LYMPHOCYTE predominance Hodgkin's disease (LPHD) represents approximately 5% to 10% of all cases of HD and is a clinically indolent disorder that mainly affects men. Patients with LPHD usually present with stage I or II disease and the condition often remains stable for years, even without treatment. Histologically, LPHD resembles the other subtypes of HD by the presence of scattered large atypical cells, the Reed-Sternberg cells and their variants, admixed with a variety of inflammatory cells. Based on the architecture of the affected lymph node, nodular and diffuse variants of LPHD are recognized. The Reed-Sternberg cell variant and putative neoplastic cell of LPHD, the "L&H" cell, has a large polyploid nucleus, small nucleoli, and a small amount of cytoplasm, which distinguish this cell from the so-called "diagnostic Reed-Sternberg cell" that is present in the other subtypes of HD. In the last decade, with the development of novel antibodies and the improvement of immunohistochemical techniques, a number of investigators have shown that the L&H cell expresses several B-cell-associated molecules and, therefore, may be of B-cell lineage. However, the question of whether LPHD represents a monoclonal or a polyclonal B-cell disorder remains unresolved. Southern blot analysis and, more recently, polymerase chain reaction (PCR) analysis of DNA extracts of tissues involved by LPHD have not shown clonal Ig gene rearrangements in most studies. However, such results are not conclusive because these techniques can only detect a monoclonal population that constitutes at least 1% of the total cell population in the tissues; the number of L&H cells in most cases of LPHD is below the threshold of detection when the Southern blotting technique is used. Although the PCR technique is very sensitive when clone-specific primers are used for amplification, detection of clonal rearranged Ig genes using consensus primers is far less sensitive because the products of a small clonal population may be obscured by those derived from the numerous nonneoplastic B cells also present in the tissues. In situ analysis of Ig light chain protein or mRNA expression, which provide conclusive evidence for the monoclonality of most B-cell lymphomas, has yielded conflicting results with respect to the clonality of the L&H cells of LPHD.

To confirm the B-cell origin and to shed more light on the issue of clonality of the L&H cells of LPHD, a highly sensitive single-cell assay was developed. To allow the study of a number of cases representative of the disease and to have better morphologic preservation of single cells, a method was developed to isolate single L&H cells from readily available formalin-fixed, paraffin-embedded tissue. We then used a PCR technique to detect Ig heavy chain (IgH) gene V-D-J rearrangements in single L&H cells. The PCR products that comprise the CDR3 were then compared to cell clone-specific. The PCR products were size-fractionated by polyacrylamide gel electrophoresis and representative region 3 (CDR3) of the Ig heavy chain (IgH) gene, which is B-cell clone-specific. The PCR products were size-fractionated by polyacrylamide gel electrophoresis and representative products were directly sequenced. Single T cells and small B cells were also isolated from the tissues and used as negative and positive controls, respectively. In all four cases of LPHD, the IgH CDR3 of single L&H cells could be amplified. Within each case, the IgH CDR3 of single L&H cells was found to be of different length or of different sequence. Therefore, our results provide strong evidence for the B-cell origin of the L&H cells and the polyclonal nature of LPHD.

MATERIALS AND METHODS

Materials

Patients diagnosed with LPHD were selected from the files of the Lymphoma Registry at the University of Nebraska Medical Center. We selected four cases with the following characteristics for the study. First, the morphology of the lymph node was considered typical of either nodular (cases no. 1 and 3) or diffuse LPHD (cases no. 2 and 4) by three of the authors (W.C.C., D.D.W., and J.D.). Second, the L&H cells expressed epithelial membrane antigen (EMA), as evaluated by immunohistochemistry on formalin-fixed, paraffin-embedded tissue. EMA has previously been reported to be expressed by L&H cells and was a useful L&H cell marker in
this study because, with the exception of a few EMA-expressing plasma cells, it was exclusively expressed by L&H cells in the diseased tissues. Two control cases of LPHD were also selected in which the L&H cells did not express EMA. Third, a 280-bp fragment of the β-tubulin gene could be amplified from DNA extracts of each case. This product is longer than the expected amplified IgH CDR3 product using our primers, thus assuring that the formalin-fixed, paraffin-embedded tissues contained DNA that was not extensively degraded and was useful to our studies. Finally, only those cases of which frozen tissue samples were also available for the purpose of DNA extraction and Southern blotting were selected for this study.

**Immunohistochemistry**

Five-micron paraffin sections and 4-μm cryostat sections were cut and stained with a three-step, peroxidase-labeled method. After dewaxing and before staining, paraffin-embedded tissue sections were heated in citrate buffer for 5 minutes using a microwave oven. Cryostat sections were fixed for 10 minutes in acetone before staining. The primary antibodies anticleucocyte common antigen (anti-CD45), L26 (anti-CD20), polyclonal anti-κ Ig light chain, polyclonal anti-λ Ig light chain, BerH2 (anti-CD30), and anti-EMA were purchased from Dako Corp (Carpinteria, CA) and anti-LeuM1 (anti-CD15) was purchased from Becton Dickinson (San Jose, CA). These antibodies were used for staining paraffin-embedded sections. The antibodies anti-Leu12 (anti-CD19) and anti-Leu14 (anti-CD22) were also obtained from Becton Dickinson and were applied to the cryostat sections.

**Southern Blot Analysis**

DNA extraction from frozen tissue samples, electrophoresis, blotting, and probing was performed as previously described.

**Single-Cell Suspension**

Fifty-micron paraffin sections were cut. The knife of the microtome was extensively cleaned before cutting a new paraffin block. The tissue was put in a microfuge tube, dewaxed in xylene and alcohol, and rehydrated in phosphate-buffered saline (PBS; pH 7.4). To obtain a single-cell suspension, the tissue was subsequently digested with a mixture of collagenase (0.3%, type II; Sigma, St Louis, MO) and trypsin (0.25%, type XI; Sigma) dissolved in PBS (pH 7.4). The digestion was performed for 16 hours at room temperature in a 1 mL volume using microfuge tubes. The remaining undigested tissue was removed with a pipette tip. The cell suspension was washed twice by pelleting the cells with an Eppendorf centrifuge 5415C (Brinkmann Instruments Inc, Westbury, NY) at 5,000 rpm for 10 minutes and then resuspending the cells in PBS.

**Identification and Isolation of Single Cells**

**Immunocytochemistry.** In contrast to Truemper et al, who previously identified Reed-Sternberg cells in single-cell suspensions by the absence of B- or T-cell markers, we chose to positively select Reed-Sternberg cells. This is possible because L&H cells often express EMA, which is not expressed by an other major cell population in lymph nodes involved by the disease. In addition, in our experience, it is difficult to recognize L&H cells with certainty by phase contrast microscopy alone. Single-cell suspensions of all four cases and of two control cases were stained with anti-EMA (dilution, 1:200) to detect the L&H cells. Another part of the single-cell suspension of two cases (cases no. 1 and 2) was also stained with anti-CD3 (polyclonal rabbit antibody, dilution 1:100; Dako Corp) to detect T cells or with anti-mb-I (antibody HM57, undulid culture supernatant; kindly provided by D.Y. Mason, Oxford, UK) to detect B cells. The T and B cells were then used as negative and positive controls in PCR amplifications, respectively. Biotinylated rabbit antirat-mouse or goat antirabbit antibodies (Dako Corp) were used as linker antibodies. All antibodies were diluted in PBS containing 10% horse serum. Subsequently, peroxidase-conjugated avidin-biotin-complex (Dako Corp) was added, and 3,3'-diaminobenzidine and H2O2 were used as peroxidase substrates. All incubations were performed for 30 minutes, except the incubations with anti-EMA and anti-CD3, which were performed for 1 hour, and the incubation with anti-mb-I, which was performed for 2 hours.

**Isolation of single cells.** Depending on the cell density, 20 to 100 μL of each stained cell suspension was diluted in 6 mL of sterile PBS and left to settle to the bottom of a 60-mm Petri dish (Corning Glass Works, Corning, NY). Using a Nikon Diaphot inverted microscope, single EMA-positive L&H cells could be clearly recognized as very large, black-brown staining cells (Fig 1). Very few small EMA-staining cells were seen that may have been plasma cells. No EMA-positive large cells were seen in the single-cell suspensions derived from the two cases of LPHD in which the L&H cells did not express EMA, as evaluated by immunocytochemistry on tissue sections. As expected, the cells staining for CD3 and mb-I were mostly small cells. However, the large L&H cells were also mb-I--positive, as previously reported. Single EMA-positive L&H cells (which were at least 10 times the size of the unstained small lymphocytes in the preparation; Fig 1), CD3+ T cells, or mb-I--positive B cells were manually drawn into a glass micropipette. To control that no other cells were submitted for PCR amplification, the cell was ejected into another Petri dish that contained only sterile PBS. Subsequently, the cell was drawn into a new glass micropipette and observed with the inverted microscope for the absence of any other contaminating cells (Fig 2). The cell was then dropped into 25 μL of PCR buffer containing 200 μg/mL of proteinase K ( Gibco BRL, Gaithersburg, MD). Finally, the micropipette was again inspected with the inverted microscope to ascertain that the cell had cleared the pipette. All single cells were selected after inspection and approval by two of the authors (A.T. and J.D.).

**PCR Amplification**

The IgH CDR3 of DNA of single cells, as well as dewaxed paraffin-embedded tissue sections, of the four cases of LPHD was amplified using a seminested PCR method, thus assuring specificity of the amplified products. In both PCR rounds, a consensus primer to the 3' end of the third framework region of the VH genes was
SINGLE-CELL STUDY OF HODGKIN’S DISEASE

Fig 2. An immunostained L&H cell (arrowhead) drawn into the micropipette.

used (sequence: 5’CTGTCAAGCGGTGTATTTG3’), as reported previously by Yamada et al.49 In the first and second PCR rounds, different consensus primers to the 3’ end of the antisense JH segments were used, with the one in the second PCR round (sequence: 5’ACCATGAGAGACGG-TGAC3’) being internal to the one used in the first round (sequence: 5’ACCTGAGAGACGGTGAC3’). In our hands, use of these primers in a seminested PCR method results in the detection of rearranged IgH genes in 65% of B-cell lymphomas proven to have rearranged genes by the Southern blot technique (W.C. Chan, unpublished data). The conditions of the PCR method used for single cells was developed so that the IgH CDR3 of single Namalwa cells (a Burkitt’s lymphoma-derived cell line), which are known to have a rearranged IgH gene,19 could be amplified. The PCR method was as follows. The single cells were dropped into 25 µL of PCR buffer (50 mmol/L KCl, 10 mmol/L Tris-HCl, pH 8.3, 2.5 mmol/L MgCl2, 0.1 mg/mL gelatin, 0.45% Nonidet P40, 0.45% Tween 20) containing 200 µg/mL of proteinase K. The reaction mixture was covered with 50 µL of mineral oil and was incubated at 55°C for 2 hours. Subsequently, proteinase K was inactivated by heating the sample at 96°C for 10 minutes and 25 µL of a mastermix (400 µmol/L dNTP, 4 µmol/L of each primer, 1 U of Taq polymerase in PCR buffer) was added to the samples while at 94°C. PCR cycle consisted of annealing for 40 seconds at 55°C, extension for 40 seconds at 72°C, and denaturation for 40 seconds at 94°C. The first round of PCR was performed for 30 cycles and the second round consisted of 35 cycles. Two microliters of a 1:400 dilution of the PCR products from the first round was used as the template for the second round of PCR. All reactions were performed using an Omnigen thermocycler (Hybaid Ltd, UK). Positive controls consisted of DNA extracted from the Namalwa B-cell line; negative controls consisted of sterile water. Both controls were included with each set of reactions performed. To test whether false-positive or false-negative results were produced by the isolation and staining procedure, the single cells, single T cells and single B cells isolated from the same tissues of LPHD were used as negative and positive controls, respectively, using the same reaction conditions. These reactions also included a positive control (Namalwa DNA) and a negative control (sterile water).

DNA was also extracted from one 5-µm dewaxed, paraffin-embedded tissue section from each of the four cases of LPHD according to the method of Wright and Manos23 and was subsequently submitted for IgH CDR3 amplification. Ten microliters of all PCR products was electrophoresed on 8% polyacrylamide gels and visualized with UV light after ethidium-bromide staining.

DNA Sequencing

IgH CDR3 PCR products of all L&H cells of case no. 1 and those of similar length obtained from case no. 3 (cells L2A6 and L2C8) and case no. 4 (cells L5A3 and L5A7) were sequenced to determine the IgH CDR3 identity. For this purpose, the second round of IgH CDR3 PCR was repeated in a 100 µL volume, according to the method described above. The products were gel-purified and submitted for direct sequencing. Automated direct sequencing was performed using the same 35 shine and JH primers as described above for the second round of IgH CDR3 amplification and fluorescent deoxyxynucleotide terminators as previously described,25 using the Applied Biosystems 373A sequenator (Applied Biosystems, Foster City, CA).

RESULTS

Immunohistochemistry and Southern Blot Analysis

The results of these studies are summarized in Table 1. L&H cells expressed one or more B-cell surface antigens in all four cases. The presence of κ and λ Ig light chains was detected on the cytoplasmic membrane of small B cells in all four cases. L&H cells contained cytoplasmic κ and λ Ig light chains but whether only one of both of these proteins were present in a single cell could not be assessed with certainty. No clonal Ig gene rearrangements were detected in the Southern blots of the four cases studied.

PCR Amplification of the IgH CDR3 From Single Cells and Tissue Sections

The PCR products obtained from DNA extracts of tissue sections from the four cases of LPHD appeared as smears in the 100-bp range when electrophoresed. Such smears are typical of the presence of a polyclonal B-cell population. No prominent discrete bands indicative of a dominant B-cell clone were observed (Fig 3).

The number of positive results obtained for the single cells analyzed from each case are summarized in Table 2.

Table 1. Phenotype of the L&H Cells of Lymphocyte Predominance

<table>
<thead>
<tr>
<th>HD and Genotype of the Tumor</th>
<th>Case No. 1</th>
<th>Case No. 2</th>
<th>Case No. 3</th>
<th>Case No. 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immunohistochemistry</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD45</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>CD19</td>
<td>E</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>CD20</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CD22</td>
<td>E</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>CD15</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CD30</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>EMA</td>
<td>+</td>
<td>+</td>
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<td>+</td>
</tr>
<tr>
<td>κ</td>
<td>+</td>
<td>E</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>λ</td>
<td>+</td>
<td>E</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Southern blots*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>JH</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Jκ</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cl</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Abbreviation: E, equivocal.
* For Southern blot, (−) indicates no clonal rearrangements detected.

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Positive results from single L&H cells and B cells, as well as negative results obtained from single T cells, are illustrated in Fig 4. Single L&H cells showed one or two rearranged IgH genes. IgH CDR3 were of different lengths for different cells from the same case, suggesting that the L&H cells within any one case were polyclonal. Some single cells yielded faint rearranged bands (case no. 3, lane 7, and case no. 4, lane 1). One L&H cell from case no. 2 (lane 3) and one from case no. 4 (lane 7) showed three discrete bands. As expected, single small B cells also showed one or two rearranged IgH genes, whereas the T cells did not show any Ig gene rearrangements.

Sequencing of PCR Products

We have sequenced one of the IgH CDR3 PCR products of all cells of case no. 1 and those of the other cases that appeared to comigrate when electrophoresed on regular polyacrylamide gels. The former was performed to confirm that IgH CDR3 products derived from L&H cells and that migrated differently in the gels were composed of an entirely different sequence and, thus, were not clonally related. IgH CDR3 products that migrate differently in routine polyacrylamide gels could potentially belong to one B-cell clone that replaces its V\textsubscript{H} gene, such as occurs in B-cell precursor leukemia." Sequence analysis of comigrating IgH CDR3 PCR products was performed because these products could potentially have homologous or, in the case of somatic point mutations, almost homologous sequences and therefore might have belonged to clonally related L&H cells. Somatic point mutations affecting one B-cell clone, such as those occurring in the germinical center and follicular lymphomas, result in a variable number of nucleotide substitutions that do not affect the migration of the IgH CDR3 PCR products in regular polyacrylamide gels.

The sequences of analyzed products are given in Table 3. The 10 sequences are composed of unique combinations V, D, and J segments and contain a variable number of N nucleotides, as expected for IgH CDR3 sequences. None of the sequences obtained in any one case shows significant homology. Therefore, these sequence data do not provide evidence for a clonal origin of the analyzed L&H cells.

DISCUSSION

In the present study, we have shown by a single-cell PCR technique that individual L&H cells, the putative neoplastic cells of LPHD, have uniquely rearranged IgH genes and thus represent a polyclonal B-cell population.

Over the past 10 years, evidence supporting the B-cell origin of the L&H cell has accumulated. Poppema et al. first showed the presence of either \( \kappa \) or \( \lambda \) Ig light chains in L&H cells, and several investigators subsequently reported the expression of J chain by these cells. J chain expression is considered proof of a B-cell origin because it is present in cells that synthesize Ig and is absent in cells that may have pinocytosed Ig from the extracellular space, a phenomenon known to occur in Reed-Sternberg cells. Furthermore, L&H cells express mb-l, a protein that is part of the Ig membrane complex and is restricted to B cells. L&H cells have also been shown to express other B-cell antigens such as CD19, CD20, and CD22. Our finding that L&H cells rearrange their IgH genes is not surprising in the light of these data and strongly supports the B-cell lineage of the L&H cell. However, using our technique, we have not been able to show IgH gene rearrangements in all L&H cells of any single case. Lack of positive results in all L&H cells may be explained by the fact that the consensus Ig variable gene primer used in our study does not hybridize to all \( \text{V}_{\text{H}} \) gene families. Indeed, using this consensus \( \text{V}_{\text{H}} \) primer, we have only been able to detect IgH gene rearrangements in 65% of B-cell non-Hodgkin’s lymphomas that are known to have rearranged IgH genes by Southern blotting (W.C. Chan, unpublished data). Using consensus \( \text{V}_{\text{H}} \) primers, others have obtained comparable results. In addition, formalin fixation may not have left the IgH CDR3 intact for amplification in a number of cells. Finally, our results do not exclude the possibility that some L&H cells do not have rearranged IgH genes. However, this seems unlikely because approximately the same percentage of small B cells isolated from the same tissues yielded negative results.

Surprisingly, in two assays of single L&H cells, but in none of the single B-cell assays, three distinct bands indicative of three IgH gene rearrangements were observed. Although contamination in PCR amplifications can never be formally ruled out, the fact that all T-cell and water controls

Table 2. Number of Positive PCR Reactions for IgH CDR3 in Single Cells From LPHD

<table>
<thead>
<tr>
<th>Case No.</th>
<th>L\textsubscript{H} Cells</th>
<th>B Cells</th>
<th>T Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6/14</td>
<td>7/17</td>
<td>0/7</td>
</tr>
<tr>
<td>2</td>
<td>4/9</td>
<td>8/17</td>
<td>0/6</td>
</tr>
<tr>
<td>3</td>
<td>8/17</td>
<td>0/13</td>
<td>0/13</td>
</tr>
<tr>
<td>4</td>
<td>8/14</td>
<td>0/7</td>
<td>0/7</td>
</tr>
</tbody>
</table>

Total 26/54 (48%) 15/34 (44%) 0/13 (0%)

Values are the total number of positive single-cell PCR reactions/total number of single-cell PCR reactions performed.
Fig 4. PCR detection of IgH CDR3 in single cells obtained from four cases of LPHD. (A) through (D) show results obtained from cases no. 1 through 4, respectively. All PCR products were size-fractionated by polyacrylamide-gel electrophoresis (8%) and visualized with ethidium bromide. Discrete bands of different sizes are observed in L&H cell and B-cell lanes, whereas no products are present in T-cell lanes. (m, 100-bp DNA ladder; the small bars indicate 100 and 200 bp, respectively; N, Namalwa cell line positive control.)
were, without exception, negative and that none of the single B-cell assays yielded three bands makes contamination an unlikely possibility. Alternatively, the three bands seen in the two single L&H cell assays may be explained by cell fusion in that cell population. The hypothesis that some Reed-Sternberg cells represent fused cells has been proposed by several investigators, but has not been supported by convincing evidence in the past. Further study as to whether L&H cells may represent fused B cells is necessary. PCR analysis of the entire rearranged V_{H} genes of freshly isolated and unfixed L&H cells would be useful to determine whether L&H cells functionally rearrange their Ig genes and whether somatic point mutations occur. These data would be of great interest and may yield important information on whether the L&H cells represent functional or aberrant B cells, whether cell fusion may occur, and whether the cells are related to germinai center cells.

In view of our findings that the IgH CDR3 of L&H cells varies between cells from any one case, LPHD very likely represents a polyclonal B-cell disorder. The sequence of the CDR3 is highly specific to one mature B cell or clone and its length is determined by deletions at the ends of V_{H}, D, and J_{H} segments and by D gene fusion and random insertion of a variable number of nucleotides at the recombination junctions. Although somatic point mutations may affect the IgH CDR3 and result in differences in sequence among cells derived from one clone, they do not result in altered migration patterns of IgH CDR3 sequences in regular polyacrylamide gels. When somatic point mutation occurs in a clone, the V-D-J junction of subclones will only differ with respect to a few nucleotides that have been substituted. Moreover, sequence analysis of the V-D-J junction will easily allow the designation of mutated sequences as belonging to one clone based on their limited differences and the unique V, D, and J segments and N regions used. Although the amplified IgH CDR3 from two L/H cells from cases no. 3 and 4, respectively, comigrate when electrophoresed, sequence analysis shows no evidence of sequence homology. In contrast to somatic point mutations, V_{H} gene replacements, such as occur in acute lymphoblastic leukemias, could potentially result in IgH CDR3 products of different size when analyzing one clone. However, sequence analysis of the PCR products obtained from our cases does not indicate that L&H cells in any one case do belong to one clone that replaces its V_{H} gene. Taken together, our data strongly indicate that the L/H cells of the respective cases do not derive from a single clone. In agreement with the results obtained with the single-cell assay, no clonal IgH gene rearrangements were detected in DNA extracts obtained from tissues involved by LPHD with either the Southern blotting or PCR technique. These latter results are similar to those consistently reported by others. Although the results obtained with the Southern blotting and PCR technique used on DNA extracts support the polyclonal nature of LPHD, they cannot be regarded as conclusive evidence because the sensitivity of these techniques may be too low to detect the clonality of minor cell populations such as the L&H cells represent. Other, potentially more sensitive, techniques previously used to investigate clonality in LPHD have yielded conflicting results. Us-

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### Table 3. Nucleotide Sequences of the IgH CDR3 of L&H Cells

<table>
<thead>
<tr>
<th>Cell</th>
<th>No.</th>
<th>Case</th>
<th>V_{H} Segment</th>
<th>D Segment</th>
<th>J_{H} Segment</th>
<th>CDR3 Sequence</th>
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<tr>
<td>L3A3</td>
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<td>L271</td>
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<td>TGGCAAG</td>
<td>TACAGA</td>
<td>TGGCAAG</td>
</tr>
<tr>
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<td>1/2</td>
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<td>TACAGA</td>
<td>TGGCAAG</td>
</tr>
<tr>
<td>L3C3</td>
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<td>TGGCAAG</td>
<td>TACAGA</td>
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</tr>
<tr>
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<td>TGGCAAG</td>
</tr>
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<td>TGGCAAG</td>
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<td>TACAGA</td>
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<tr>
<td>L3L3</td>
<td>1/22</td>
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<td>L3M3</td>
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Abbreviation: (II), lower band on the polyacrylamide gel.
ing a sensitive immunohistochemical technique for demonstrating $\kappa$ and $\lambda$ Ig light chains in tissue sections, Schmid et al.\textsuperscript{2} found $\kappa$ Ig light chain restriction in at least part of the tissues in 18 of 19 cases of LPHD. However, other investigators have reported a polytypic expression of Ig light chains in LPHD.\textsuperscript{3} In the present study, we also found reactivity for both Ig light chains in L&H cells in all four cases studied. Whether these results are in agreement with the polyclonal nature of the disease, or could be attributed to absorption of Igs by L&H cells from the intercellular space, is not clear.\textsuperscript{38,39} The apparent $\kappa$ Ig light chain restriction, but lack of $\lambda$ Ig light chain restriction, in the L&H cells of all of the cases studied by Schmid et al.\textsuperscript{2} could be explained by differences in the sensitivity of the primary antibodies or differential unmasking of antigens in their tissues and, therefore, may not be indicative of monoclonality. Hell et al.\textsuperscript{40} have recently reported restricted expression of Ig light chain mRNA in four of nine cases of LPHD using an in situ hybridization technique and a large cocktail of oligonucleotide probes for both $\kappa$ and $\lambda$ light chains. However, others have been unable to show the presence of Ig light chain mRNA using a similar technique.\textsuperscript{23} Although the use of a cocktail of numerous oligonucleotide probes may increase the sensitivity of the technique, it may also increase the possibility of nonspecific hybridization, which may account for the results obtained by Hell et al.\textsuperscript{40} Further studies are needed to clarify these issues.

In the present study of four cases of LPHD, two cases (cases no. 2 and 4) represented the diffuse variant of the disease. There is as yet lack of agreement as to whether the diffuse variant of LPHD, particularly those cases in which the L&H cells express the classical Hodgkin’s cell marker CD15, should be included with the nodular variant of LPHD. Some studies have shown that cases of “LPHD” with a diffuse pattern and a classical Reed-Sternberg cell phenotype clinically behave like other subtypes of HD.\textsuperscript{47,48} whereas others have not confirmed this finding.\textsuperscript{49,50} Our study suggests that both the diffuse and nodular variants of LPHD, at least those with a B-cell immunophenotype, represent a polyclonal B-cell disorder and may represent a single biologic entity. However, more cases of both nodular and diffuse variants of LPHD will need to be studied with our single-cell assay to fully establish the relationship between these variants of LPHD.

Although we have provided strong evidence that LPHD is a polyclonal B-cell lymphoproliferative disorder, the cause of the disease and the role and function of the L&H cell are unknown. It is of interest that another form of lymphadenopathy of unknown cause, so-called “progressive transformation of germinal centers” (PTGC), mimics the histology of LPHD except for the absence of L&H cells.\textsuperscript{2} In addition, both PTGC and LPHD result in localized lymphadenopathy that tends to recur, but usually does not progress.\textsuperscript{51} In a small percentage of cases, PTGC and LPHD are associated.\textsuperscript{52,55} either synchronously or metachronously, which supports the hypothesis that the two diseases may be part of a spectrum of a peculiar, lymphoproliferative process. The recognition of LPHD as a polyclonal B-cell disorder in addition to the well-known indolent clinical course of the disease discussion may lead to reevaluation of current modes of treatment.

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REFERENCES


Lymphocyte predominance Hodgkin's disease: lineage and clonality determination using a single-cell assay

J Delabie, A Tierens, G Wu, DD Weisenburger and WC Chan