Clinical Significance of Multidrug Resistance P-Glycoprotein Expression on Acute Nonlymphoblastic Leukemia Cells at Diagnosis

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To evaluate the clinical value of the expression of multidrug resistance P-glycoprotein (P-170) on the surface of acute nonlymphoblastic leukemia (ANLL) cells, we analyzed specimens from 150 newly diagnosed patients for staining with MRK16, a monoclonal antibody (MoAb) that binds to an external epitope of P-170. Other surface markers (CD13, CD14, CD15, and CD34) were studied by the same technique. A marker was considered positive when 20% or more cells were stained. Of 150 samples, 71 were P-170-positive. These patients were more frequently P-170-positive (23/71, 32%) than in P-170-negative cases (64/79, 81%) (P < 0.01). CD34 positivity was also associated with a low remission rate (P < 0.01). Survival was shorter for P-170- and CD34-positive patients (P < 0.01). The prognostic value of both markers was confirmed in multivariate analysis. CR duration was also shorter for P-170-positive cases, but the difference is less significant (P = 0.05). It is concluded that P-170 analysis may be an important tool for predicting the outcome of intensive chemotherapy in ANLL patients.

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D RUG RESISTANCE is the major cause of treatment failure in acute nonlymphoblastic leukemia (ANLL). The mechanisms by which leukemic cells are or become resistant to chemotherapy are still unclear. In vitro studies on tumor lines have shown that one mechanism implicates the multidrug resistance gene MDR-1, which encodes for a transmembrane glycoprotein (P-170) capable of expelling cytostatic drugs from the cytosol. An increase of MDR-1 RNA or of its product (P-170) has been reported in a large series of hematological malignancies, including multiple myeloma, non Hodgkin’s lymphoma, chronic myelogenous leukemia in blast crisis, and acute lymphoblastic leukemia. In ANLL, this overexpression was frequently observed in patients resistant to relapsed disease, but also in some patients at diagnosis or even in complete remission (CR). Despite this large number of studies, few prospective data are available regarding the clinical value of MDR-1/P-170 analysis in the blasts of ANLL patients at diagnosis. In a series of 36 patients, Sato et al showed a poor prognosis for those with high levels of MDR-1 RNA transcripts, while a preliminary report by Ball et al did not confirm the prognostic value of P-170 expression as detected by flow cytometry. In this study, we show that P-170 expression is associated with stem cell (CD34+) phenotype and with poor outcome of intensive antileukemic treatment.

MATERIALS AND METHODS

Patients. One hundred fifty patients with newly diagnosed ANLL presenting between June 1986 and December 1990 and treated by intensive chemotherapy were studied. Diagnosis was made on bone marrow smears according to usual cytological and cytochemical procedures, and classification was established according to French-American-British (FAB) criteria. Leukemias secondary to previous chemotherapy or to preceding myelodysplastic syndrome (MDS) were not excluded from the analysis. All patients gave informed consent to participate in the protocol.

Antileukemic treatment differed according to age and year of diagnosis, but for remission induction all patients received daunorubicin or mitoxantrone for 3 days and cytosine arabinoside for 7 days. CR was assessed according to Cancer and Leukemia Group B (CALGB) criteria. Details on induction and postinduction treatments are given in Table 1. Sixteen patients were treated by induced leukemias were more frequently P-170-positive. CD34 and P-170 expression were significantly associated. All patients were treated by intensive chemotherapy. Complete remission (CR) rates were significantly lower in P-170-positive (23/71, 32%) than in P-170-negative cases (64/79, 81%) (P < 0.01). CD34 positivity was also associated with a low remission rate (P < 0.01). Survival was shorter for P-170- and CD34-positive patients (P < 0.01). The prognostic value of both markers was confirmed in multivariate analysis. CR duration was also shorter for P-170-positive cases, but the difference is less significant (P = 0.05). It is concluded that P-170 analysis may be an important tool for predicting the outcome of intensive chemotherapy in ANLL patients.

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FC receptors. Cells were incubated with MoAbs at 4°C for 30 minutes, washed with PBS, incubated with F(ab'), fragments, and the staining was considered positive when 20%, 71 of 150 samples studied (47%) were considered positive for P-170 expression.

Correlations with clinical and biological characteristics. There was no correlation between P-170 expression and initial characteristics such as sex, age, presence of extramedullary disease, blood counts, or marrow blast percentage. No relationship was noted to FAB subtype, with the exception of M3 subtype, where only two of 12 cases were P-170-positive (P < .05).

One hundred twenty-two ANLL cases were considered de novo, while 12 cases were secondary to preceding MDS and 16 to chemotherapy for previous malignancy. P-170 was more frequently expressed in ANLL secondary to MDS (9/12) and previous chemotherapy (10/16) than in de novo ANLL (52/122) (P < .05).

The relationships of P-170 to other surface markers were studied. CD13, CD14, CD15, and CD34 were positive in 87% of samples, in cases with hyperleukocytosis, in cases with FAB subtype, and in cases with previous chemotherapy or MDS and 16 to chemotherapy for previous malignancy. P-170 was more frequently expressed in ANLL secondary to MDS (9/12) and previous chemotherapy (10/16) than in de novo ANLL (52/122) (P < .05).

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Prognostic value of P-170 expression. CR was obtained in 87 of 150 patients (58%). The remission rate was highly predicted by P-170 expression, as 23 of 71 (32%) P-170-positive patients obtained remission versus 64 of 79 (81%) P-170-negative patients (P < 10⁻⁴). The remission rate was also influenced by age (CR rate 54/75 for patients aged 60 or less v 33/75 for patients over 60, P < 10⁻³), WBC count (CR rate 54/78 for WBC ≤ 30 × 10⁹/L v 33/72 for WBC > 30 × 10⁹/L, P < .01) and preceding MDS or therapy-induced ANLL (CR rate 77/122 for de novo ANLL v 10/28 for secondary ANLL, P < .01). The influence of P-170 remained significant within each of these prognostic categories, in younger (P < .005) and older (P < 10⁻³) patients, in cases with (P < 10⁻³) or without hyperleukocytosis (P < 10⁻³), and in de novo ANLL (P < 10⁻³).
CD34 expression was also predictive of induction treatment outcome, as 28 of 83 (34%) CD34+ patients went into remission, versus 59 of 67 (88%) CD34- patients \( (P < 10^{-3}) \). By combining both markers, it was possible to define a subgroup with a very poor prognosis (both markers positive, CR rate 9/50) and a subgroup with very good prognosis (both markers negative, CR rate 45/46).

Factors influencing survival were first studied by univariate analysis. Survival was significantly shorter for CD34+ \( (P < 10^{-3}) \) and P-170-positive patients \( (P < 10^{-3}) \) (Fig 2). Age 60 or less was also significant \( (P = .01) \). In multivariate analysis, the significant variables were P-170 \( (P < .005) \), and age \( (P < .01) \). The presence of preceding myelodysplastic syndrome or secondary leukemia did not significantly influence survival, nor did initial counts, FAB classification, or other surface markers.

The remission duration was studied in 87 patients by univariate analysis, there was no significant factor, but patients with de novo disease, and with P-170-negative disease showed a trend toward longer remission \( (.05 < P < .1) \). In multivariate analysis, P-170 expression significantly influenced CR duration \( (P = .05) \).

DISCUSSION

The expression of MDR-1 products has already been reported in a large number of ANLL patients, mainly in refractory or relapsed disease, but also at diagnosis. The percentage of untreated cases expressing MDR1 obviously depends on the method used for detection. In our study using MRK16 MoAb and the staining technique described by Hamada and Tsuuo, we observed 47% positive cases. This is consistent with the results of Sato et al, who reported a high expression of MDR-1 RNA transcripts in 36 untreated patients. This percentage is much higher than that reported by Kennitz et al on a larger series of patients using a polyclonal antibody and an immunocytochemical technique. Like Sato et al, we did not observe a correlation with initial characteristics such as age, hematological parameters, or FAB classification (with the exception of FAB M3), and we confirmed the higher expression in therapy-related leukemias. This last finding might be explained by previous exposure to chemotherapy, but we also observed a high expression in ANLL secondary to MDS, which confirms the observations of Holmes et al. It is possible that P-170 is expressed more in leukemias arising from poorly differentiated cells. The partial correlation with CD34 is consistent with this hypothesis, and was recently reported by List et al in a series of 45 cases with MDS and therapy-induced ANLL.

High levels of MDR-1/P-170 in malignant cells have been associated with clinical resistance to chemotherapy in a variety of malignancies, including ovarian cancer, neuroblastoma, myeloma, and acute leukemia. However, few prospective studies have been conducted in patients with newly diagnosed ANLL. In the series of Sato et al, a higher CR rate and a longer remission duration were observed in patients with low levels of MDR-1 transcripts. We also observed a significant difference between P-170-positive and -negative patients with regard to CR achievement and survival. The difference was less significant for CR duration, but this may be explained by the fact that there were too few patients in the P-170-positive group in remission, and by the heterogeneity of postinduction treatments. Our results are in contradiction with those of Ball et al, although we used the same MoAb to detect P-170. Detailed analysis of this last study is needed to understand this discrepancy. Our study also confirmed the prognosis value of CD34 that we and others have already reported.

Although our data indicate that P-170 expression is highly correlated with therapy outcome, MDR is certainly not the only factor of drug resistance in ANLL. However, in vitro data show that drug resistance of ANLL cells to daunorubicin may be reversed by P-170 blockers such as verapamil. Our study contributes to the identification of patients with poor prognosis, but also suggests that therapeutic trials using P-170 blockers in addition to chemotherapy might be of interest, at least for patients with MDR-1-positive blast cells.

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