CHROMOSOMAL ABNORMALITIES IN HODGKIN'S DISEASE ARE NOT RESTRICTED TO HODGKIN/REED-STERNBERG CELLS

MAURICE P. H. M. JANSEN1*, ANTON H. N. HOPMAN2, ANNICK M. HAASEVOETS3, INGE A. F. GENNOTTE2, FREDRIK J. BOT3, JAN WILLEM ARENDS3, FRANS C. S. RAMAEKERS2 AND HARRY C. SCHOUTEN1

1Department of Internal Medicine, University Hospital Maastricht, The Netherlands
2Department of Molecular Cell Biology and Genetics, University of Maastricht, The Netherlands
3Department of Pathology, University Hospital Maastricht, The Netherlands

SUMMARY

Hodgkin and Reed-Sternberg cells are considered to represent the malignant fraction in Hodgkin's disease. Several studies have shown that the Hodgkin and Reed-Sternberg cells are chromosomally abnormal, but genetic data about the morphologically normal cell population in Hodgkin's disease are very limited. This latter cell population has therefore been examined for chromosomal aberrations, using the in situ hybridization (ISH) procedure, making use of DNA probes for chromosomes 1, 7, 8, 9, 11, 12, 15, 17, and 18. Nuclei were isolated from freshly frozen (10 cases) and paraffin-embedded (16 cases) biopsy samples and 1000 nuclei per case were evaluated. The cases of Hodgkin's disease were compared with reactive lymph nodes, which show aberrant chromosome copy numbers in less than 1% of the cells. Using strict scoring criteria, nuclei in the tumour were found to show an abnormal genotype, in the range of 1-12% per cent, with trisomies occurring most frequently. No characteristic numerical chromosome abnormality was observed. ISH on 4 μm thick paraffin sections of six cases of Hodgkin's disease revealed numerical aberrations for chromosome 1 in cells which appeared to be morphologically normal. The genomically abnormal nuclei did not differ in morphology or size from the nuclei of morphologically normal cells, but differed considerably in size when compared with the nuclei of Hodgkin/Reed-Sternberg cells after the ISH procedure. Three of these six cases revealed a population of apparently normal cells with an aberrant copy number which differed notably from the fraction observed in reactive lymph nodes. It is concluded, therefore, that a subset of morphologically normal cells, next to the Hodgkin/Reed-Sternberg cells, are chromosomally aberrant and may participate in the malignant cell fraction of Hodgkin's disease. © 1998 John Wiley & Sons, Ltd.


KEY WORDS—Hodgkin's disease; Hodgkin/Reed-Sternberg cells; morphologically normal cells; in situ hybridization; cytogenetics

INTRODUCTION

Hodgkin's disease (HD) consists of an apparently small population of malignant cells, the morphologically distinguished Hodgkin/Reed-Sternberg cells, as well as background cells such as lymphocytes, histiocytes, and macrophages, which are considered to be normal (reactive) cells. The proportion of the malignant mononucleated Hodgkin cells and multinucleated Reed-Sternberg cells varies from 1 to 3% per cent of the total cell population. These cells express the activation marker antigens CD15 and CD30, molecules that are established markers for the immunocytochemical detection of the malignant cells in Hodgkin's disease.1

The genotype of Hodgkin's disease has been extensively analysed by karyotyping2–6 and by flow cytometry.7–10 Aneuploidy was observed with both techniques and hyperdiploidy was often detected. Structural and numerical chromosome aberrations were discovered by karyotyping, but a specific chromosome abnormality has not so far been characterized in Hodgkin's disease.1

*Correspondence to: Maurice P. H. M. Jansen, Department of Internal Medicine, University Hospital Maastricht, P.O. Box 5800, 6202 AZ Maastricht, The Netherlands. E-mail: maurice.jansen@molecub.unimaas.nl

A method that allows the simultaneous study of the karyotype and the immunophenotype revealed an aberrant karyotype in CD30-positive Hodgkin/Reed-Sternberg cells.11–13

In this study, we have applied the in situ hybridization (ISH) technique for the chromosomal analysis of interphase nuclei. The ISH procedure has been used to detect specific numerical chromosome abnormalities and to determine the frequency of abnormal cells in several other types of malignant tissues.14–17 Large populations of interphase nuclei can be evaluated relatively easily for numerical and structural chromosome aberrations by ISH using specific DNA probes.14

ISH combined with immunophenotyping has been applied before to detect the chromosome copy number in CD30-positive Hodgkin/Reed-Sternberg cells.18 It was reported that in these cells, additional chromosome abnormalities were detected with ISH which were not observed by karyotype analysis.19

To our knowledge, hardly any data are available on the ploidy status of the morphologically normal cells in Hodgkin's disease.10,11,20 These cells, however, may constitute part of the malignant population, as was suggested by the finding of a high aneuploid fraction in some cases of Hodgkin's disease.10 Genotyping of
the morphologically normal cells can therefore help to specify further the malignant cell population in Hodgkin’s disease and to clarify the relationship of these cells to Hodgkin/R eed–Sternberg cells.

MATERIALS AND METHODS

Tissue samples

Paraffin-embedded (n=16) and frozen (n=10) biopsy specimens from 21 patients with Hodgkin’s disease and paraffin-embedded (n=6) and frozen (n=10) biopsy specimens of reactive lymph nodes were analysed by single-target ISH. These cases of Hodgkin’s disease were histologically classified as nodular sclerosis (n=12), nodular sclerosis/lymphocyte-depleted (n=2), mixed cellularity/lymphocyte-depleted (n=1), mixed cellularity (n=4), and lymphocyte-depleted (n=2).

Preparation of nuclei from freshly frozen biopsies

Nuclei were isolated from 50 μm thick sections of frozen biopsy material. The sections were digested with 100 μg/ml pepsin from porcine stomach mucosa (2500–3500 units per mg protein; Sigma, St Louis, MO, U.S.A.) in 0.01 N HCl for 20 min at 37°C. The digest was cytospinned (Cytospin3, Shandon Scientific Limited, Astmoor, U.K.) for 5 min at 700 rpm onto poly-l-lysine (MW 150 000; Sigma)-coated glass slides and air-dried. A cidic dehydration of the slides was performed with 70 per cent ethanol/0.01 N HCl to preserve nuclear morphology. The slides were post-fixed in 1 per cent HCl at 4°C for 5–20 min, as described by Hopman et al.21 and Schutte et al.22 The sections were enzymatically digested for 20 min at 37°C with 4 mg/ml pepsin in 0.2 N HCl. The digest was cytospinned for 5 min at 700 rpm onto poly-l-lysine-coated glass slides and air-dried. Acidic dehydration of the slides was performed with 70 per cent ethanol/0.01 N HCl to preserve nuclear morphology. The slides were post-fixed in 1 per cent paraformaldehyde/0.1 M phosphate buffer (pH 6.0) for 10 min at 4°C and were subsequently washed in phosphate-buffered saline (PBS), pH 7.4, dehydrated in an ascending ethanol series and air-dried.

Sample preparation from paraffin-embedded biopsies

Isolation of nuclei from 50 μm thick sections of paraffin-embedded biopsy samples was performed by a method modified from Hedley et al.21 and Schutte et al.22 The sections were enzymatically digested for 20 min at 37°C with 4 mg/ml pepsin in 0.2 N HCl. The digest was cytospinned for 5 min at 700 rpm onto poly-l-lysine-coated glass slides and air-dried. Acidic dehydration of the slides was performed with 70 per cent ethanol/0.01 N HCl to preserve nuclear morphology, and they were subsequently washed in PBS.

The 4 μm thick paraffin sections on Super Starfrost Plus slides (M enzel Gläser, Braunschweig, Germany) were treated with 1x sodium thiocyanate at 80°C for 10 min and digested with 4 mg/ml pepsin in 0.1 N HCl at 37°C for 5–20 min, as described by Hopman et al.23

DNA probes

Probes utilized in the ISH analysis hybridize to the (peri) centromeric regions of human chromosomes 1 (pUC1.77, 1.77 kb24), 7 (p7T1, 0.68 kb25), 8 (D8Z2, 2.55 kb26), 9 (pH U R 98, 0.16 kb27), 11 (pC11A, 0.85 kb28), 12 (pa12H8, 1.35 kb29), 15 (D15Z1, 1.80 kb30), 17 (p17H8, 2.7 kb31), and 18 (L1-84, 0.68 kb32). These probes were labelled by a standard nick-translation reaction with biotin-11-dUTP (Sigma) according to the supplier’s instructions. Probes for chromosomes 1 and 8 were also labelled with FITC-11-dUTP (Boehringer, Mannheim, Germany) for the double-target ISH procedure.

In situ hybridization and immunocytochemistry

The DNA probes were diluted with hybridization buffer (60 per cent formamide, 2 × SSC, pH 7.0; 10 per cent dextran sulphate, 0.2 mg/ml hering sperm DNA as carrier DNA; and 0.2 mg/ml yeast tRNA as carrier RNA) to a final concentration of 0.4 ng/μl for pUC1.7 and 1 ng/μl for all the other autosomal probes. Ten microlitres of hybridization mixture was added to the slides under a coverslip. Samples from frozen biopsy material were then denatured in a moist chamber at 70°C for 3 min, while samples from paraffin-embedded material were denatured at 80°C for 5 min. Hybridization was performed overnight at 37°C. After hybridization, samples were washed twice for 5 min in 60 per cent formamide, 2 × SSC, pH 7.0 at 42°C; twice for 5 min in 2 × SSC, pH 7.0 at 42°C; and once in 4 × SSC/0.05 per cent Tween 20 buffer for 5 min at room temperature. The biotinylated probes in the single-target FISH were detected according to the procedure of Pinkel et al.,33 in subsequent incubations with fluorescein isothiocyanate (FITC)-conjugated avidin (AvFITC; Vector, Brunswick Chemie, Amsterdam, The Netherlands), biotin-conjugated goat anti-avidin (BioGaA; Vector), and again FITC-conjugated avidin, all for 20 min at 37°C in a 1:100 dilution. Nuclei were counterstained with propidium iodide (PI; Sigma; 0.5 μg/ml) or 4,6-diaminidino-2-phenylindole (DAPI; Sigma; 1-25 ng/μl), both diluted in glycerol/isotonic 2.3 per cent 1,4-diazabicyclo-(2.2.2)-octane (DABCYO; Sigma). The biotinylated probes in the double-target ISH were detected by subsequent incubations with Texas Red-conjugated avidin (Vector), biotin-conjugated goat anti-avidin, and Texas Red-conjugated avidin. FITC-labelled probes were enhanced with rabbit anti-FITC (DAKO A/S, G rostrup, Denmark) and swine anti-rabbit conjugated FITC, DAKO A/S). Afterwards, nuclei were counterstained with DAPI. In parallel, the ABC method34 was applied for detection, with peroxidase activity being visualized with a diaminobenzidine (DAB; Sigma) staining reaction. These slides were counterstained with haematoyxlin, dehydrated, and mounted in Entellan (M erck, Darmstadt, Germany). CD30 staining was performed with the anti-CD30 antibody Ber-H2 on 4 μm paraffin sections (DAKO A/S). Sections were proteolytically digested with 0.1 per cent trypsin (Sigma) in 0.1 per cent CaCl2 for 30 min at 37°C before Ber-H2 was applied in a dilution of 1:20. The antibody was detected with biotin-conjugated horse anti-mouse (Vector) and the ABC method and demonstrated with a DAB reaction.
**Results**

**ISH on isolated nuclei**

Ten frozen reactive lymph nodes and ten frozen samples of Hodgkin's disease were analysed with nine different chromosome probes to determine the frequency of chromosomally abnormal cells. Nearly all nuclei in the reactive lymph nodes could be evaluated, showing a fraction of nuclei with an increased chromosome copy number varying between 0·1 and 0·2 per cent, producing cut-off limits ranging from 0·3 to 0·8 per cent for the individual chromosomes.

Using the ISH scoring criteria as described, the frequencies of nuclei with aberrant chromosome copy numbers in Hodgkin's disease varied from 1 to 5 per cent on average, with two cases showing even higher frequencies for chromosome 1, i.e. 7 and 12 per cent (Figs 1a and 2a). All cases of Hodgkin's disease exhibited a gain for at least four chromosomes, with trisomies for these chromosomes being detected most frequently. Figure 1 also shows that the frequency of cells exhibiting abnormal copy numbers varied for the different chromosomes in individual cases. For example, the two cases with high frequencies of abnormal nuclei for chromosome 1, i.e. 7 and 12 per cent, showed aberrations for chromosome 8 in only 4 and 2 per cent of the cells, respectively, and in only 5 and 3 per cent of the cells for chromosome 11, respectively. Furthermore, the former case was analysed with double-target ISH combining probes for chromosomes 1 and 8, 1 and 11, and 8 and 11. Double-target ISH showed different chromosome constitutions in the abnormal nuclei (Figs 1a–1f). Some nuclei had an aberrant copy number for only one chromosome (Figs 1a–1f), whereas others displayed abnormalities for both chromosomes (Figs 1g–1i), but a proper determination of the frequencies of abnormal nuclei in the double-target ISH was not possible owing to the low frequencies of the subfractions.

Since all cases of Hodgkin's disease showed a numerical aberration for chromosome 1 in a relatively large fraction of nuclei isolated from frozen tissue sections, the cell types in which these chromosome aberrations occur were examined in more detail. However, not all nuclei of the Hodgkin/Reed–Sternberg cell population could be evaluated in the suspensions obtained from frozen biopsies, because of overlap or loss of morphology (Fig. 2b). ISH was therefore, performed on nuclear suspensions isolated from paraffin-embedded biopsy material, in an attempt to circumvent the loss of morphology. Five reactive lymph node samples and seven cases of Hodgkin's disease were investigated. The cut-off limit for nuclei with a gain for chromosome 1 was established at 1 per cent on basis of the frequency of numerically aberrant cells in nuclear suspensions from the reactive lymph nodes. Furthermore, in these samples, more than 95 per cent of the nuclei could be evaluated for ISH results, because nuclear overlap and truncation were not hampering the analyses. Nuclear suspensions of six HD cases revealed fractions of nuclei with abnormal chromosome copy numbers ranging from 2 to 10 per cent, which exceeded the threshold. However, nuclei from Reed–Sternberg cells might have been excluded in these evaluations as a result of nuclear overlap.

Based on the results on isolated nuclei from frozen and paraffin-embedded biopsies, chromosome abnormalities in morphologically normal cells were anticipated. To prove that 'normal' cells were chromosomally aberrant, ISH was performed on tissue sections to correlate cellular morphology—normal versus tumour cells—with chromosome constitution.

**ISH on paraffin-embedded biopsies**

CD 30 staining and bright-field ISH for chromosome 1 were performed on parallel paraffin sections of 16 cases of Hodgkin's disease to characterize further the chromosome copy numbers in tumour cells and morphologically normal cells. Determination of the cut-off limit in paraffin sections of reactive lymph nodes was complicated by nuclear truncation and nuclear overlap. For this reason, we only evaluated recognizable individual nuclei, without any overlap. Following this approach in reactive lymph node sections, only very few aberrant nuclei were observed (always less than 1 per cent). It seemed realistic, therefore, to use the cut-off level which was also used in nuclear suspensions.

Nine cases with typical examples of large (multi-)nucleated Hodgkin/Reed–Sternberg cells, CD 30-positive in the serial sections, had tumour cells with copy number gains for chromosome 1. Figure 2d illustrates CD 30-positive Hodgkin/Reed–Sternberg cells and the same area in Fig. 2e demonstrates cells with trisomies for chromosome 1, which appear to be morphologically normal cells. Reed–Sternberg cells with multiple copies for chromosome 1 are presented in Figs 2e, 2f and 2f.
Moreover, as shown in Fig. 2f, nuclei from Hodgkin/Reed–Sternberg cells, with an average nuclear surface area of 195.6 \( \mu m^2 \) (n=15), were considerably larger than the nuclei of normal cells, with an average nuclear surface area of 24.6 \( \mu m^2 \) (n=300). In six cases, a gain in chromosome 1 copy number was observed in a fraction of cells with an average nuclear surface area of 30.6 \( \mu m^2 \) (n=26), i.e., morphologically normal cells (Figs 2c, 2e-2g and 2i). It is obvious that in normal cells, nuclear truncation in 4 \( \mu m \) thick tissue sections is limited by comparison with Hodgkin/Reed–Sternberg cells. Consequently, the detected chromosome copy number represents more accurately the true copy number of chromosomes in morphologically normal cells than in tumour cells.

The results from the paraffin sections of the six cases with chromosome abnormalities in morphologically normal cells are described in detail below.

**Case 1**—CD 30 staining of paraffin sections detected 0.3 per cent of CD 30-positive Hodgkin/Reed–Sternberg cells. Based on morphological criteria, 0.2 per cent Hodgkin/Reed–Sternberg cells were identified after ISH on paraffin sections and evaluation of 6777 nuclei. In 50 per cent of these tumour cells, three and four ISH signals per nucleus (55 and 45 per cent, respectively) were observed for chromosome 1. Moreover, a numerical chromosome abnormality was observed in 0.5 per cent of morphologically normal cells.

**Case 2**—ISH evaluation of 1600 nuclei in paraffin sections exhibited 1.2 per cent Hodgkin/Reed–Sternberg cells. A numerical chromosome abnormality was observed in 65 per cent of these cells, with three, four, and more ISH signals per nucleus in a frequency of 77, 15, and 8 per cent, respectively. Morphologically normal cells with three ISH signals constituted 0.7 per cent of the total cell population.

**Case 3**—The frequency of CD 30-positive Hodgkin/Reed–Sternberg cells after CD 30 staining was 0.8 per cent. Evaluation of 4443 nuclei after ISH demonstrated 0.6 per cent Hodgkin/Reed–Sternberg cells on the basis of morphology, with 42 per cent of these cells having a numerical chromosome abnormality. Three and more ISH signals per nucleus were observed in 80 and 20 per cent of these latter cells, respectively. Three ISH signals were also observed in 1 per cent of the cells which appeared to be morphologically normal.

**Case 4**—CD 30 staining identified 0.7 per cent of CD 30-positive Hodgkin/Reed–Sternberg cells. Five thousand nuclei were evaluated for ISH signals, which revealed 0.8 per cent Hodgkin/Reed–Sternberg cells according to nuclear morphology. More than two signals were observed in 33 per cent of these tumour cells and the cells had three or four ISH signals per nucleus (77 and 23 per cent, respectively). A fraction of 1.2 per cent of morphologically normal cells showed three ISH signals in their nuclei.

**Case 5**—Evaluation of 2000 nuclei after ISH exhibited 1.4 per cent Hodgkin/Reed–Sternberg cells, based on morphological criteria. More than two signals were observed in 79 per cent of these tumour cells; three, four, or more ISH signals per nucleus were detected in a frequency of 48, 22, and 30 per cent, respectively. In 1.9 per cent of morphologically normal cells, three ISH signals were observed.

**Case 6**—CD 30 staining identified 2.2 per cent Hodgkin/Reed–Sternberg cells. After evaluation of 3054 nuclei in paraffin sections by means of ISH, 1.9 per cent of Hodgkin/Reed–Sternberg cells were detected. In 74 per cent of these Hodgkin/Reed–Sternberg cells, three, four, or more ISH signals per nucleus (64, 21, and 14 per cent, respectively) were observed. Morphologically normal cells with three signals for chromosome 1 constituted 2.3 per cent of the total cell population.

**DISCUSSION**

Many studies using different techniques have demonstrated that the malignant Hodgkin/Reed–Sternberg cells in Hodgkin's disease are chromosomally aberrant. The chromosome status of the morphologically normal cells, however, has been less clear until now. Teerenhovi et al. reported that only normal karyotypes were observed in B- and T-lymphocytes of Hodgkin's disease. However, using flow cytometry, Erdkamp et al. demonstrated that the aneuploid cell fraction in some cases of Hodgkin's disease was larger than the frequency of Hodgkin/Reed–Sternberg cells, thus implying that morphologically normal cells might also belong to the aneuploid population. Weber-Matthiesen et al., combining FISH with CD 30 staining on fresh tumour material, were the first to report that a few of the apparently normal lymphocytes had lost the Y chromosome, but they did not regard these lymphocytes as being chromosomally abnormal. Nolte et al., using the same technique on paraffin sections, reported that chromosome aberrations were limited to the Hodgkin/Reed–Sternberg cells.
In the present study, we have focused on the numerical chromosome constitution of the morphologically normal cells in Hodgkin's disease. In nuclei isolated from frozen and paraffin material, 1–12 per cent of chromosomally abnormal nuclei were observed in the single-target ISH. However, not all nuclei of the Hodgkin/Reed-Sternberg cell population could be evaluated by the criteria employed, because of overlap or loss of morphology. Despite this fact, the number of abnormal nuclei detected in the suspensions from

---

Fig. 2—(a, b) Nuclei isolated from a frozen biopsy sample of Hodgkin’s disease (nodular sclerosis). In a, the arrow points to a nucleus with a trisomy for chromosome 1. The arrow in b indicates a nucleus that was not evaluated because of the loss of morphology or overlap. This nucleus or nuclei may, however, belong to a Hodgkin or a Reed-Sternberg cell. (c) A 4 μm thick paraffin section of Hodgkin’s disease (nodular sclerosis) after ISH with chromosome 1 showing a small nucleus with a numerical chromosome abnormality (arrow). (d, e) CD 30-positive Hodgkin/Reed-Sternberg cells in 4 μm thick serial paraffin sections of Hodgkin’s disease (nodular sclerosis). Arrow-heads indicate Reed-Sternberg cells (d), some of which are also seen in e. The black arrows in e, however, show nuclei from morphologically normal cells (see d) with a numerical aberration for chromosome 1. (f-i) Bright-field and fluorescence ISH on paraffin sections of mixed cellularity Hodgkin’s disease. Clear differences in morphology and size between nuclei from a Reed-Sternberg cell and a morphologically normal cell (arrow) are illustrated in f. Both nuclei have multiple copies for chromosome 1. g shows other nuclei from morphologically normal cells with trisomies for chromosome 1. Finally, h and i demonstrate chromosomally abnormal nuclei after FISH from Hodgkin/Reed-Sternberg cells (arrow-head) (h) and a morphologically normal cell (arrow) (i), respectively. × 400.
Hodgkin’s disease exceeded the expected number of Hodgkin/R eed–Sternberg cells in virtually all cases. Furthermore, it cannot be excluded that the frequency of numerical aberrations may be considerably higher, since chromosomes not yet tested may also be numerically altered and abnormal chromosomes lacking the centromere may not become visible with the technique applied. A relatively large fraction of chromosomally abnormal nuclei may therefore be ascribed to the morphologically normal cell population in Hodgkin’s disease.

To prove this assumption, 4 μm thick paraffin sections were investigated with I SH and serial sections stained with CD30. Clear evidence was obtained that a proportion of cells surrounding Hodgkin/R eed–Sternberg cells contained numerical chromosome aberrations. When genomically abnormal CD 30-negative cells were found in the neighbourhood of CD 30-positive Hodgkin/ R eed–Sternberg cells, their differences in immunophenotype and size were clearly demonstrated. The Hodgkin/ R eed–Sternberg cells revealed high copy numbers for chromosome 1 after I SH, but the genuine chromosome copy number of these cells is difficult to assess in paraffin sections due to nuclear truncation, which results in an underestimation. This nuclear truncation is correlated with the size of nuclei and the section thickness. Thus, nuclei from normal cells are less affected by truncation than large nuclei from Hodgkin/R eed–Sternberg cells, which are about eight times the size of the normal nuclei, because the size of nuclei from normal cells is in the range of the section thickness. In addition, the observed chromosome copy number in morphologically normal cells represents more accurately the genuine copy number of the chromosomes.

The frequency of morphologically normal cells with an aberrant copy number for chromosome 1 in paraffin sections of Hodgkin’s disease ranged from 0·5 to 2·3 per cent. These cases clearly showed normal cells with chromosome abnormalities on the basis of a distinguished morphology and ISH-signal distribution. In three cases, the frequency of 1·2, 1·9, and 2·3 per cent of cells with an aberration for chromosome 1 was higher than the cut-off limit (1 per cent) assessed in nuclear suspensions of reactive lymph nodes. The cut-off limit for paraffin sections, however, may differ from the threshold for suspensions of nuclei. An underestimation of the cut-off limit will occur in paraffin sections because of loss of signals as a result of truncation. For this reason, a statistical analysis was difficult to apply in sections.

The observed genomic changes in morphologically normal cells may be clonal, but may also result from genetic instability. Weber-M athiesen et al showed that Hodgkin/R eed–Sternberg cells also exhibit chromosome instability, as these cells have different abnormal copy numbers for particular chromosomes. Our single-target ISH results from nuclear suspensions suggest that nuclei with numerous chromosome constitutions may be present. This chromosome instability was confirmed in our double-target ISH, in which nuclei with various combinations of numerical chromosome abnormalities were present.

Our finding that morphologically normal cells in Hodgkin’s disease undergo genomic changes is supported by the data of K app et al and Wolf et al, who observed that Hodgkin’s disease-like lesions in SCID mice may have been derived from EBV-infected bystander cells. Furthermore, these authors found that a cell line with Hodgkin/R eed–Sternberg cell characteristics can be obtained from peripheral blood mononuclear cells. This also suggests that precursors of the malignant Hodgkin/R eed–Sternberg cells are present amongst the morphologically normal cells.

These morphologically normal cells are randomly scattered throughout the tissue section and are sometimes located in the vicinity of Hodgkin/R eed–Sternberg cells, suggesting that they might originate from T- or B-lymphocytes, but more studies should be undertaken in order to characterize their immunophenotype and immunoglobulin (Ig) gene rearrangements. Immunophenotyping is necessary to define their cellular origin, whereas Ig gene rearrangement analysis should determine whether Hodgkin/R eed–Sternberg cells are derived from these chromosomally abnormal cells. So far, however, we have not been able successfully to combine in situ hybridization with immunocytochemistry using enzymatic reactions for bright-field microscopy, due to immunocytochemical shielding of the nuclei by the precipitated reaction product.

Although we realize that our data may be at variance with other reports in the literature, we believe that we have presented compelling evidence to support the idea that the malignant cell population in these lesions is not limited to the Hodgkin/R eed–Sternberg cells, but also includes a subset of morphologically normal cells.

REFERENCES


