CORRESPONDENCE

LEVELS OF EXPRESSION OF MDR-3 AND GLUTATHIONE-S–TRANSFERASE GENES IN CHRONIC LYMPHOCYTIC LEUKEMIA LYMPHOCYTES

To the Editor:

Multidrug resistance involves the simultaneous development of resistance to a range of anticancer drugs and is due to the presence of elevated levels of a 170-Kd membrane glycoprotein termed P-glycoprotein, which is thought to act as an energy-dependent drug efflux pump[1] and is encoded by the mdr-1 gene. A second human gene, mdr-3, encodes a closely related protein.[2] Although it has been commonly believed that mdr-3 does not play a role in multidrug resistance, Nooter et al[3] have shown that increased expression of mdr-3 in prolymphocytic leukemia is associated with cyclosporin-induced increase in drug accumulation. In a recent issue of *Blood,* Sonneveld et al[4] have reported elevated levels of expression of both mdr-1 and mdr-3 in 29 of 31 chronic lymphocytic leukemia (CLL) patients mdr-3 expression was significantly higher in advanced stages of CLL and decreased in some patients with therapy-induced down-staging.

We feel that these findings require further amplification, particularly in view of the newer therapeutic strategies being developed for CLL. The finding that increased expression of mdr-3 is associated with lower intracellular levels of anthracyclines and that this may be reversed by cyclosporin and verapamil assumes importance in the context of increasing application of anthracyclines in the treatment of CLL. Many of our patients receiving "conventional" treatment with alkylating agents are potential candidates for alternative combination regimens containing anthracyclines (with or without Vinca alkaloids). Of particular interest is the possibility that the response to such treatments may actually be enhanced by prior or concurrent alkylating agent therapy, with its associated reduction in mdr-3 level.

We have now examined the level of expression of the mdr-3 gene in the lymphocytes of a further series of CLL patients and studied the possibility of its correlation with the level of expression of the glutathione-s–transferase (GST) pi gene. This gene is thought to be involved in some cases of resistance to alkylating agents, and its level of expression has previously been found to correlate with mdr gene expression in some systems.[5,6] Therefore it is of interest to define its level of expression in relation to mdr-3 in CLL.

With the exception of 1 patient, all of the patients in our study were diagnosed as having typical B-cell CLL, all Binet stage A or B. Ages ranged from 46 to 82 years, with a median of 68. Of the CLL patients, 3 had received no treatment. The other patients had received chemotherapy over varying periods of time, principally chlorambucil either alone or with prednisolone, and 2 patients had also received cyclophosphamide. One other patient had CLL in prolymphocytic transformation and had been treated with COP (cyclophosphamide, vincristine, and prednisolone) and splenic irradiation.

The leukaemic cells were obtained from peripheral blood samples. Erythrocytes were hypotonically lysed and mononuclear cell fractions were obtained by density centrifugation (Lymphoprep; Nyegaard, Oslo, Norway) of edetic acid anticoagulated blood. Cell viabilities exceeded 90% in all cases. The fractionated lymphoid cells showed Leu 12 or SIg/CIg light chain restriction in at least 80% of cells. RNA was isolated from the cells by the guanidinium thiocyanate/caesium chloride method, denatured, and slot blotted. After hybridization with ^{32}P dCTP random-primed–labeled cDNA probes, the final wash was with 0.1× SSC/0.1% sodium dodecyl sulfate at 65°C for 30 minutes. The cDNA mdr-1– and mdr-3–specific probes, HepG 2-26 and HepG 2-16,[2] were the gift of Prof P. Borst (Netherlands Cancer Institute, Amsterdam) and their specificity was confirmed by hybridizing plasmid DNA containing the mdr-specific cDNAs with the radiolabeled mdr-1 and mdr-3 probes. The GST pi cDNA probe was a gift of Dr J.A. Moscow (National Institutes of Health, Bethesda, MD). Blots were quantified by laser densitometery. To correct for any possible inaccuracies in RNA loadings, each filter was reprobed with an actin probe and RNA loading of each sample was normalized to the actin mRNA level.

The level of expression of the mdr-3 gene in 16 CLL patients was expressed relative to the mean value for 6 control individuals, which was set at 1 U (standard deviation, 0.34) and is shown in Fig 1. The mean of the patient values was 2.6 (range, 0.7 to 6.3; standard deviation, 1.3). Three of the patients had mdr-3 RNA values close to 1, whereas the remaining 13 had elevated levels. Eleven of these 13 patients had between 1.8 and 3.2 times control values, but two patients had very high values of 4.2 and 6.3 times controls. mdr-1 expression was also elevated in several patients. These results support those of Sonneveld et al.[4]

The level of mdr-3 gene expression in the one patient with prolymphocytic transformation was found to be indistinguishable (1.1

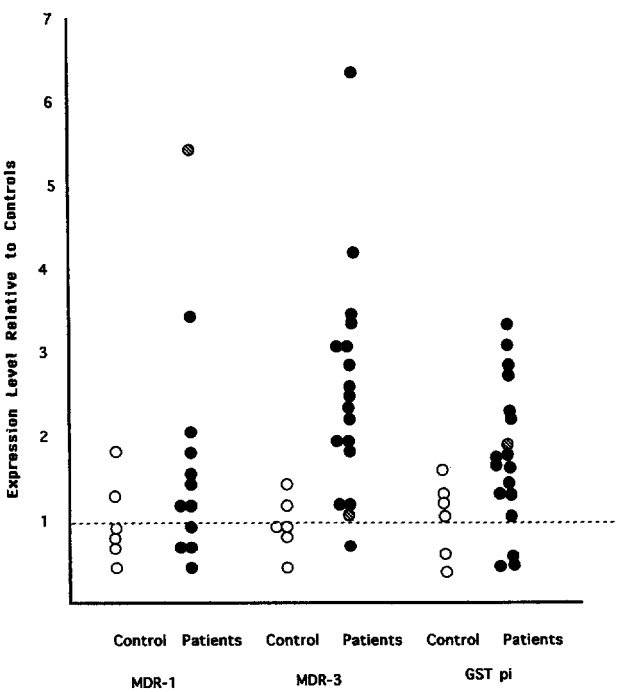

Fig 1. Expression of mdr-1, mdr-3, and GST genes in patients with hematologic malignancies. (○) Controls; (●) CLL; (◎) CLL in prolymphocytic transformation.

times) from control values. This patient had a high level of mdr-1 expression (5.4 times control).

The mean value of GST pi gene mRNA in all of the patients described above was 1.76 times the control mean (significantly different from the control value at the 0.1% level, t = 4.4), with a range of 0.5 to 3.3 times the control value. However, there was no correlation between GST expression and mdr-3 expression (correlation coefficient, −.17). This finding will be of interest in the context of assessing the overall pattern of expression of drug resistance-related genes in CLL. There is now a clear need to explore further these factors and others that may influence the response to treatment in patients with CLL.

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