ASPARAGINE SYNTHETASE ACTIVITY OF THE LEUKOCYTES IN PATIENTS WITH ACUTE LEUKEMIA

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The asparagine synthetase (AS) activity was studied in the leukocytes of healthy subjects and patients with acute leukemia. AS activity in normal leukocytes ranges between 0.01 and 2.0 nmole/mg protein/h. No AS was found in many of the donors. AS is completely absent from the leukocytes of patients with various forms of acute leukemia. Increased AS activity after asparaginase therapy was observed in a patient with a lymphoblastic type of acute leukemia resistant to the antitumor action of asparaginase. The content of free asparagine in leukemic cells was a little lower than in healthy human leukocytes.

An important condition of the clinical application of asparaginase is its strictly selective and specific action on certain tumor cells [1, 2]. Animal tumors in which asparagine synthesis, catalyzed by the enzyme asparagine synthetase (AS), have been shown to be sensitive to the action of asparaginase [7]. Tumors resistant to asparagine, like normal cells, synthesize asparagine in sufficient amounts to maintain their own basic metabolic functions. Although the mechanism of the antitumor action of asparaginase has not been completely explained, it is presumed that the selectivity of its action on certain types of human leukemia is connected with absence or a deficiency of AS in leukemic cells.

This investigation was devoted to the study of AS activity and the asparagine content in leukocytes of healthy subjects and of patients with various types of acute leukemia.

TABLE 1. Asparagine Synthetase Activity in Leukocytes of Healthy Donors and Patients with Leukemia

Source of leukocytes	Number of cases	AS activity (in nm/mg protein/h)
Donors	50 (27)	0,01—2,0
Patients with acute leukemia:		
lymphoblastic	20 (0)	0
myeloblastic	8 (2)	0,001—0,002
histiomonocytic	3 (0)	0
undifferentiated forms	12 (0)	0
Patients with chronic lymphatic leukemia	4 (0)	0

Note. Number of cases in which enzyme activity was found in shown in parentheses.

EXPERIMENTAL METHOD

Aspartic acid-C^{14} was used. Since the commercial preparation contained 15% of asparagine-C^{14}, the aspartate-C^{14} was purified by chromatography on an alumina column. The highly purified preparation of aspartate-C^{14} was used in appropriate dilutions to determine AS activity. The incubation sample (1 ml) contained supernatant of fragmented leukocytes (4-10 mg protein), aspartate-C^{14} (10×10^6 pulses/min), unlabeled aspartate (2 μmoles), L-glutamine (20 μmoles), ATP (10 μmoles), and $MgCl_2$ (10 μmoles). Incubation was carried out in 0.1 M Tris-buffer, pH 7.8, for 1 h at 37° C. The control sample contained the same components and a suspension of leukocytes inactivated by boiling. The reaction was stopped by the addition of 0.5 ml 0.8 M $HClO_4$ which was then neutralized with 7 M KOH. Unlabeled asparagine was added to the supernatant and the asparagine-C^{14} formed as a result of the reaction was separated from the aspartate-C^{14} on alumina columns. Samples were taken

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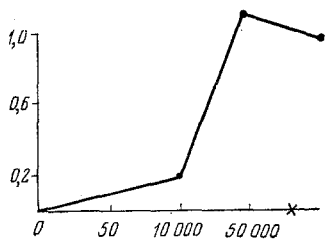

Fig. 1. Dynamics of asparagine synthetase (AS) activity in leukocytes of a patient with acute lymphoblastic leukemia. Abscissa, asparaginase activity (in i.u.); ordinate, AS activity (in nmoles/mg protein/h). End of asparaginase administration is marked by a cross.

from the eluate (10 ml) as follows: 0.5-1.0 ml to determine radioactivity and 30-50 μmoles for identification of asparagine by electrophoresis on paper. The unit of AS activity adopted was the number of nanomoles of asparagine formed per milligram protein per hour.

Free asparagine was determined by an enzymic method [3] based on the conversion of asparagine into aspartate, subsequent transamination of the aspartate into α-ketoglutarate, and measurement of the optical density of NAD reduced as a result of conversion of the oxaloacetic acid formed into malate. The supernatant of leukocytes obtained after boiling and centrifugation was used. Under experimental conditions equilibrium of the reactions was completely shifted toward malate formation. The asparagine content (in μmoles) was calculated by the formula

$$\frac{\Delta E_{360} \cdot V}{\varepsilon \cdot L}$$

where ΔE_{360} represents the difference in optical density of samples hydrolyzed by asparaginase and without the addition of asparaginase; V the volume of the sample; ε the coefficient of molar extinction; and L the cell layer thickness.

EXPERIMENTAL RESULTS

AS activity was investigated in the leukocytes of healthy human donors and patients with acute leukemia. The results of these experiments are given in Table 1.

Table 1 shows that AS activity is healthy human leukocytes is very low and varies within wide limits. In many donors (23 of 50) no enzyme whatever was found. Considerable fluctuations in AS activity in human lymphoid tissue and bone marrow have been noted by other workers [6, 9]. The present observations showed that in most cases AS was totally absent. Of eight cases of acute myeloblastic leukemia AS was found in the leukocytes of only two patients, and the level of the enzyme was at the limit of sensitivity of the method. Because of differences between the relative proportions of white blood cells under normal conditions and in leukemia, some of the patients were studied in a stage of clinical and hematological remission, when blast cells were absent from the sternal marrow and peripheral blood. Both in the acute period of the disease and in the stage of remission no AS was detected.

In the study of asparagine synthesis in a patient with the lymphoblastic type of leukemia resistant to asparaginase therapy no AS was found in the blood cells. After injection of asparaginase the level of the enzyme was increased, and it remained high throughout the subsequent tests although asparaginase had soon to be discontinued because of the development of drug-induced cytopenia. Dynamics of AS activity in this patient's leukocytes is illustrated in Fig. 1. AS activity is known to be controlled by a negative feedback mechanism through the repression of synthesis of the enzyme by asparagine and through asparagine inhibition. Removal of asparagine from the blood after administration of asparaginase stimulates AS synthesis both in normal tissues and in tumors resistant to asparaginase [5]. In tumors sensitive to asparaginase the mechanism of AS regulation is disturbed. The absence or a very low level of AS in normal tissues and in some tumors can evidently be partially controlled by the asparagine present in them. Results indicating the participation of asparaginyl-tRNA in the regulation of AS synthesis have recently been obtained [4]. The repression and derepression of AS synthesis in human and mouse leukemic cells has been shown to be connected with the asparaginyl-tRNA level in the cells and also with changes in its acceptor activity. It is also possible that in the intact organism other regulatory mechanisms which have not been fully studied may also operate [10].

Determination of free asparagine showed that its level in leukemic cells, especially in the myeloblastic form of leukemia, is somewhat lower (22.4 nmoles/mg) than in healthy donors (38 nmoles/mg protein). These results indirectly indicate differences in the metabolism or synthesis of asparagine in normal and leukemic cells. However, the connection between the AS level in human leukemic cells and their asparagine content has not yet been fully explained. By contrast with mouse leukemia, in human leukemic

cells there is no precise correlation between the free asparagine content, the AS activity, and sensitivity to asparaginase [5, 6]. The absolute asparagine requirement of normal and leukemic cells likewise has not been established [8].

The results indicate definite differences both in the synthesis of asparagine and in its concentration in the leukocytes of healthy donors and patients with acute leukemia. Despite the selectivity of action of asparaginase, no difference in AS activity was found in patients with different types of acute leukemia. No correlation likewise was observed between the content of the enzyme in the cells of these patients and the course of the disease. The levels of free asparagine and AS activity in leukemic cells evidently cannot completely define the dependence of leukemic cells on asparagine and, consequently, they do not provide sufficient basis for the clinical application of asparaginase. The study of the regulatory mechanisms of asparagine synthesis could provide more reliable and promising criteria for the evaluation of the carcinostatic action of asparaginase.

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