with C3H mouse serum or peritoneal ascitic fluid. In contrast, the other component was significantly inactivated with the mouse humoral fluids, and lacked tumor-inhibitory activity. While the differences in the pH optimum and the stability of the two components may account in part for the difference in tumor-inhibiting properties, they felt that the true reason was not yet known. In addition they stated it seems that the use of an enzyme derived from an organism closely related genetically to the recipient would lessen the possibility of eliciting an immune response in the host, and also that treatment with larger doses of the enzyme over a shorter period of time would appear to be highly desirable in tumor therapy.

Various phases of research on L-asparaginase had by this time been going on. Dolowy et al became interested in finding

out if L-asparaginase was capable of affecting an intracerebral tumor.[21] Partially purified preparations of L-asparaginase from guinea pig serum and E. coli were tested on intracerebral 6C3HED tumors. Both guinea pig serum L-asparaginase and E. coli asparaginase when given in sufficient doses were found to be effective in causing prolonged regression of intracerebral 6C3HED lymphomas actively growing in C3H mice. The authors felt it was not known whether the "drug" penetrated the meninges or the brain substance directly.

In another vein, other authors were working toward explaining a mechanism of action. Sebin and Kidd found that heated guinea pig serum injected intraperitoneally in mice in which lymphoma 6C3HED-OG (of Gardner's original line) were growing in the peritoneal cavity, quickly induced an alteration in the protein metabolism of the proliferating lymphoma cells, as shown by studies with radioactive valine.[22] Also, they noted that alterations in nucleic acid metabolism became manifest in the asparagine-dependent cells after long exposures to the guinea pig serum in vivo, perhaps, they suggested, as a result of a primary inhibition of protein synthesis.

About the same time, Mashburn and Wriston presented evidence that the regression of lymphosarcoma 6C3HED induced by L-asparaginase was preceded by an increase in the alkaline ribonuclease of the post-mitochondrial cell fraction of the tumor, while the acid ribonuclease was increased later during the regression.[23] They felt that since RNase is a possible regulatof of protein synthesis, its increased activity

could lead to cell destruction. In addition, they stated the cytotoxic effect of asparaginase on 6C3HED cells *in vitro* reported by Berrela and workers[24] was accompanied by a reduction in the amount of tritiated cytidine taken up by the cells, and suggested that such a decrease could be caused by an increased rate of RNA destruction by RNase which had been activated by asparaginase as well as by a decrease in RNA biosynthesis.

Continuing in the line of Roberts and co-workers[20], Broome and his associates found that, depending upon the conditions of growth, *E. coli* cells possessed one or two asparaginase activities.[25] This was due to two distinct enzymes which differ in a number of properties, most significantly in their affinities for L-asparagine, and they found the enzyme with the lower affinity to be ineffective. Agouti serum, which contains an L-asparaginase with an affinity intermediate between those of the two enzymes from *E. coli*, inhibited tumors less than did the *E. coli* enzyme with the higher affinity. They concluded that the affinity of asparaginase for its substrate was related to its degree of effectiveness against sensitive tumors.

At the same time, Berrela and his group were studying the anti-tumor activity of guinea pig serum fractions *in vivo* and *in vitro* by a variety of methods including bright field, fluorescence, and electron microscope observations, and by dye permeability and cytidine-^{3}H incorporation in 6C3HED lymphosarcoma cells.[26] In C3H mice bearing the ascites tumor treated with the L-asparaginase fraction, they found a decrease in the total number and mitoses of tumor cells, a reduction in

RNA, and a rise in the number of macrophages and phagocytosis of tumor cells. Anti-6C3HED isoantibodies were not detected in their sera. The L-asparaginase fraction was also found to have an *in vitro* cytotoxic activity, this fraction acting directly on the tumor cells. Hence, they concluded that the *in vivo* host macrophage response and phagocytosis of structurally normal cells appeared to be a secondary, nonspecific phenomenon.

It remained until December of 1966 for an article to appear in which the effects of L-asparaginase on tumors in higher animals, including man, were described. Dolowy *et al* showed the enzyme to be effective and active against early (day 0) through late (day 24) 6C3HED subcutaneous tumors in C3H mice, but whole guinea pig serum in the same doses was not effective against intracerebral tumors comprising the same cells.[27] Whole guinea pig serum caused no acute toxic effects in mice with small tumors, and no toxic effects were seen after administration of a partially purified L-asparaginase (PPLAFI) in C3H mice with small tumors from 0-2 days or in monkeys during nine days of observation. Profuse diarrhea was observed in mice with massive tumors after PPLAFI treatment, during tumor regression.

In the same article it was reported that PPLAFI was given IV to an eight year old boy with a three year history of acute lymphoblastic leukemia. He had been previously treated with prednisone, 6-MP, methotrexate, Cytoxan, irradiation to the central nervous system and right testis, and vincristine. After 33 months, however, he became refractory to all of these agents, in frank relapse, with progressive disease. Consent was obtained to try L-asparaginase on this boy. During the

infusion of 390 ml. the boy's temperature rose from 99 to 104 degrees F., his pulse from 150/min. to 190/min., and the respiratory rate from 36 to 60/min. at two hours of infusion. His blood pressure fell from 120/80 to 90/60 at five hours, and the hematocrit from 21 to 15 in four hours with evidence of marked hemolysis which necessitated the cessation of L-asparaginase administration. Ten hemorrhagic bowel movements had occurred during the infusion. In the next seven days, his wbc's decreased from 18,000/mm^3 to 4700/mm^3, lymphoblasts from 67% to 14%, liver size decreasing, as did the size of the tumorous testis. The patient died with pulmonary hemorrhage ten days after treatment. Leukemic infiltrates were found at necropsy to be present in the meninges, brain, liver, spleen, marrow, and testis. The authors concluded by saying that further work was needed to ascertain the reason for the hemolysis, whether treatment of humans without prior infiltration of the central nervous system by tumors would result in significant remissions, whether human lymphoblasts are dependent on L-asparagine, what blood levels of L-asparaginase occur in mice after therapeutic doses, and whether these blood levels of L-asparaginase could cause remission of acute leukemias in hu ans.

Other investigators continued to be motivated. Boyse et al compared the activity of guinea pig serum in suppressing certain leukemias (e.g. radiation-induced EARAD-1) with the activity of asparaginase EC-2 from E. coli.[28] The latter enzyme was found to be considerably more active in experiments with EARAD-1 leukemia, provided it was used in treating established leukemia, and not administered as a single dose at the time of inoculation of the disease. Permanent cures were ob-

tained with the aid of 2000 or more "units" of EC-2. The fact that it was possible to re-inoculate the disease seemed to show that immunologic factors did not contribute to the cure. Re-inoculated survivors were shown to be successfully retreated with EC-2 L-asparaginase.

Meanwhile, Boyse et al compared "delayed" testing with "concurrent" testing for the evaluation of chemotherapeutic agents using samples of L-asparaginase as test materials.[29] A large difference in the detection of the anti-tumor activity of the agents was found, depending upon the method of testing. Guinea pig serum L-asparaginase was quite effective in repressing tumor development when administered within an hour of tumor inoculation, while an L-asparaginase (EC-2) from E. coli was not. The "delayed" test (treatment given seven days after tumor inoculation) however, showed that EC-2 was able to induce complete remission of an established transplant of each of the three lymphoid tumors tested (EARAD1, 6C3HED, and P1798). These authors concluded that their results point to the value of the "delayed" test for the screening of potential chemotherapeutic agents. This conclusion was in direct opposition to the view of Jameson et al[4] set forth several years earlier, but can be explained on the fact that Jameson and his workers were not dealing with a purified substance, but with "whole" guinea pig serum.

In a direct attempt to measure the toxicity of L-asparaginase to normal and leukemic human lymphocytes, Delewy and his group designed a study to determine what, if any, cytocidal effect would occur.[30] Their quantitative in vitro tests showed that purified preparations of the L-asparaginase from

E. coli were more toxic to blood lymphocytes from 12 of 15 patients with chronic lymphocytic leukemia than to lymphocytes from 25 persons with normal hemograms. Incubation for seven days with 10u/ml. killed, on the average, 77% of leukemic and 34% of normal lymphocytes. The reagent produced appreciable toxicity to leukemic lymphocytes after two days of incubation. Although some tumor cells are known to require L-asparagine, the authors concluded, the requirements of normal and leukemic human lymphocytes for L-asparagine remained "not known".

An interesting finding was introduced shortly thereafter by Prager and co-workers when they reported observing long-lived immunity to the 6C3HED ascites lymphosarcoma in C3H/HE mice following treatment of the tumor-bearing animals with *E. coli* L-asparaginase.[31] This tumor, which had its origin in C3H mice, is normally lethal for this strain. Immunity of C3H/HE mice was demonstrated by 1) tumor rejection, 2) neutralization of tumor cells by immune serum and lymphoid extract and 3) *in vitro* cytotoxicity of the same preparations. No information as to the processes at work in this novel phenomenon were available at the time of publication, but a combination of factors was felt to be responsible to explain the acquisition of active immunity to the 6C3HED tumor cells.

In an attempt to explain some of the earlier reported discrepancies, Broome and Schwartz published another investigation which indicated that resistant lymphoma cells differed from sensitive ones in their greater ability to synthesize asparagine.[32] Moreover, it appeared that the resistant cells responded to deprivation of exogenous asparagine by increas-

ing their rate of asparagine synthesis, producing sufficient amino acid for normal protein synthesis even when substantial amounts were lost to the medium. In a rather unique set-up, Broome and Becker were able to show a similar result.[33] They found that L-asparaginase in agouti serum and in extracts from E. coli inhibited the early wave of mitosis occurring in rat liver approximately 30 hours after hepatectomy, but even with continued treatment of the animal, the later wave of mitosis at 50 hours was not inhibited. This result did differ from the permanent inhibition of growth which asparaginase causes in various tumors. These authors felt that the reduced amount of asparagine in the liver after treatment with asparaginase must be critically low for processes essential for cell growth, particularly for the synthesis of large amounts of new protein. However, after a delay, sufficient asparagine apparently must be available for growth, the most likely source being an increased rate of synthesis in the liver cells themselves.

Delewy et al have remained interested in the cytotoxic effects of L-asparaginase.[34] They found the 6C3HED lymphoma, which was susceptible to E. coli in vivo, also susceptible in vitro. The 6C3HED-ECLAR1 lymphoma, resistant to E. coli L-asparaginase in vivo, was likewise resistant in vitro. At two days of incubation, these cells responded to L-asparaginase concentrations of 1000-10,000-fold of that needed to exert a cytotoxic effect upon the susceptible cell line. Spleen cells were found to be about as resistant as the 6C3HED-ECLAR1 lymphoma. Thymus cells were more susceptible than spleen cells but more resistant than the susceptible tumor cell line.

Old et al returned to the study of higher mammals.[35]

Three dogs with advanced lymphosarcoma were treated with L-asparaginase from E. coli. All three responded by marked regression of lymph nodes and dramatic improvement in general condition without evidence of toxicity. Two dogs returned temporarily to normal health and were clinically free of disease; the other showed a partial remission. One remained in good health for 50 days after treatment was discontinued, although the disease began to show signs of recurrence. Early relapse in the other two cases was attributed to insufficient dosage of L-asparaginase. The authors did show that the occurrence of sensitive tumors is not peculiar to laboratory rodents and that sensitivity is not restricted to the transplanted tumors that have been used in past studies of L-asparaginase. They were encouraged in the belief that asparaginase-sensitive tumors will be found in man, and that toxicity for man would present no real problems. They suggested that the selection of patients for treatment would be facilitated by the development of a simple test, as that described by Sobin and Kidd[18], to indicate asparagine-dependence of human lymphoma cells or other human tumor cells in vitro.

At the same time, Hill et al were studying the effects of L-asparaginase therapy on spontaneous lymphosarcoma in two dogs and on three humans with acute lymphatic leukemia.[36] The two dogs with advanced lymphosarcoma showed rapid and complete regression of lymph node masses following the L-asparaginase therapy. One of the dogs was given the enzyme IV for three days, after which time the therapy was discontinued for lack of enzyme. Subsequently, the tumor returned and the dog died four months later. The second dog was given

a larger dose and it is still alive and doing well five months after beginning treatment, although the authors report a recent enlargement of cervical nodes for which the same treatment was being resumed.

The first of their human patients was a seven year old white male who "was in a terminal stage with rapidly resistant neoplastic cells". He was eventually given 3300IU of the enzyme over a five day period, in conjunction with a daily dosage of 30 mg. prednisolone, which was decreased to 15 mg. and then 10 mg. during treatment with L-asparaginase. Leukocytes decreased from 5000/mm^3 to 900/mm^3 and the lymphoblasts from 32% to 8%. Thereafter the patient deteriorated, and expired 18 days after the last treatment. At necropsy, typical findings of terminal acute leukemia were observed, but, with the possible exception of "fatty metamorphosis" in the liver, "no changes were observed that could be related to the L-asparaginase therapy". The second patient was an 18 year old white male who was given IV a total of 80,020 IU of L-asparaginase over a six day period. An immediate clinical and laboratory remission was observed and the boy "left the hospital shortly thereafter feeling well". Due to a depletion of enzyme supply the same treatment was unable to be reinstituted some three months later when the boy began having a laboratory relapse, and he was therefore given maintenance therapy with standard agents.

Their last patient was a nine year old white male who was chosen because of his poor response to standard therapy, and rapid deterioration of his clinical condition. He was

given massive doses (exact amount not specified) over a more prolonged period of time and enjoyed a relatively good remission until he developed a terminal septicemia from E. coli and moniliasis some four plus months following the onset of the enzyme therapy.

These authors were encouraged by the lack of side effects observed in their study and felt that the disadvantages of amino acid depletion therapy appear to be few. They concluded by intimating that although this whole concept of treatment is attractive, it is not possible to truly evaluate the therapeutic implications of this treatment until more extensive investigations have been accomplished.

The most recent study reported that was reviewed by the author was the result of investigations done by Oettgen et al.[37] Clinical trials with E. coli L-asparaginase EC-2 w re conducted in eleven patients for the purpose of determining whether responsive lymphomas and leukemias in fact do occur in man. Daily dosages ranged from 50-200 IU/kg IV for 6-112 days, with total dosages ranging from 1050-6300 IU/kg. Favorable prompt responses occurred in five patients with acute lymphoblastic leukemia and in one patient with acute myeloblastic leukemia. Bone marrow remissions, lasting up to 15 monkswere observed in three children with acute lymphoblastic leukemia on maintenance therapy. Two patients with acute myeloblastic leukemia and two with lymphosarcoma did not respond. These authors observed side effects of fever, nausea, and weight loss. They report a positive correlation between response in vivo and predictive tests indicating asparagine-dependence of leukemia cells in vitro, and they suggest that

this could provide a means for selection of suitable patients.

In summary, this has been an outline of the majority of these research endeavors concerning L-asparaginase, including its discovery and subsequent clinical trials, both on laboratory animals and on human leukemia patients. The results to date have been encouraging but not conclusive. It appears that further probing into the metabolic and nutritional differences between neoplastic and normal cells, as exemplified by the trials with L-asparaginase, offers a truly promising area of cancer research.

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