L-ASPARAGINASE EFFECTS ON INTACT MURINE LEUKEMIA CELLS AND ON ISOLATED CELL PLASMA MEMBRANES

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Summary The enzyme L-asparaginase catalyzes solubilization of a minor portion of the total glycoprotein of isolated plasma membranes from the L5178Y murine leukemia. Such a solubilization was not found using intact L5178Y cells, or using cells or plasma membranes of the asparaginase-resistant L5178Y/ASP cells. The mechanism of asparaginase action therefore involves inhibition of glycoprotein and protein synthesis required for maintenance of membrane structure, rather than a direct lytic action on preformed membranes of the intact cell.

The enzyme L-asparaginase has marked anti-tumor and immunosuppressive activity, as summarized in recent reviews (1-3). Enzyme-catalyzed depletion of exogenous supplies of L-asparagine leads to a rapid inhibition of protein synthesis in cells lacking asparagine synthetase. We have reported evidence that L-asparaginase has a selective and early inhibitory effect on synthesis of asparaginyl-glycoproteins (4,5). Furthermore, the enzyme was found to catalyze hydrolysis of two high molecular-weight asparagine derivatives, fetuin (4) and asparaginyl-tRNA (6). A recent report (7) describing substantial solubilization of cell membranes by L-asparaginase has prompted the present examination of effects of the enzyme on intact cells and on isolated membranes; the asparaginase-sensitive L5178Y and the resistant L5178Y/ASP cell lines were employed here.

Methods The procedures for maintaining L5178Y and L5178Y/ASP have been described, along with methods for isolating these cells (5). Plasma membranes were prepared as described by Dods et al. (7). Where indicated, cells were labeled *in vivo* by intraperitoneal injection of 250 μCi of ^{3}H-L-fucose 12 hours, and again 2 hours before collection of cells from

tumor-bearing animals. Our methods for measuring anthrone-reactive carbohydrate, fucose, and sialic acid, together with the procedure for isolating cell surface glycoprotein bound by papain-sensitive linkages, have been reported (8).

Plasma membrane preparations containing glycoprotein labeled with ^{3}H-L-fucose were prepared from cells exposed to this radioactive precursor in vivo as described above. These membranes were incubated in 0.1 M Tris buffer at pH 7.2 for 30 min with the specified concentration of L-asparaginase (EC-2, derived from Escherichia coli, and supplied by Merck). Solubilization of radioactivity, and of anthrone-positive material and protein was measured. Cells were centrifuged at 105,000 x g and the supernatant was analyzed for released ^{3}H-fucose radioactivity, anthrone positive material, and protein.

Intact cells, previously labeled with ^{3}H-L-fucose in vivo, were suspended in Earle's salts (5×10^{7} cells/10 ml) and treated with 10 or 100 U/ml of enzyme for 30 min. The cells were collected by centrifugation, and acid-insoluble radioactivity of the cell pellets was measured. The supernatant fluid was concentrated in vacuo and fractionated on a 1 x 45 cm column of BioRad P-100 gel. The effluent was monitored for radioactivity.

Results When L5178Y or L5178Y/ASP cells containing labeled glycoproteins were treated with asparaginase in vitro, no significant release of radioactive material into the supernatant fluid was found (Table 1). Analysis of the supernatant fluids showed that the released high molecular-weight radioactivity had an average molecular weight of 100,000. In control or treated tubes, more radioactivity was released from L5178Y cells, a finding which may be related to the higher level of cell-surface glycoprotein in these cells as compared with L5178Y/ASP (Table 2). This finding of lower levels of cell surface glycopeptides in the resistant cells compared to the sensitive cells indicates that L-asparaginase resistance is a consequence of lower

Table 1. Results of treatment of ^{3}H-fucose labeled intact cells with asparaginase.

Cell line	Enzyme level (U/ml)	Cell pellet (counts/min)	High mol. wt. radioactivity in the supernatant fluid (counts/min)
L5178Y	0	35,000	600
	10	36,500	575
	100	34,575	610
L5178Y/ASP	0	21,600	310
	10	20,100	295
	100	20,700	305

Cells (50 mg, wet weight) were incubated with specified levels of enzyme for 30 min; the cells were then collected and acid-insoluble radioactivity associated with cell pellets was measured. The supernatant fluid was desalted on P100 gel columns and the high molecular weight radioactivity (average mol. wt. 10,000) was measured. Low molecular weight material (mol. wt. under 500) represented approximately 3000 counts/min from each supernatant, and was chromatographically identified as fucose.

Table 2. Cell-surface glycoprotein in papain-sensitive linkages

Cell Line	Anthrone (μg/g)	Fucose (μg/g)	Sialic acid (μg/g)
L5178Y	480	175	206
L5178Y/ASP	130	56	80

Cells (300 mg wet weight) were treated with papain (8), and release of glycoprotein into the medium was measured. Units are in terms of galactose equivalents (anthrone test) and micrograms of fucose or sialic acid released per gram of cells, wet weight.

levels of plasma membrane glycoproteins, the exact opposite of the finding for actinomycin D resistant cells, which have higher levels of plasma membrane glycoproteins (8,9). The lower levels of surface glycoproteins in the

Table 3. Effect of L-asparaginase on murine leukemia cell isolated plasma membranes. Data from 2 experiments.

Cell Line	Enzyme (U/ml)	Anthrone (μg/g)	Protein (Lowry) (μg/g)
L5178Y/ASP	0	2	0
	1	4	0
	10	2	0
	100	3	0
L5178Y	0	2	0
	1	12	11
	10	26	20
	100	23	15
L5178Y/ASP	0	0	1
	1	0	1
	10	0	2
	100	0	1
L5178Y	0	6	1
	1	22	32
	10	46	46
	100	34	33

Isolated plasma membranes were incubated with specified levels of enzyme for 30 min, and release of anthrone-reactive material (galactose units) and protein was measured. Units are in terms of micrograms of galactose and micrograms of protein released per gram of wet cells from which the membranes were derived.

L-asparaginase resistant cell line may be the result of adaptation or selection for plasma membranes which do not require asparaginyl-glycoproteins. That is, the lower amounts of anthrone-positive material and fucosyl and sialyl glycopeptide released by papain from the L5178Y/ASP cells (Table 2) may reflect the fact that the L-asparaginase resistant cell plasma membranes are essentially devoid of asparaginyl-glycoproteins since it is the synthesis of these glycoproteins which L-asparaginase inhibits (4,5).

Treatment of isolated plasma membranes from L5178Y cells with asparaginase resulted in the release of small amounts of anthrone-positive material and protein into an acid-soluble fraction (Table 3). This result was not found using isolated plasma membranes from L5178Y/ASP cells. Furthermore,

Table 4. Effect of L-asparaginase on ^{3}H-fucose labeled cell plasma membranes

Enzyme Level (U/ml)	L5178Y (counts/min)	L5178Y/ASP (counts/min)
0	290	284
1	430	285
10	630	273
100	810	285

Isolated plasma membranes from cells labeled *in vivo* with ^{3}H-L-fucose were incubated with asparaginase for 30 min; release of radioactivity into the supernatant fluid was measured. Each experiment represents approximately 1 mg of plasma membranes per tube; total radioactivity was 23,000 counts/min/mg of plasma membrane protein.

treatment with asparaginase of fucose-labeled plasma membranes isolated from L5178Y solubilized some radioactivity, a result not found using L5178Y/ASP (Table 4). We could not detect any alterations in the L5178Y plasma membranes using phase-contrast microscopy (1000X) after treatment of either intact cells or isolated plasma membranes with 100 U/ml of L-asparaginase for 30 minutes.

Discussion The data presented here show that minor amounts of glycoprotein are released from isolated L5178Y membranes by the action of L-asparaginase. L5178Y/ASP membranes were not so affected by the enzyme, nor could such a release be found using intact L5178Y cells. The rapid cell death resulting from treatment of L5178Y *in vivo* with asparaginase (10) cannot, therefore, be attributed to an immediate cell lysis resulting from an action of the enzyme on preformed membrane structures.

We have previously postulated that treatment with asparaginase results in a preferential and early inhibition of synthesis of asparaginyl-glycoproteins (4,5). Jasin and Prager (11) have confirmed this observation and have now reported that treatment with asparaginase impairs synthesis of

immunoglobulin IgG, an asparaginyl-glycoprotein, by spleen. Inhibition of synthesis of other asparaginyl-glycoproteins which play an important role in rapidly turning over plasma membrane integrity would be expected to result in early cell lysis. Thus a mechanism of action of L-asparaginase may be inhibition of synthesis of asparaginyl-glycoproteins which in turn inhibits membrane synthesis in rapidly turning over cell plasma membranes. The fact that neoplastic cells are somewhat more responsive to L-asparaginase may result from their more rapid division, the lesser likelihood of neoplastic cells to be in the G_0 phase of growth, and the more rapid membrane turnover of neoplastic cells.

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