To the Editor:

In the July 1, 1991 issue of Blood, Trainor et al. reported the results of their method for detecting gene rearrangement in B- and T-lymphoproliferative disorders by using the polymerase chain reaction (PCR). The study is based on the assumption that monoclonality of B cells is characterized by a fragment length homogeneity of the amplified third-complementarity-determining-regions (CDR III), i.e., a discrete DNA band on electrophoresis, whereas polyclonality is characterized by DNA fragment length heterogeneity, i.e., a broad band or a smear on electrophoresis. They also reported that their amplification is lineage-specific (75% of specificity for B-cell tumors) showing a 2% to 5% sensitivity.

Although more sensitive and specific assays for detecting monoclonality in B-cell malignancies are already available, we would like to comment on some technical aspects of Trainor’s method: (1) The separation of DNA fragments on agarose gel, also using an appropriate concentration for fragment size, cannot discriminate differences of few bases. This is commonly seen, e.g., in the pattern of migration of the molecular weight markers commercially available. Because many B-cell clones have a quite similar number of nucleotides in the CDR III, they will give a discrete band on electrophoresis, mimicking a band composed by only one clone. (2) The efficiency of the PCR is affected by many factors: primer concentration, annealing temperature, buffer composition, number of cycles, and so on. Hence, any variation in the stringency of the reaction can influence the appearance of a DNA band on agarose gel. (3) Starting with a similar approach we amplified the complete assembled variable region (VDJ) by using leader sequence degenerate primers and a JH consensus sequence primer. The known leader sequences showed highly conserved regions and then could be used as 5′ priming site in the VDJ amplification.

When our method was applied to B-cell malignancies a discrete band was always obtained on electrophoresis, but after PCR product cloning and sequencing a mixture of different clones was always detected. From five patient analysis three different results emerged: (1) several identical sequences (tumor VDJ) and polyclonal sequences; (2) polyclonal sequences only; (3) clusters of identical sequences, not corresponding to the neoplastic VDJ later identified with a different method.

All of these data supported the hypothesis of a technical artifact, and one possible explanation was that a preferential priming, for some VDJs, could originate during the annealing phase, giving rise to a biased amplification of clones that were not necessarily the neoplastic one (i.e., the more represented in the specimen).

Based on these findings we elaborated a modified anchor-PCR strategy to avoid the troubles due to the preferential amplification that can occur using consensus sequence or degenerate primers.

Finally, also excluding the possibility of a preferential priming during the reaction, the presence of a discrete band on electrophoresis cannot be considered a proof of monoclonality unless confirmed by sequence analysis.

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REFERENCES

The letter by Corradini et al raises several separate issues.

(1) Preferential priming of VDJ sequences is a real phenomenon and it results in amplification of only a proportion of the sequences that are present. However, we have not found it to be a problem when amplifying polyclonal DNA extracted from peripheral blood or lymph nodes as so many VDJ sequences are present that amplification of even only a proportion of them still results in a heterogeneous smear on electrophoresis. We have, at times, found it to be a problem when only a few cells are obtained so that the number of VDJ sequences is limiting, as in occasional lymph node aspirates (Sykes PJ, Neoh SH, Brisco MJ, Hughes E, Condon J, Morley AA: Quantitation of targets for the polymerase chain reaction by use of limiting dilution. [submitted]). In this circumstance one or only a few bands may be observed and a mistaken diagnosis of monoclonality or oligoclonality may be made. However, this phenomenon will be recognized if a second aliquot of material is amplified, as this results in a band of different size being obtained. Therefore, we routinely study all node aspirates in duplicate.

We, too, have M13 cloned and sequenced the amplified discrete material obtained at diagnosis from patients with monoclonal lymphoid disorders. Although we have observed occasional, presumably polyclonal sequences, we have always observed an identical sequence in nearly all clones obtained from any one patient. More recently we have used direct sequencing and again have observed a discrete single ladder, except in one patient in whom amplification produced two bands that were inadvertently extracted into the same reaction mixture for sequencing. We have no clear explanation for the frequency of the technical artefact observed by Corradini et al except to suggest that their polymerase chain reaction (PCR) conditions may be suboptimal or that the length of their amplified product, which stretches from the leader sequence to the J region, results in less efficient amplification.

(2) We agree that agarose gels give suboptimal resolution and we now tend to use small polyacrylamide gels. However, unless the number of VDJ targets is limiting, the lack of resolution of agarose gels matters little, as there is enough size heterogeneity of amplified product to overcome any lack of resolution.

(3) We agree that the efficiency of the PCR is affected by many factors that can influence the final result. The reaction does need to be carefully optimized and we now use an annealing temperature of 63°C for the TCRγ primers and 50°C for the IgH primers, as preferential priming at low DNA concentrations seems to be lessened at these temperatures.

(4) Corradini et al have erred in not distinguishing the use of PCR to detect monoclonality from the use of PCR to detect and quantitate specific clones. Detection of monoclonality is mainly of value at diagnosis. It has a sensitivity of 2% to 10%, although this can be increased by a factor of 10 by several modifications, and this limited sensitivity means that detection of monoclonality is of limited value in detecting minimal residual disease. Once monoclonality has been detected at diagnosis, several groups, including ourselves, have shown that it is possible to proceed further, eg, by sequencing or by production of labeled probes, and detect the disease clone at very high sensitivity during the course of the disease. Thus, detection of monoclonality and detection of the disease-specific clone are not directed toward the same end, as implied by Corradini et al, but are complementary.
Analysis of immunoglobulin heavy chain gene rearrangement using the polymerase chain reaction [letter; comment]

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