To the Editor:

In their recent report, Weber-Matthiessen et al. used the technique of simultaneous fluorescence immunophenotyping and interphase cytogenetic analysis (FICTION) to determine the incidence of several numerical chromosome abnormalities within CD30+ cells of Reed-Sternberg, the presumed malignant cells of Hodgkin’s disease. Although this study is interesting and shows the feasibility of FICTION, the results seem to be overinterpreted. Because Reed-Sternberg cells have two nuclei, each CD30+ cell is expected to have four copies of each somatic chromosome. Therefore, finding four copies of any chromosome is expected and does not represent clonal aberration. On the other hand, finding three copies could be simply due to inefficient hybridization. Similarly, finding five copies could be due to nonspecific hybridization fluorescent signal. Therefore, it is imperative to define the false-positive and false-negative background signals in control cells before considering any abnormality as the true one. In our previous studies of non-Hodgkin’s lymphoma, up to 12% of normal control lymphocytes had aberrant chromosome numbers (depending on the chromosome and the labeling technique). Although it is understood that normal control cells for Reed-Sternberg cells can be found only in the wildest dream of a Hodgkin’s disease scientist, it is important to point out the limitations of this interesting study.

REFERENCES


Response: Truth by FICTION

We feel that Dr Younes’s criticism might be based on some misunderstandings that can easily be eliminated. He correctly points out that binucleated cells are expected to contain two regular diploid chromosome sets, one in each nucleus. Being aware of this fact, we did of course examine the signal number in every individual nucleus separately, no matter if one (Hodgkin cells) or several (Reed-Sternberg cells) nuclei were present within the CD30+ cells with tumor cell morphology. The figures presented in our report show supernumerary chromosome signals in mononucleated Hodgkin cells. Moreover, we also mentioned the signal constellation determined in the...
multinucleated Reed-Sternberg cells: "Remarkably, the different nuclei of multinucleated Reed-Sternberg cells always contained identical hybridization signals." To document this phenomenon, we here enclose two additional photographs taken from case no. 29 of our study both showing the same binucleated Reed-Sternberg cell. Figure 1A shows the red CD30 immunophenotype of the cell and green and blue hybridization signals for chromosomes 12 and 17, respectively. Simultaneous phase contrast analysis on the same cell (Fig 1B) allowed unequivocal affiliation of the signals to the individual nuclei. The nuclei of this cell contained each three signals for chromosomes 12 and 17 (one of the blue chromosome 12 signals in the right nucleus is not in focus). This signal constellation was present in the nuclei of all CD30⁺ tumor cells in this case. We have mentioned in our report that, taking into account the detection limit of FISH, CD30⁻ surrounding cells did not contain supernumerary signal numbers. In our hands, the detection limit is less than 1%. This finding is in line with those of most other experienced FISH laboratories.

Using the FICTION technique, we are able to study the chromosomes selectively within the immunophenotypically and morphologically defined tumor cells. Therefore, the risk of distorting interphase cytogenetic data by unintentional evaluation of normal lymphocytes is eliminated. In this respect, FICTION creates facts.

Klaus Weber-Matthiesen
Jens Deerberg-Wittram
Brigitte Schlegelberger
Department of Human Genetics
University of Kiel
Kiel, Germany
Numerical chromosome aberrations in CD30+ cells of Hodgkin's disease: truth or FICTION [letter; comment]

A Younes