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Letter to the Editor

Oncogene Amplification and Clonal Evolution in Acute Leukaemia

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IMMUNOGENOTYPING by use of antigen receptor gene probes offers a sensitive means to determine clonality in human leukaemias of B- and T-cell phenotype [1–6]. By this analysis, acute leukaemias usually present as monoclonal expansions, whereas lymphomas sometimes show evidence of oligo-clonality [7, 8]. We have recently studied a 16-year-old cALL patient with evidence of bi-clonality as determined by DNA analysis. The leukaemic blasts uniformly expressed HLA–DR, CALLA, and TdT. Analysis of DNA extracted from the leukaemic blasts using a probe derived from the joining region of the immunoglobulin heavy (IgH) chain gene [9] revealed the presence of 2 leukaemic clones (Fig. 1a). An extended immunogenotyping analysis revealed germ-line configurations for immunoglobulin light chain genes and the T-cell receptor (TCR) α and β chain genes. However, a monoclonal rearrangement at the TCR γ chain gene locus was detected with a TCR γ joining gene region probe [10] (Fig. 1b). Quantitative scanning densitometry analysis of the autoradiographs is compatible with the presence of 2 clones: the minor clone comprises about 20–30% of total cell mass and has 2 rearranged IgH alleles, and germ-line configuration at the TCR γ gene locus. About 60% of cell mass is occupied by the major clone, characterised by a rearranged and a deleted allele at the IgH locus in addition to a rearrangement at the TCR γ gene locus. The latter rearrangement can be viewed as evidence for lineage infidelity, which is not uncommon in acute leukaemia [11, 12]. Since the IgH rearrangements in the major clone could have arisen by further rearrangements in the minor clone, we suggest that clonal evolution had occurred in this patient characterised by rearrangements at both IgH and TCR γ chain genes.

The amplification of oncogenes is related to the progression of several malignancies [13, 14]. We therefore sought to determine whether this patient also had evidence for oncogene amplification. Amplifications of *c-myc*, *c-mos*, *bcr* , *c-abl*, *c-Ha-ras*-1, *c-N-ras*, and *c-Ki-ras*-2 were not found, however, this patient had evidence for *c-myb* amplification (6–8-fold) as indicated in Fig. 1c). This analysis was done by simultaneous hybridisation with the oncogene probe [15] and an unrelated sequence (the β-globin gene [16] in this particular instance), the latter being used to calibrate for the amount of DNA available for hybridisation on the filters.

Oncogene amplifications are rare in untreated acute lymphoblastic leukaemia [17, 18]. The same is true for oligo-clonality in this disease, which we have observed only once in a series of 14 ALL patients (Boehm TLJ, unpublished), a finding similar to those of others [19]. Although we cannot determine which clone carries the *c-myb* amplification, this unusual coincidence of bi-clonality and oncogene amplification in an untreated acute leukaemia may be pertinent to the role of oncogene amplifications in clonal evolution and progression of leukaemia. Expression of *c-myb* in acute leukaemias was recently shown to be highest in pre-B-ALL patients [20]. It is thus reasonable to speculate that *c-myb* amplification conferred a growth advantage to the original leukaemic clone.

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To our knowledge, this is the first report which relates oncogene amplification to clonal evolution in human leukaemia. It remains to be seen whether oncogene amplifications are the cause or a by-product of clonal evolution in human leukaemia. Nevertheless, it is possible that the presence of oncogene amplifications and/or signs of clonal evolution at diagnosis define a clinically relevant subgroup of patients with acute leukaemia.

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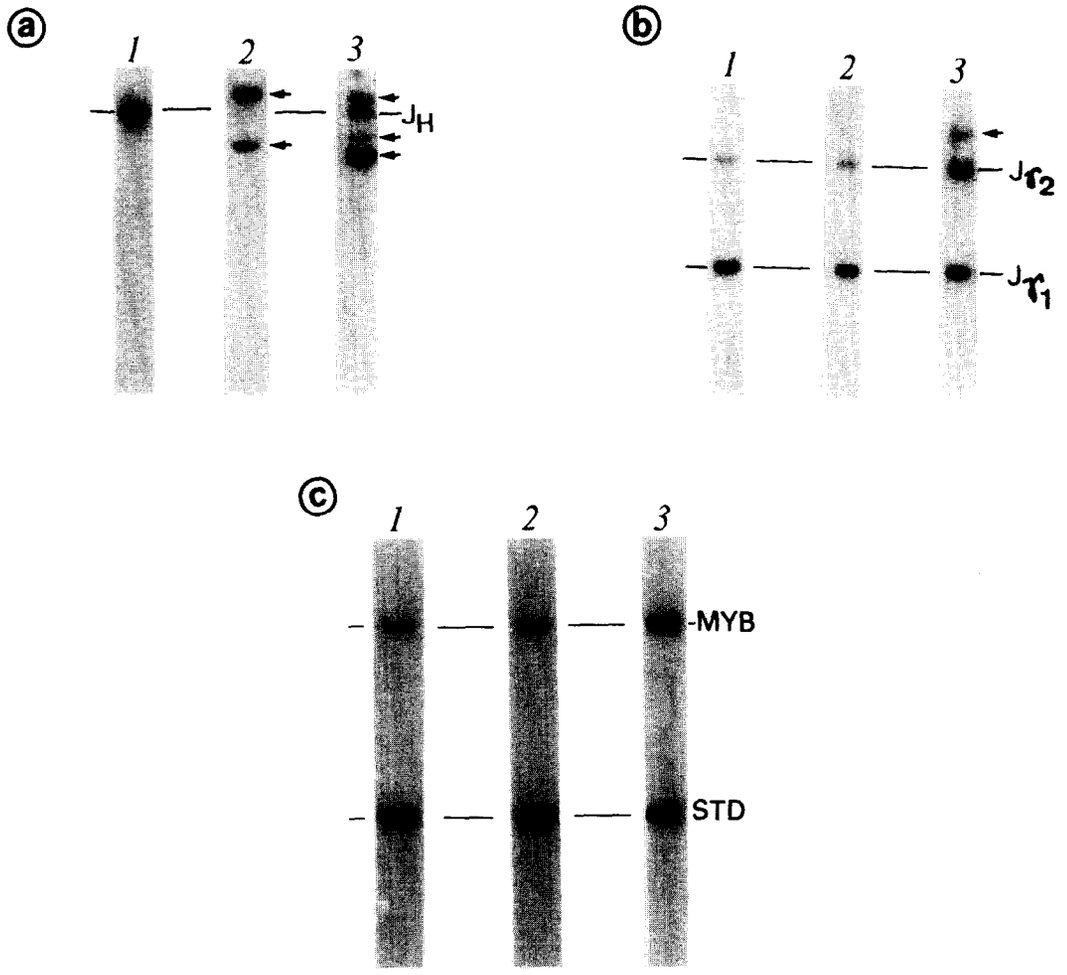

Fig. 1. *Autoradiographs for IgH* (*a*) *and TCR* γ (*b*) *chain gene rearrangements, and* c-myb *amplification* (*c*) *in placenta DNA* (*lanes labeled* 1), *and DNA extracted from leukaemic blasts of two ALL patients* [2, 3]. *DNAs were extracted, cleaved with EcoRI* (*a,b*) *and Bam HI* (*c*) *restriction enzymes, electrophoresed through agarose gels, blotted onto nylon membranes, and hybridised with the indicated radiolabeled gene probes* [11]. *The germ-line configuration is indicated by a bar, rearranged alleles are indicated by arrowheads. In* (*c*), *STD denotes the hybridisation signal of a* β*-globin gene probe used as an internal standard for hybridisation efficiency and the amount of DNA on the filters. Qualitatively similar results were obtained after digestion of DNA with BamHI and HindIII restriction enzymes, respectively. This rules out trivial explanations such as incomplete digestion, somatic mutation, or restriction fragment length polymorphism. No evidence for more than* 2 *chromosomes* 14 *was obtained by dosage analysis with chromosome* 14 *gene probes* (*T-cell receptor* α *chain gene probes*).