In vitro Cytocidal Effect of L-Glutaminase on Leukaemic Lymphocytes

Mouse lymphomas, such as 6C3HED, undergo complete regression after one or more injections of L-asparaginase¹, and remissions have been reported in some patients with acute leukaemia after treatment with L-asparaginase²⁻⁴. Roberts *et al.*⁵ found that purified bacterial L-glutaminase inhibited the growth of Ehrlich mouse carcinoma. Before considering this enzyme for therapeutic studies in man, it is necessary to study *in vitro* effects of the enzyme on human blood cells. Previous work⁶ demonstrated that the cells of 6C3HED lymphoma do not survive incubation with very low concentrations of L-asparaginase (1.7 mIU/ml.), while the cells of the *in vivo* resistant variant of 6C3HED were not killed by 170 mIU of enzyme/ml. The blood lymphocytes from patients with chronic lymphocytic leukaemia were found to be more sensitive than normal lymphocytes to incubation with 170 mIU of L-asparaginase/ml. but not as sensitive as 6C3HED mouse lymphoma which responds *in vivo* to the enzyme. In this study, the slide chamber method⁷ was used to compare the sensitivity of normal and leukaemic lymphocytes to glutaminase.

The blood cells tested were obtained from five persons with normal haemograms, from eight patients with chronic lymphocytic leukaemia, and from three patients with acute leukaemia. Suspensions of the blood cells plus varying amounts of L-glutaminase were incubated at 37° C for 7 days. The suspensions were then examined in a special slide chamber and the surviving cells were counted. The criteria of viability depended on the morphological integrity of the cells under phase microscopy⁷.

The L-glutaminase was derived from bacteria and was described as preparation GA : 1.2 in a previous report⁵. The preparation also had L-asparaginase activity equal to 80% of the glutaminase activity. In all experiments, control tests were done with equivalent amounts of purified L-asparaginase to evaluate toxic reactions due to L-asparaginase.

A summary of the findings is given in Table 1. Most of the normal lymphocytes survived incubation with 1,700 mIU of glutaminase/ml. The cells of one patient (M.U.) with chronic lymphocytic leukaemia had approximately the same resistance to the enzyme as normal lymphocytes. In the case of the other seven patients with chronic lymphocytic leukaemia and of three

patients with acute leukaemia, very few cells survived incubation with 5 to 0.5 mIU of enzyme/ml.

The media of the incubated cells were analysed to determine the effect of the enzyme on the concentration of glutamine, ammonia and glutamate. As would be expected, the enzyme caused a decrease in glutamine (to <0.02 mmol) in suspension of both the leukaemic and normal lymphocytes. The ammonia and glutamate increased correspondingly to 1.0 and 0.8 mmol, respectively. In further experiments, the addition of 1 mmol of NH₄Cl or 0.8 mmol of sodium glutamate or both had no effect on the survival of leukaemic lymphocytes. These findings suggest that the decrease of glutamine in the media killed leukaemic but not normal lymphocytes *in vitro*.

According to our findings, the leukaemic lymphocytes from seven of eight patients were killed directly or indirectly by L-glutaminase, usually at the level of 1.7 mIU/ml. It is noteworthy that this dose of L-asparaginase was cytotoxic to cells of 6C3HED mouse lymphoma⁶ which undergoes remission *in vivo* with L-asparaginase therapy.

Normal lymphocytes resisted the concentration of 1,700 mIU of L-glutaminase/ml. although leukaemic lymphocytes usually responded to 1.7 mIU/ml. Previous work from this laboratory⁸ showed that the leukaemic lymphocytes are also more sensitive *in vitro* than normal cells to other reagents including L-asparaginase, prednisolone, dimethyl sulphoxide and heat. For example, leukaemic lymphocytes were sensitive to prednisolone at the level of 1 µg/ml. and to L-asparaginase at 170 mIU/ml. Glutaminase, however, acted at a much lower concentration than the other reagents and showed a much greater difference in its effect on normal and leukaemic cells.

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Table 1 Percentage of Lymphocytes or Blasts that survived Incubation at 37° C for 7 Days with Varying Amounts of L-Glutaminase

Source of cells	Cells surviving (%)							
	0	1,700	170	17	5	1.7	0.5	0.17
Normal persons (5)	53	33	35					
Patients with chronic lymphocytic leukaemia								
M. U.	32	18	11					
P. K.	46	—	3	3	3	13	33	59
K. O.	48	—	—	2	1	2	1	38
S. T.	39	—	—	0	0	1	5	29
C. S.	43	—	0	1	0	2	—	—
K. R.	76	—	0	4	1	3	—	—
H. U.	42	—	0	0	0	0	—	—
L. A.	65	—	4	8	11	7	43	66
Patients with acute leukaemia*								
P. H.	55	—	0	—	0	1	—	—
T. K.†	45	—	3	12	35	34	—	—
W. M.†	27	—	2	2	1	2	10	—

* P. H. had acute plasma cell leukaemia; T. K. had acute myelomonocytic leukaemia; W. M. had acute lymphoblastic leukaemia.
† Cells were incubated for 5 days.

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Inequality of Inspired and Expired Gaseous Nitrogen in Man

IN studies of animal respiration, it is conventional to assume that the amounts of nitrogen inspired and expired are equal, and this assumption has passed from one textbook to another¹⁻¹². The argument is that there is no known metabolic reaction of the human body involving molecular nitrogen, and