CLONAL INSTABILITY IN EARLY B-LINEAGE ACUTE LYMPHOBLASTIC LEUKEMIA

To the Editor:

In the January 1, 1992 edition of Blood, Wasserman et al reported data on clonal instability in acute lymphoblastic leukemia (ALL) by assessing the sequence of the third complementarity determining region (CDR-III) of the Ig heavy chain (IgH) gene at presentation and relapse. Only one case, with a 10-year remission before relapse, showed a complete clonal change from a total of 12 patients studied.1 In the January 15, 1992 edition of Blood, Tycko et al2 described a case of ALL with an early pre-B phenotype that showed clonal instability as assessed by the T-cell receptor (TCR) \( \gamma \) gene, but with a stable IgH gene sequence between presentation and subsequent relapse 5 years later. Both reports commented on the implications of these findings on monitoring minimal residual disease (MRD) by IgH or TCR gene amplification with the polymerase chain reaction (PCR) and hybridization with clone-specific oligonucleotide probes.1,2 We describe the case of an infant, also with an early pre-B phenotype, who showed evidence of complete clonal change based on assessment of the IgH gene between presentation and subsequent relapse 10 months later.

This male infant presented at 2 months of age with gross hepatosplenomegaly and a total leucocyte count of 169 \( \times 10^9/\text{L} \). Immunophenotypic analysis showed ALL of an early pre-B stage: TdT+, CD19+, CD34+, DR+, CD1-, CD7-, CD10-, CD13-, CD33-, and cytoplasmic \( \mu \). Cytogenetic analysis showed a normal male karyotype: 46:X, Y. Despite intensive chemotherapy, this infant suffered an isolated marrow relapse 10 months later. At relapse, the leukemic cells had an identical immunophenotype, but showed t(4;11)(q21;q23) on cytogenetic analysis. After further chemotherapy, a second relapse (with identical phenotypic and karyotypic findings) occurred, followed by death. Southern blot analysis showed a one-allele IgH gene rearrangement of different size from presentation to first relapse; the TCR genes were in the germline configuration throughout. The IgH CDR-III gene sequence was obtained from presentation and relapse marrow samples by PCR-based amplification using consensus variable (V) and joining (J) region primers and a direct sequencing technique.3 This was consistent with the Southern blot assessment showing the presence of completely different IgH gene rearrangements from presentation to first relapse (Fig 1). Clone-specific oligonucleotide probes were designed to both the presentation and relapse sequences. After radiolabeling, these probes can then be used to detect MRD at a level of 1 leukemic cell in \( 10^4 \) to \( 10^5 \) normal cells.4 In the case described, samples taken at presentation, during

Fig 1. (A) IgH CDR-III gene amplification products at presentation (P) and first relapse (R1). The PCR was performed using consensus V and J primers. After polyacrylamide gel electrophoresis, the size of the amplified IgH gene rearrangement can be assessed by comparison to a size marker (SM). The band obtained from the first relapse sample was identical in size to that from the second relapse (not shown). (B) Clone-specific probe hybridization. Presentation (P) and relapse (R) sequences were obtained by a direct sequencing technique. Clone-specific probes (underlined) were designed to the junctional areas of the IgH CDR-III sequences and synthesized. After radiolabeling, these oligonucleotide probes can then be used to detect MRD. Serial dilution of leukemic DNA into normal marrow DNA showed a sensitivity of detection of 1 leukemic cell in \( 10^4 \) normal cells for both the P and R probes (not shown). Both these probes were then hybridized with PCR products obtained from the amplification of marrow samples at presentation (P) and both relapses (R1 and R2) with the consensus V and J primers. Intervening “remission” samples were also tested at 1, 2, 3, 4, and 11 months. All samples tested were taken from the bone marrow (BM), except for one additional blood sample (VB) at 2 months. Clone-specific probes P hybridized only to the presentation sample. Clone-specific probe R hybridized only to the relapse samples and one intervening “remission” marrow sample. Neither probe hybridized to samples between presentation and first relapse.
morphologic remission (1, 2, 3, and 4 months into treatment), and at both subsequent relapses were tested with both presentation and relapse clone-specific probes. The results are shown in Fig 1. This case supports the hypothesis of Tycko et al that ALL of an early pre-B or stem cell stage may be prone to clonal progression. This may be particularly so for congenital or infant ALL. Analysis of 12 older children with ALL of a more mature B-cell phenotype (either common ALL or cytoplasmic μ+ pre-B ALL) by IgH CDR-III PCR and sequencing showed no other examples of total clonal change between presentation and subsequent relapse (unpublished data). Three additional points of interest are present in the case described. Firstly, the interval between presentation and relapse was only 10 months. This shows that, unlike the cases previously described, clonal change can occur as an early event within the initial treatment period. Secondly, the absence of hybridization of either clone-specific probe to the intervening “remission” marrow samples suggests that clonal change may have occurred via a third or intermediate IgH gene rearrangement not detected by PCR. Thirdly, the occurrence of the t(4;11) at relapse but its absence at presentation may suggest that this translocation is not the initiating genetic event in leukemogenesis. Alternatively, this translocation may have been present at diagnosis, but below the threshold of detection of the relatively insensitive technique of cytogenetic analysis.

In summary, we present additional information on the occurrence of clonal instability in childhood ALL as assessed by study of the IgH gene. This could cause a false negative assessment of MRD. However, as most examples of clonal instability do not involve loss of all the presenting IgH rearranged alleles, PCR-based amplification of the IgH gene CDR-III represents a useful and generally applicable technique for monitoring MRD. We agree with the conclusions of Tycko et al that analysis by two or more different techniques (eg, IgH and TCR γ or δ gene PCR) is recommended in ALL of an early pre-B or stem cell stage, although in the case described here this was not possible due to the germline configuration of the TCR genes.

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REFERENCES