To the Editor:

Weiss et al. investigated the translocation t(2;5) in Hodgkin’s disease (HD), a translocation primarily thought to be specific for anaplastic large cell lymphoma, CD30. They tested 34 cases and were not able to detect t(2;5) in a single case. This is in sharp contrast to the results we described recently. We were able to demonstrate t(2;5) in about 70% to 80% of cases of HD. These discrepancies may be most probably caused by technical factors and different methods used. The following considerations seem thus to be important:

1. Although we used fresh-frozen material, Weiss et al. used formalin-fixed and paraffin embedded tissue, for which it was shown earlier that most RNA molecules (>90%) are cross-linked or degraded and therefore not available for cDNA synthesis and subsequent PCR amplification. These observations correspond well with our studies on the influence of formalin fixation and subsequent RNA-analysis by reverse transcriptase-polymerase chain reaction (RT-PCR) (unpublished data, May 1995). Where less than 10% of the RNA obtained from fresh material was recovered from formalin-fixed tissue. Moreover, RNA from formalin-fixed tissue was an inefficient template generating hardly less than 1/100, if any amplification product at all compared with that of fresh material.

2. Even if it might be possible to amplify house-keeping genes, eg, β-actin, β-globin, or highly expressed genes like npm, which are amplifiable from every cell, it does not mean that lower expressed genes are detectable.

3. Weiss et al. ensured the adequate preservation of viable RNA within the paraffin blocks by documenting a normal 185-bp npm RT-PCR product in all cases. This seems to be insufficient under two aspects. First, npm might be expressed significantly higher than npm/alk either due to npm-expression in each cell opposite to npm/alk which is detectable in rare Hodgkin- and Sternberg-Reed (HD- and SR-) cells only or due to different transcriptional/posttranscriptional control mechanisms. Second, the investigators have not excluded simple DNA amplification of npm, which cannot easily be ruled out, because the exon/intron structure of the npm gene at the genomic level is not yet known. Therefore, the primers may either amplify genomic DNA or RNA/cDNA. It seems to be technically difficult to purify DNA from RNA from fixed and cross-linked material and the investigators have not shown DNA digestion controls or other controls or data concerning these problems.

4. Moreover, they prepared controls by the use of RNA from cells of a t(2;5)-positive cell line. This is by no means an adequate control comparing apples with pears. We have already convincingly shown that it is possible to amplify a 1,500-bp product of npm/alk from a single cell carrying the translocation; therefore, the investigators should detect even easier the small
175-bp product; however, as discussed earlier, the primers they used for npmlalk amplification are not optimal. They may allow amplification of npmlalk when abundant transcripts are available as in cases of anaplastic large cell lymphoma (ALCL), where almost every cell should be positive, but they may fail for lower gene expression as it has to be expected in HD.

Furthermore, the investigators mentioned mixing experiments, which should hold a sensitivity of 1 in 106 cells, but they neither documented how these mixing experiments were performed (fresh cells or fixed and paraffin embedded cells) nor did they show results in figures or pictures supporting their own results.

(5) Importantly, the investigators mentioned a discrepancy between results obtained by Southern analysis, and their own RT-PCR results. Southern analysis should on the one hand be free of contamination and amplification pitfalls and on the other hand documents individual cases with higher number of cells with t(2;5). This can best be compared with the rearrangement studies of T-cell receptor or IgH chain genes in HD which is also found only in a small number of cases by Southern analysis because of the low sensitivity of this latter method.

In our report we have speculated on the number of t(2;5)-positive cells in cases of HD, which may have a broad spectrum (from only few to all HD cells). This observation could indicate that the t(2;5) is not the first hit in a multistep model of tumorigenesis of HD, but more likely one (later) step responsible for the transition to higher malignancy (HDNS, HDMC into HDNSI, HDLD or ALCL).

(6) In addition we could recently demonstrate a positive signal for t(2;5) in one case of HD using Northern blot analysis of an extracted frozen sample and could show npmlalk positive HD and SR cells in HD using a polyclonal antiserum against a domain in the alk kinase (Figs 1 and 2).

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REFERENCES

Response

Drs Orscheschek and colleagues raise a number of theoretical concerns about the validity of our negative findings. We still believe our work to be valid, despite their concerns. Although there is no doubt that there may be degradation of RNA in formalin-fixed, paraffin-embedded tissues, previous studies have shown that RNA may still be easily amplified. We believe our own sensitivity studies also address this issue. Our positive control cell line was made into a cell pellet, fixed in formalin, and embedded in paraffin to simulate our specimens as closely as possible. Our polymerase chain reaction (PCR) studies were positive to 1 in 106 cells, which should be adequate to detect expression in Reed-Sternberg cells, which comprise approximately 1% of tumor tissue. Of course, we cannot completely rule out the possibility that the expression of the alk/npm transcript in Reed-Sternberg cells is several orders of magnitude lower than our control cell line; however, we believe this is an unlikely possibility. Similarly, we agree with Orscheschek that there is no perfect control to ensure amplifiable RNA in the tissue. Nonetheless, we chose one, the normal npm reverse transcriptase-PCR product, which we thought most closely approximated detection of the alk/npm product. Since there is about a 1,000-hp intron between the primers (M. Raffeld, unpublished data), the product we detected at 185 bp should reflect only mRNA and not DNA.

We believe that the burden of proof of results lies with Dr Orscheschek and colleagues. Their demonstration of t(2;5) in 70% to 80% of cases of Hodgkin’s disease has not been confirmed by multiple studies of large numbers using a variety of tissues (including fresh-frozen tissues) using a variety of techniques, with 152 cases studied by reverse transcriptase-PCR, including over 63 cases studied with fresh frozen tissue; 77 cases studied by in situ hybridization’; and 114 cases studied immunohistochemistry, totalling 244 negative cases without one positive. Of course, these results do not rule out the possibility that rare cases of Hodgkin’s disease, such as Orscheschek and colleagues report above, may truly possess the t(2;5). However, it is well known that false-positive results due to
inadvertent contamination are much more common than false negative results due to inadequate sensitivity when PCR is used.

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REFERENCES
Presence of the t(2;5) in Hodgkin's disease [letter; comment]

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