Molecular Genetic Techniques for Detection of Minimal Residual Disease in Acute Lymphoblastic Leukemia: Possibilities and Limitations

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Introduction

Despite impressive advances in the treatment of acute lymphoblastic leukemia (ALL), disease relapse following successful remission induction still poses a significant clinical problem (Champlin and Gale 1989). Since most recurrences originate from neoplastic cells escaping the therapeutic intervention, the development of methods to monitor individual responses of patients, to detect impending relapses prior to clinical manifestation, or to determine the quality of a bone marrow scheduled for autologous transplantation represents a major challenge of today's oncology.

Until recently, the methods used for the identification of residual disease, including Southern blot analysis, have not been sensitive enough to demonstrate less than 1%–10% neoplastic cells in a sample being examined, a detection level also achieved by morphological examination. However, the use of double-color immunofluorescence and polymerase chain reaction (PCR) strategies has opened new avenues to the analysis of minimal residual leukemia by allowing the identification of as few as $10^{-6}$ malignant cells (Campana et al. 1990a; Saiki et al. 1988). In the following, I will briefly summarize our experience with the application of PCR methods in diagnosis and monitoring of ALL Patients.

Clonospecific T-Cell Receptor $\delta$ Probes

The genes encoding immunoglobulin (Ig) and T-cell receptor (TCR) chains are all assembled from multiple segments which recombine during B- and T-cell differentiation. Accordingly, leukemia cells of every ALL patient exhibit a distinct immunogenotype that can readily be demonstrated by Southern blot analysis. Based on the unique pattern of Ig and/or TCR gene rearrangements, a variety of PCR methods have been proposed for the
evaluation of therapeutic efficacy (D’Auriol et al. 1989; Hansen-Hagge et al. 1989; Yamada et al. 1989). A strategy developed in our laboratory proceeds from the immunogenotypic characterization of 497 ALL patients enrolled in the German multicenter ALL trials BFM (children) and BMFT (adults). This study revealed that a TCRδ rearrangement and/or deletion is present in 97% of T-cell acute lymphoblastic leukemia (T-ALL) cases and also in 88% of common cell acute lymphoblastic leukemia (cALL) cases (Hansen-Hagge et al. 1989; Yokota et al. 1991). Even more remarkable is the observation of a preferential recombination pattern depending on the immunophenotype. Thus in T-ALL a prevalence of $V_{51}D_{J51}$ (29%), $V_{82}D_{J51}$ (11%), and $D_{62}J_{51}$ (19%) recombinations contrasts with a predominant rearrangement of $V_{82}D_{83}$ (52%) and $D_{62}D_{83}$ (16%) in B-precursor leukemias.

Despite this limited repertoire of recombination events occurring in ALL, the respective TCRδ loci show enormous junctional diversity due to imprecise joining and insertion of N-region nucleotides. The amplification of TCRδ junctional regions thus leads to the generation of clonospecific probes which can be used for the identification of minimal residual leukemia. The detection limit of each probe varies (Table 1), but they allow the identification of $10^{-4}$–$10^{-6}$ neoplastic cells in the vast majority of cases. In the few exceptions, sequence analyses of the junctional regions and subsequent synthesis of oligonucleotide probes finally provided us with tools of sufficient sensitivity.

Thus far we have analyzed bone marrow (BM) or peripheral blood (PB) samples obtained from 52 pediatric and 11 adult ALL patients, including 40 cALL and 23 T-ALL cases (Table 2). Since PB samples contained significantly fewer residual cells in all instances where both PB and BM specimens from a patient were tested, only data from the latter source are presented. In a group of 47 patients studied during complete clinical/hematological remission, bone marrow samples obtained during consolidation therapy exhibited remaining neoplastic cells at a level of $10^{-2}$ to $10^{-4}$ in most cases (Table 2). Remarkably, a significant number of patients still showed minimal residual leukemia at frequencies of $10^{-3}$–$10^{-6}$ during the phase of maintenance therapy. By contrast, patients generally lacked evidence of ALL cells after termination of treatment. However, it is important to note that the result obtained from a single PCR analysis bears only limited prognostic relevance. More important appears to be the determination of the kinetic behavior of a neoplastic cell population through serial PCR analyses. Thus, a steady increase of blasts may indicate an imminent relapse, while a continuous, albeit prolonged decline of neoplastic cells may

<table>
<thead>
<tr>
<th>Number of ALL cells</th>
<th>$10^{-2/-3}$</th>
<th>$10^{-3/-4}$</th>
<th>$10^{-4}$</th>
<th>$10^{-5}$</th>
<th>$10^{-6}$</th>
</tr>
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<td>Number of cases</td>
<td>3</td>
<td>4</td>
<td>21</td>
<td>27</td>
<td>8</td>
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