An investigation into the mechanism of L-asparaginase resistance in L5178Y murine leukemia cells

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Summary. Resistance of leukemia cells to L-asparaginase is presumed to be due to increased expression of asparagine synthetase activity by resistant cells, so they are no longer dependent on an exogenous source of L-asparaginase for growth. The mechanism by which cells acquire the ability for increased enzyme expression, however, has not been clearly defined. Evidence presented here indicates that genomic alterations in the form of translocations, gene amplification, or increased P-glycoprotein expression, do not account for the phenotypic transformation from L-asparaginase sensitivity to L-asparaginase resistance. Instead, both sensitive and resistant L5178Y cells contain immunoreactive material detected by Western blotting with an antiserum prepared against bovine pancreatic asparagine synthetase. This suggests that the mechanism of resistance might involve modification of asparagine synthetase in L-asparaginase-resistant cells by an as-yet-unidentified mechanism or by inhibition of enzyme activity in the L-asparaginase-sensitive cells.

Keywords: Amino acids – Asparaginase – Leukemia – Resistance – Asparagine synthetase – Chemotherapy

Abbreviations: CHO: Chinese hamster ovary; DMEM: Dulbecco’s modified Eagle medium; EtBr: ethidium bromide; I.U.: international unit; PBS: 0.14 M NaCl, 0.01 M KCl, 0.02 M phosphate, pH 7.4; SDS: sodium dodecyl sulfate.

Introduction

The development of resistance to chemotherapeutic agents is one of the major obstacles to the successful treatment of cancer. Fundamental processes operative
in generating drug-resistant mutants cover a broad spectrum of mechanisms at the level of DNA modifications, membrane changes, and specific metabolic alterations. The seemingly inherent ability of cancer cells to adapt to chemotherapeutic agents can perhaps best be understood if examined within the context of a well-defined system in which acquisition of resistance occurs concomitantly with a detectable change in cell function.

In mammalian cells asparagine synthetase is responsible for the ATP-dependent synthesis of asparagine from glutamine and aspartic acid (Kartner et al., 1983; Mehlhaff et al., 1985). In contrast to the situation in most normal cells, asparagine synthetase activity in certain tumor cells is undetectable (Cooney and Handschumachar, 1970; Horowitz et al., 1968; Patterson and Orr, 1967; Pragar and Bachynsky, 1968a, 1968b), making them dependent on an exogenous source of asparagine for survival. The enzyme L-asparaginase, by catalyzing the hydrolysis of asparagine to aspartic acid and ammonia, deprives the malignant cells of the asparagine available from extracellular fluid, resulting in cell death (Boyse et al., 1967).

The purpose of this study was to investigate the basis of resistance of L5178Y murine leukemia cells to L-asparaginase, in light of the mechanisms previously described as mediating chemotherapeutic resistance in other systems. These included gene amplification, P-glycoprotein expression, chromosomal rearrangements that might alter regulation of asparagine synthetase expression, and production of an inactive gene product by L-asparaginase-sensitive cells. The results indicated that immunoreactive material can be detected in L-asparaginase-sensitive L5178Y cells by Western blotting with an antiserum prepared against bovine pancreatic asparagine synthetase. This suggests that the absence of enzymatic activity in the sensitive cells is most likely effected at the level of the translation product of the asparagine synthetase gene.

Materials and methods

Cell lines

L5178Y cells, originally derived from a methylcholanthrene-induced lymphoma in DBA/2 mice (Fischer, 1957), were obtained from the Division of Cancer Treatment, National Cancer Institute (DCT Tumor Bank), Frederick, MD. They are Thy-1.2-positive, surface immunoglobulin-negative, nonspecific esterase-positive, and L-asparaginase-sensitive (Yang et al., 1981). Cells were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% heat-inactivated, undialyzed fetal calf serum, 0.34 mM asparagine, 2 mM L-glutamine, and gentamicin sulfate (50 μg/ml). L-asparaginase-resistant cells were maintained in the same medium without the asparagine supplement. Cells were grown at 37°C in a humidified 10% CO₂-90% air atmosphere.

Derivation of L-asparaginase-sensitive and -resistant L5178Y cells

The genealogy of the L5178Y sensitive and resistant cells was unknown, since they were both originally obtained from the DCT Tumor Bank. Therefore, it was possible that genetic anomalies unrelated to L-asparaginase sensitivity or resistance could have accumulated in the cell lines and could obscure any results obtained in subsequent analyses. To circumvent this problem, it was necessary to derive sensitive and resistant cells that had recently