Determination of the DNA Content of the Reed-Sternberg Cell of Hodgkin’s Disease by Image Analysis

By Marian M. Haber, Jiguang Liu, Daniel M. Knowles, and Giorgio Inghirami

The nature of the Reed Sternberg (RS) cell, the malignant cell of Hodgkin’s disease (HD), remains unknown. Cytogenetic studies have yielded ambiguous results regarding the chromosomal profile of this cell. In an attempt to further clarify the ploidy status of the RS cell, we analyzed the DNA content of CD30-positive RS cells and RS cell variants in HD lesions from 32 patients using an image analysis system. A diploid and/or near-diploid (DNA index [DI], 1.0 ± 0.2) and a tetraploid (2.0 ± 0.2) RS cell population were identified in 9 and in 11 of the 32 cases examined, respectively. An aneuploid RS cell population was identified in 8 of the 32 cases examined. The remaining four cases contained two RS cell subpopulations with different DNA content, each one representing more than 15% of the total RS cell population. There was no significant correlation between the DNA content of the RS cells and the category of HD. Furthermore, analysis of multiple biopsies of an individual patient taken from different lymphoid organs at the same or different time periods showed a constant DNA profile. Our data indicate that RS cells can express variable DNA content and suggests that multiple subpopulations of RS cells with different DNA content may simultaneously coexist within the same HD lesion in some patients. In addition, the RS cell population within each patient appears to express a specific DNA content profile, possibly representing unique clones. These highly individualized profiles potentially may be useful as markers to follow the clinical course of patients with HD.

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HODGKIN’S DISEASE (HD) represents a malignant neoplasm derived from the hematopoietic-lymphoid system whose origin and nature remains controversial. In large part, this is due to difficulty in characterizing the malignant cell of HD, the Reed Sternberg (RS) cell. In an attempt to understand its variable clinical and pathologic features, some investigators have suggested that HD actually represents a syndrome rather than a single disease process. Immunophenotyping and clonal analysis of the separate morphologic categories of HD support this conclusion in so far as lymphocyte predominance (LP) HD may represent a neoplasm of B-cell origin distinct from the other categories of HD. Analyses of nodular sclerosis (NS), mixed cellularity (MC), and lymphocyte depletion (LD) HD have suggested both a B- and a T-cell origin.

In 1967, Seif and Spriggs provided evidence that a clonal aneuploid cell population exists within HD and concluded that the RS cells represent this population. Since then, numerous cytogenetic studies of HD have been performed to determine the karyotypic status of the RS cell. Nevertheless, the chromosomal content of these cells remains unclear due to the nature of HD, which limits cytogenetic analysis. Foremost is the paucity of neoplastic cells relative to benign cells in HD lesions. Furthermore, the RS cells have a low mitotic index and poor-quality banding pattern combined with a complex karyotype. Monoclonal antibodies (Mab) Ki-I and Ber H2, which recognize the CD30 antigen expressed by RS cells and RS cell variants, addresses to some extent the difficulty in identifying a small neoplastic cell population present amidst a large nonneoplastic lymphoid population.

Recently, recognition of the utility of Mab Ber H2 led to its use to define those cells on which cytogenetic analysis was performed. However, the low number of cells analyzed in this study suggests that improvement in the ability to reliably identify RS cells does not significantly obviate the problem of performing cytogenetic studies on cells with a low mitotic rate. Therefore, a technique other than cytogenetic analysis is necessary to investigate the ploidy status of RS cells.

The computerized microscope image analysis system represents an ideal approach to evaluate the phenotypic and biologic properties of individual cells within a tissue sample. Unlike flow cytometry, the cell analysis system allows the operator to morphologically select and manually collect the sample population, optimizing yield while reducing contaminants. Furthermore, disassociation or culturing procedures that alter the tissue and potentially cause loss of fragile aneuploid cell populations are not required.

For these reasons, we analyzed the DNA content of RS cells and RS cell variants in HD lesions from 32 patients with HD. Monoclonal antibody Ber H2 was used to identify the neoplastic cells and a Feulgen stain was performed for DNA content. We quantitated the nuclear DNA content of the individual RS cells and RS cell variants using image analysis. The double-staining procedure in conjunction with morphologic identification of the sample population enabled us to collect information on a highly purified population of RS cells in situ.

MATERIALS AND METHODS

Patients. Thirty-two patients diagnosed with HD and subclassified by standard morphologic criteria (slides reviewed by D.M.K.) and corroborated with immunophenotypic analysis were included in this study. The 32 patients were selected to produce a panel in which the four morphologic categories of HD were represented and in which an adequate amount of tissue was available to perform the additional studies outlined here.

Pathological specimens. Biopsy specimens were collected during the course of standard diagnostic procedures under sterile conditions.

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patients, more than one sample was examined, so that the total number of samples investigated was 38. One representative portion of each specimen was routinely processed for histopathology and another portion was embedded in a cryopreservative solution (OCT compound, Miles, Elkhart, IN) and stored for varying periods at -70°C. In addition, tissue imprints were prepared in 11 of the 38 HD biopsy specimens. The tissue imprints were performed on silane precoated slides, air-dried, fixed in acetone/chloroform, and stored for varying periods at -70°C.

**Immunohistochemical and Feulgen procedures.** Immunohistochemical staining was performed using Mab Ber H2 (Dako Corp, Carpenteria, CA), which detects the RS-cell-associated antigen CD30,10 on 6-µm acetone/chloroform-fixed frozen tissue sections and/or tissue imprints, and developed using a modified alkaline phosphatase antialkaline phosphatase (APAAP) technique.14 The percentage of Ber H2-positive cells was quantitatively determined by two independent investigators (M.H. and G.I.) by subjective assessment. The tissue sections and imprints were fixed in 10% buffered formalin for 30 minutes and stained by the Feulgen method (Quantitative DNA staining kit, Cell Analysis Systems [CAS], Lombard, IL). Briefly, the fast-fixed samples were placed in 5N HCL for 60 minutes to hydrolyze DNA. The slides were then stained with the Schiff reagent for 1 hour and rinsed in three changes of “rinse solution” (Quantitative DNA staining kit) for 1, 5, and 10 minutes, respectively. Following dehydration in 70% acid alcohol for 5 minutes and further dehydration in 100% ethanol for 3 minutes, the slides were placed in xylene for 3 minutes and then cover-slipped.

**Image analysis.** Quantitative investigation with a computerized image analyzer (CAS-200, Cell Analysis System, Elmhurst, IL) was used to evaluate the DNA content of a population of nonneoplastic cells in addition to CD30 antigen-positive cells exhibiting the cytological features of RS cells and RS cell variants. Calibration of the computer was performed by measuring at least 20 predeposited control cells, rat hepatocytes, with a known nuclear mass (7.18 pg) from which the mode of the summed optical density per cell was obtained. The computer calculated a K value from this data by the linear equation: cell mass = K(ΣOD). This K value was then used by the system to compute the nuclear mass for each test cell and to report the modal value of the primary peak in picograms. The DI can then be obtained by either dividing the modal value of the test cell by the DNA mass of the reference cell or by dividing the test value by the DNA mass obtained from a known normal, i.e., diploid, population of cells.15 In our study, a population ranging from 10 to 136 cells, either lymphocytes or fibroblasts, were analyzed in each sample to provide a normal value in picograms for use in the calculation of DNA indices. This was done in part to control for any effect of our double-staining procedure and the possible chromatic interference of APAAP substrate with Feulgen staining.16 However, the latter possibility was not anticipated, since Mab Ber H2 preferentially stains a nonnuclear antigen. The total number of cells counted and the DNA mass for each individual cell were displayed on a CAS-generated histogram (X axis, DNA mass/DNA index; Y axis, number of cells counted).

**Sample collection.** In each case, the entire tissue section and/or imprint slide was scanned for malignant cells. The number of cells counted reflected not only the number of CD30-positive RS cells and variants present, but also the staining quality of the nuclei. In some RS cells, particularly the “popcorn” variant, the nuclei displayed central clearing of the chromatin and yielded aberrant values indicative of artificial loss of nuclear material. Those RS cells and variants in which the staining was deemed inadequate were excluded from analysis. Only those RS cells and variants which met the histologic, immunophenotypic, and staining quality criteria were accepted as the neoplastic cell population of HD. Therefore, there was no significant difference between the number of cells collected from the different morphologic categories of HD (Table 1).

The DNA contents of the RS cells and variants, which are generally accepted as the neoplastic cell population of HD, was more heterogeneous. A single diploid population with or without a tetraploid population in all of the RS cells and variants (Fig 1). The entire tissue section and/or tissue imprint was scanned for malignant cells. The number of cells counted reflected not only the number of CD30-positive RS cells and variants present ranging from approximately 1% to 20%. The total neoplastic cell count measured for each patient ranged from 13 to 251 cells (mean, 92.5) in frozen sections and from 25 to 198 cells (mean, 76) in tissue imprints. Since Mab Ber H2 staining aided in the recognition of these cells, the actual collection was more dependent on tissue size and the quality of the section and/or tissue imprint, rather than on the category of HD. Therefore, there was no significant difference between the number of cells collected from the different morphologic categories of HD (Table 1).

The normal resting lymphocytes showed a diploid DNA content (DI, 1.0 ± 0.2) in all 38 frozen tissue sections and 11 tissue imprints examined. On the other hand, the DNA content of the RS cells and variants, which are generally accepted as the neoplastic cell population of HD, was more heterogeneous. A single diploid population with or without a tetraploid population in all of the RS cells and variants examined was found in nine of 32 cases. To confirm these findings, more than one tissue sample of two patients (no. 12 and 13) was examined and each sample of the case displayed an exclusively diploid profile. Histologically, these seven patients presented with LP (2), NS (2), MC (3), and LD (2) HD.

When the RS cells and variants were examined in the 23 remaining cases, a single aneuploid population (DI, 1 ± x,
CD30-positive cells were evaluated in frozen tissue sections using Mab Ber H2 using a modified APAAP technique. CD30 positivity manifested as diffuse red cytoplasmic staining. DNA content was assessed using Feulgen staining (see Materials and Methods). Examples of RS cells (A) and RS cell variants including giant cell variant (B) and mononuclear cell variants (C) are shown.

2 ± x, and 4 ± x with x > 0.2) with a primary mode comprising at least 15% of the total RS cells was identified in eight cases and a tetraploid (DI, 2 ± x) population was observed in 11 cases. The aneuploid population(s) was accompanied by a diploid or tetraploid population that comprised more than 10% of the total RS cells in only two cases. In addition, an octoploid (<10% of the total RS cells) population was seen in 18 of the 32 cases. Interestingly, the remaining four HD cases contained RS cells and variants that displayed two different and independent DNA primary modes. Furthermore, each primary mode was associated with repeat populations demonstrating a 1, 2, 4, and 8 pattern containing less than 15% of the total neoplastic cell population.

The DNA content analysis of intact nuclei can be easily obtained using tissue imprints, in contrast with frozen tissue section preparations where the nuclei are variably cut across. In particular, since RS cells and variants have large and often convoluted nuclei, varying portions of RS nuclei will be obtained in a 6-μm frozen tissue section. Thus, using this approach, the DNA content analysis of RS cells could be potentially incorrect. Therefore, we decided to evaluate and compare the DNA findings derived from tissue imprints and frozen tissue sections obtained from the same specimen in 11 different cases of HD. We were able to demonstrate that the primary mode obtained with tissue imprints and frozen tissue sections were similar or identical in each of the 11 cases that we evaluated (Table 2).

However, when the coefficient of variation (CV) of a given curve was evaluated, those obtained from frozen tissue sections were considerably larger than those derived from tissue imprints (Fig 2). In addition, using tissue imprints, we were also able to confirm the existence of multiple RS cell subpopulations with different DNA content, comprising at least 15% of the total RS cells, within the same HD lesion (Fig 3).

The ploidy status of an individual cell was not clearly related to its cytologic appearance. No discernible difference was seen when measurements were made exclusively on mononuclear RS cell variants, rather than on classical RS cells. Giant (multinucleated) RS cell variants were invariably nondiploid. Interestingly, in one case (Table 2, no. 11) we were able to evaluate the DNA content of two distinct nuclei within the same Ber H2-positive RS cells (five RS cells, 10 total nuclei) and found that each nucleus possessed a similar DNA content (1.4 ± 0.2) representing one half of that found in the principal Ber H2–positive RS cells (primary node, 2.7).

The need to examine the reproducibility of our method and the effect of time and chemotherapy led us to perform multiple analyses on a variety of samples. In four samples representing three different patients, two sections were cut from the same block, stained in separate batches, and cells collected on separate days. The graphs generated were remarkably similar in each sample (data not shown). Multiple blocks of lymphoid tissue obtained either at one time or within the same month were examined in two patients. Multiple samples collected over an interval of at least 9 months were analyzed in an additional two patients (Table 3). The histograms generated from different biopsies showed similar profiles; identical DNA peaks were invariably found in the multiple samples from a given
peak and the adjacent nadir differed among the samples patient. However, the percentage of cells comprising the accounting for slight differences in modal data. In cases no. 12 (three samples) and 13 (two samples), the populations

Table 1. Clinical Characteristics and Image Analysis Data From 32 Patients With HD

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Morphologic Subtype</th>
<th>% CD30 (Ber H2)-Positive Cells</th>
<th>No. of Cells Counted</th>
<th>Primary* Site</th>
<th>Primary* Mode</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>M/53</td>
<td>LP</td>
<td>&lt;2</td>
<td>105</td>
<td>Cervical LN</td>
</tr>
<tr>
<td>2</td>
<td>F/53</td>
<td>LP</td>
<td>&lt;5</td>
<td>67</td>
<td>Scalene LN</td>
</tr>
<tr>
<td>3</td>
<td>F/69</td>
<td>LP</td>
<td>&lt;5</td>
<td>70</td>
<td>Inguinal LN</td>
</tr>
<tr>
<td>4</td>
<td>M/15</td>
<td>LP</td>
<td>&lt;5</td>
<td>66</td>
<td>Cervical LN</td>
</tr>
<tr>
<td>5</td>
<td>M/54</td>
<td>LP</td>
<td>&lt;5</td>
<td>62</td>
<td>Cervical LN</td>
</tr>
<tr>
<td>6</td>
<td>M/77</td>
<td>NS</td>
<td>&lt;15</td>
<td>103</td>
<td>Chest Wall</td>
</tr>
<tr>
<td>7</td>
<td>F/27</td>
<td>NS</td>
<td>&lt;5</td>
<td>50</td>
<td>Cervical LN</td>
</tr>
<tr>
<td>8</td>
<td>F/29</td>
<td>NS</td>
<td>&lt;5</td>
<td>57</td>
<td>Supraclavicular LN</td>
</tr>
<tr>
<td>9</td>
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<td>126</td>
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<td>&lt;2</td>
<td>38</td>
<td>Cervical LN</td>
</tr>
<tr>
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<td>NS</td>
<td>&lt;10</td>
<td>100</td>
<td>Cervical LN</td>
</tr>
<tr>
<td>12</td>
<td>F/25</td>
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<td>&lt;15</td>
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</tr>
<tr>
<td>13</td>
<td>M/77</td>
<td>NS</td>
<td>&lt;15</td>
<td>100</td>
<td>LN</td>
</tr>
<tr>
<td>14</td>
<td>M/7</td>
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<td>76</td>
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<td>15</td>
<td>M/5</td>
<td>NS</td>
<td>5</td>
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<td>54</td>
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<td>&lt;10</td>
<td>63</td>
<td>LN</td>
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<td>18</td>
<td>F/26</td>
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<td>5</td>
<td>82</td>
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<td>19</td>
<td>F/27</td>
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<td>5</td>
<td>61</td>
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<td>50</td>
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<td>&lt;2</td>
<td>42</td>
<td>LN</td>
</tr>
<tr>
<td>22</td>
<td>M/18</td>
<td>MC</td>
<td>&lt;15</td>
<td>53</td>
<td>Cervical LN</td>
</tr>
<tr>
<td>23</td>
<td>F/38</td>
<td>MC</td>
<td>&lt;5</td>
<td>104</td>
<td>Inguinal LN</td>
</tr>
<tr>
<td>24</td>
<td>M/11</td>
<td>MC</td>
<td>&lt;5</td>
<td>233</td>
<td>Inguinal LN</td>
</tr>
<tr>
<td>25</td>
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<td>MC</td>
<td>&lt;15</td>
<td>120</td>
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</tr>
<tr>
<td>26</td>
<td>F/57</td>
<td>MC</td>
<td>&lt;2</td>
<td>192</td>
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<tr>
<td>27</td>
<td>F/77</td>
<td>LD</td>
<td>&lt;5</td>
<td>50</td>
<td>Paratracheal LN</td>
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<tr>
<td>28</td>
<td>M/69</td>
<td>LD</td>
<td>&lt;15</td>
<td>75</td>
<td>Lung</td>
</tr>
<tr>
<td>29</td>
<td>M/52</td>
<td>LD</td>
<td>&lt;5</td>
<td>56</td>
<td>Cervical Mass</td>
</tr>
<tr>
<td>30</td>
<td>M/54</td>
<td>LD</td>
<td>&lt;5</td>
<td>145</td>
<td>LN</td>
</tr>
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<td>31</td>
<td>M/26</td>
<td>LD</td>
<td>2</td>
<td>42</td>
<td>LN</td>
</tr>
<tr>
<td>32</td>
<td>M/64</td>
<td>LD</td>
<td>2</td>
<td>50</td>
<td>Inguinal LN</td>
</tr>
</tbody>
</table>

Clinical, pathologic, and CAS data from 32 cases of HD. Modes were extrapolated from histograms generated using the CAS data. Primary modes (*) were defined as the DI with the greatest peak and comprising at least 15% of the total cells counted.

Table 2. DI and Cell Cycle of CD30 (Ber-H2)-Positive Cells Using Tissue Imprint and Frozen Tissue Section Samples

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Morphologic Subtype</th>
<th>Tissue Imprint</th>
<th>Frozen Tissue Section</th>
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<td></td>
<td>Primary Mode</td>
<td>G0/G1 (%)</td>
<td>S (%)</td>
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<tr>
<td>9</td>
<td>NS</td>
<td>1.90</td>
<td>64</td>
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<td>11</td>
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<td>2.7</td>
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<td>14</td>
<td>NS</td>
<td>2.0</td>
<td>72</td>
</tr>
<tr>
<td>18</td>
<td>NS</td>
<td>2.0</td>
<td>83</td>
</tr>
<tr>
<td>16</td>
<td>NS</td>
<td>2.06</td>
<td>76</td>
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<tr>
<td>17</td>
<td>NS</td>
<td>2.37</td>
<td>76</td>
</tr>
<tr>
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<td>NS</td>
<td>2.48</td>
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<tr>
<td>19</td>
<td>NS</td>
<td>1.94</td>
<td>78</td>
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<td>2.12</td>
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<td>MC</td>
<td>0.96</td>
<td>70</td>
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<tr>
<td>28</td>
<td>LD</td>
<td>1.22</td>
<td>70</td>
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</table>

Fig 2. The DNA content of RS cells and RS cell variants using image analysis data in tissue imprint and frozen tissue section preparations. DNA content of CD30 (Ber H2)-positive cells stained by the Feulgen method (see Materials and Methods) in tissue imprints (A, B, and C) and frozen tissue sections (A', B', and C') were computed with an image analyzer (CAS 200) equipped with a Quantitative nuclear antigen software package. DNA indices and the number of RS cells are represented on the x axis and y axis, respectively.

Fig 3. DNA histograms from tissue imprint preparations in HD patients. DNA profiles of CD30-positive RS cells (see Materials and Methods), derived from tissue imprint preparations of patients no. 6 and 20 (A' and B', respectively), were computed with an image analyzer (CAS 200) equipped with a Quantitative nuclear antigen software package. (A and B) DNA profiles of the normal lymphocytes. DNA indices and the number of RS cells are represented on the x axis and y axis, respectively.
variants in HD lesions in order to obtain information
with their corresponding diploid populations, or alterna-

tively that a major defect occurs in the cytokinesis of RS

cells; (3) multiple stem-cell lines present in some pathologic

 specimens that comprise at least 15% of the total RS cells

represent a cytogenetic enigma. In fact, analysis of HD

studies described here, we used image analysis to

investigate the DNA content of RS cells and RS cell

variants can be found in approximately 10% of all HD

cells; (4) neoplastic RS cells with a tetraploid DNA content

represent the most frequent (primary mode) cell population

among the 32 cases of HD studied, indicating that tet-

raploid RS cells may have a biological advantage compared

with their corresponding diploid populations, or alterna-

tively a major defect occurs in the cytokinesis of RS cells;

Table 3. Clinical Characteristics and CAS Data From Four Patients With Multiple Biopsies

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Sex/Age</th>
<th>Morphologic Subtype</th>
<th>% CD30 (Ber H2)-Positive</th>
<th>No. of Cells Counted</th>
<th>Site</th>
<th>Date</th>
<th>Chemo-therapy</th>
<th>Primary Mode</th>
<th>Secondary Mode</th>
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<td>M/27</td>
<td>NS</td>
<td>&lt; 10</td>
<td>100</td>
<td>Cervical LN</td>
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<td>No</td>
<td>2.8</td>
<td>3.4</td>
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<td>12</td>
<td>F/26</td>
<td>NS</td>
<td>&lt; 15</td>
<td>115</td>
<td>Supraclavicular LN</td>
<td>10/22/90</td>
<td>No</td>
<td>1.0</td>
<td>1.6</td>
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<td>13</td>
<td>M/??</td>
<td>NS</td>
<td>&lt; 15</td>
<td>100</td>
<td>LN</td>
<td>8/5/87</td>
<td>?</td>
<td>2.2</td>
<td>1.2</td>
</tr>
<tr>
<td>23</td>
<td>F/38</td>
<td>MC</td>
<td>&lt; 15</td>
<td>104</td>
<td>LN</td>
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<tr>
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<td>F/38</td>
<td>MC</td>
<td>&lt; 15</td>
<td>104</td>
<td>LN</td>
<td>2/8/89</td>
<td>No</td>
<td>.6</td>
<td>1.2</td>
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<tr>
<td>23</td>
<td>F/38</td>
<td>MC</td>
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<td>LN</td>
<td>2/8/89</td>
<td>No</td>
<td>.6</td>
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DISCUSSION

In the studies described here, we used image analysis to

investigate the DNA content of RS cells and RS cell

variants in HD lesions in order to obtain information
regarding their ploidy status. We found that (1) a significant

percentage of RS cells and variants within HD lesions

exhibit a shared ploidy status, supporting the concept that

these cells represent a malignant clonal population; (2)

neoplastic RS cells with a tetraploid DNA content repre-

sent the most frequent (primary mode) cell population

among the 32 cases of HD studied, indicating that tet-

raploid RS cells may have a biological advantage compared

with their corresponding diploid populations, or alterna-

tively a major defect occurs in the cytokinesis of RS cells;

(3) multiple stem-cell lines present in some pathologic

specimens that comprise at least 15% of the total RS cells

and variants can be found in approximately 10% of all HD

cases (4 of the 32 cases); and (4) the RS cell population

within each patient appears to express a specific DNA

content profile, possibly representing unique clones. These

highly individualized profiles potentially may be useful as

markers to follow the clinical course of patients with HD.

Previous cytogenetic studies have demonstrated that RS
cells represent a cytogenetic enigma. In fact, analysis of HD

typically shows cases with only diploid, cases with an

admixture of diploid and aneuploid, and cases exhibiting

solely aneuploid metaphases.17 In addition, no pathogno-
monic chromosomal aberration has been found. These

contradictory findings are difficult to interpret, particularly

in view of the fact that technical problems and method-

ologic approaches have precluded a conclusive statement

regarding the cytogenetic properties of RS cells.

Cytogenetic studies, the main method of chromosomal

study, require metaphases originating from single cells and

at least short-term tissue culture techniques. Furthermore,
in no previous studies has an attempt been made to obtain a

pure population of RS cells, and therefore the origin of the
cells analyzed in metaphase could not be certain. Despite

this lack of information, the majority of investigators have

interpreted the diploid metaphases as derived from the

normal activated lymphocytes and not from a subpopula-
tion of RS cells with a diploid DNA content.18 However, we

should keep in mind that using image analysis, genetic

abnormalities, such as balanced chromosomal transloca-
tions and even loss of an entire chromosome, cannot be

identified due to the present limits of resolution of this

particular technique.

Several considerations should be kept in mind to objec-
tively evaluate this controversy. Using image analysis, we

were able to study a considerably larger population of RS

cells in each patient compared with that previously col-

lected in cytogenetic studies. Furthermore, the use of

simultaneous immunohistochemical and quantitative DNA

analysis allowed us to measure exclusively the DNA content

of the cell population expressing the RS-cell–associated

antigen CD30 and fulfilling the morphologic criteria for RS

cells and RS cell variants. Mab Ber H2 is not exclusive to

HD; this Mab also reacts with benign activated lymphoid

cells present in the interfollicular areas of peripheral

lymphoid tissues. The CD30 antigen can also be expressed

in a clinicopathologically distinct category of non-Hodgkin’s

lymphoma (NHL) designated Ki-1–positive anaplastic large-
cell lymphoma (ALCL), as well as some categories of T-

and B-cell NHL.19 Based on the fact that it is sometimes
difficult to distinguish HD from Ki-1–positive ALCL, only
cases that were well characterized as HD based on his-
topathologic, phenotypic, and molecular genetic criteria

were used in this study. In view of these criteria, the

presence of a discrete diploid population ranging from 6%
to 42% of the total RS cells in all of the cases is unlikely to
be coincidental. In addition, the presence of tetraploid (4n)
and octoploid (8n) RS cells in 96% and 50% of HD

patients, respectively, supports the idea that this subpopu-
lation (2n) of cells represents the neoplastic cell population,
rather than the benign reactive surrounding lymphoid cell

population. However, in our study, it was rare for the
diploid cell subpopulation to exist without a coexistent
aneuploid population within the same lesion. Therefore, we

believe that multiple subpopulations of RS cells with

different DNA content coexist simultaneously within the

same lesion.

As previously suggested, image analysis is probably the

most powerful technique available at the present time to
evaluate cells with an unusually high DNA content. Image

analysis requires no mechanical or enzymatic manipula-
tions to evaluate the DNA content of any given cell

population, contrary to flow cytometry, so that the destruc-
tion of large and fragile cells does not occur. This is a

particularly important feature to consider with respect to

RS cells and variants, since they have convoluted nuclei and
abundant cytoplasm, which renders them particularly susceptible to destruction when subjected to such techniques. Based on these same cytologic features of RS cells and the method of investigation, the finding of large tetraploid subpopulations (4n and or 4 [n ± x]) in our study is not surprising. Although the technology used in this study does not allow us to explain this finding, two possible hypotheses may be proffered. One, tetraploid cells may have a biological advantage compared with their corresponding diploid populations. Two, a major defect in the cytokinesis of RS cells could account for this phenomenon.

Image analysis appears to be the most powerful technique currently available to study and characterize the phenotypic and morphological features of a specific cell population. However, we should keep in mind that this technique also has certain limitations. In particular, evaluation of the DNA content of large and/or proliferating cells with convoluted nuclei such as RS cells can be particularly difficult, especially when frozen tissue section preparations are used. Based on this consideration, we decided to investigate the DNA content of CD30 (Ber H2)-positive cells using tissue imprint preparations. Our data clearly indicate that, despite the theoretical technical limitations of frozen tissue sections, when precise criteria in the acquisition and evaluation of DNA data are used, the derived information is correct and can be generalized. Furthermore, using tissue imprints, which allow a better discrimination of different subpopulations of cells (smaller CV compared with those obtained using frozen tissue sections), we were able to confirm the presence of multiple subpopulations of RS cells and RS cell variants with different DNA content within the same pathologic sample. These data suggest that multiple neoplastic subclones may coexist in the same IHD lesion, as previously demonstrated for other human neoplasms.

Despite the fact that the nature of HD remains largely unknown, it is still considered a neoplasm and therefore, presumably, results from the clonal expansion of an aberrant tumor cell. If this hypothesis is correct then we should be able to identify clonal populations for each individual patient. The evaluation of the DNA content of RS cells in multiple biopsies obtained from the same patient represents an ideal model to potentially identify a clonal marker of HD. Our findings support this hypothesis. In fact, unique and specific profiles can be found in every patient and these appear to be a stable marker, unaffected by therapy, for a given patient.

The presence of an aneuploid cell population suggests the presence of chromosomal abnormalities. However, with image analysis it is difficult to identify a common pattern present in all cases of HD suggestive of a specific chromosomal aberration. Furthermore, our definition of an aneuploid population (groups of cells with a 2, 4, 8(n ± x), or a single peak 2 (n ± x), 4(n ± x), 8(n ± x) comprising ≥20% of the cells) potentially misinterprets a large proliferating pool (S phase) as an aneuploid population. This latter interpretation is suggested by studies demonstrating a large growth fraction in HD of all categories. However, the well-defined peaks, repetitive patterns, and previous cytogenetic studies demonstrating an aneuploid population in cases of HD encourages an interpretation of these populations as aneuploid. Since classic cytogenetic analysis has failed to demonstrate a pathognomonic chromosomal abnormality in RS cells, alternative technology should be applied to identify specific chromosomal deletions and/or translocations. Using interphase cytogenetic techniques, we are currently evaluating the presence of specific chromosomal aberrations and the potential involvement of oncogenes and/or tumor suppressor genes in HD.

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Determination of the DNA content of the Reed-Sternberg cell of Hodgkin's disease by image analysis

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