Aberrant Expression of T-Cell–Associated Antigens on B-Cell Non-Hodgkin Lymphomas

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Key Words: Lymphoma; T-cell antigens; CD2; CD8; Chronic lymphocytic leukemia; Flow cytometry

Abstract

We describe 9 well-characterized cases of B-cell non-Hodgkin lymphoma (NHL) that showed aberrant expression of T-cell–associated antigens by 2-color flow cytometry. Cases were as follows: chronic lymphocytic leukemia/small lymphocytic lymphoma, 4; follicle center cell lymphoma, 2; mantle cell lymphoma, 1; and diffuse large B-cell lymphoma, 2. CD2 was the most commonly expressed antigen (5 cases). CD8 and CD7 were identified in 2 cases each, including 1 case that expressed both CD7 and CD4. The disease course and response to treatment were compatible with the type and stage of lymphoma. No unusually aggressive behavior was noted in any case. A control group of 59 cases of benign lymph nodes analyzed during the same period showed no aberrant expression of T-cell–associated antigens; thus, such expression is not a feature of benign lymphoid proliferations. Study of these B-cell lymphomas may prove invaluable to study aberrant activation of silent or repressed T-cell differentiation genes. CD2-expressing B-cell NHLs may represent clonal expansion of CD2+ B lymphocytes that normally constitute a small fraction of peripheral B lymphocytes and should not be confused with composite B- and T-cell lymphomas. Unless aggressive behavior is noted consistently, no aggressive treatment is justified.

Aberrant expression of T-cell–associated antigens on B-cell non-Hodgkin lymphomas (NHLs) other than CD43 is a known but uncommonly observed phenomenon.1-3 Aberrant expression of CD2, CD3, CD4, CD7, CD8, and CD45RO (UCHL-1) has been reported.4,5 Chronic lymphocytic leukemia (CLL)/small lymphocytic lymphoma (SLL) is the most cited B-cell malignant neoplasm with aberrant coexpression of T-cell–associated antigens.6-8 Other B-cell lymphomas also have displayed this phenomenon.9

Many of the earlier studies reporting on the dual expression of B- and T-cell antigens by B-cell NHL used anti–B cell and anti–T cell serum assays and/or the demonstration of rosette formation of lymphocytes with sheep RBCs (SRBCs).10-14 However, these techniques are rarely used today for that purpose and frequently are fraught with technical problems.15 Furthermore, rosette formation with SRBCs implicated only CD2 as the T-cell antigen aberrantly expressed on B-cell NHL, and the antisera assays did not reveal the identification of specific T-cell antigens.

The use of immunofluorescence in subsequent years not only identified the specificity of the aberrantly expressed T-cell antigen but also revealed that rarely more than 1 T-cell–associated antigen can be expressed by a single B-cell lymphoma.4 Few studies, however, have used multiparameter flow cytometry to characterize these cases, and only rare reports describe such cases in any substantial detail.16,17 The obvious questions relating to the incidence, biology, and clinical behavior of such lymphomas remain to be answered. We studied the clinicopathologic details of these rare cases to identify any unusual clinical behavior and facilitate understanding of the biology of these neoplasms.
Methods and Materials

Case Selection

All cases were identified from the files of the Lauren V. Ackerman Laboratory of Surgical Pathology, Washington University School of Medicine, St Louis, MO, from January 1993 through June 1997. Only cases with a full panel of B- and T-cell antigens and with sufficient clinical details were included. The specimens include lymph nodes (LNs), extranodal tissue (EN), bone marrow (BM), and peripheral blood (PB). Cases of B-cell NHL diagnosed by fine-needle aspiration biopsy were excluded. All cases of posttransplant lymphoproliferative disorders and T-cell–rich B-cell NHL also were excluded. We included 59 consecutive cases of benign lymph nodes in which full B- and T-cell panels were run from January 1996 through June 1997. All 9 patients received standard treatments according to various protocols. Any statistical comparison for the survival of these patients was deemed inappropriate because of the small number of patients.

Flow Cytometric Evaluation

Fresh tissues were transported immediately in RPMI solution to the flow cytometry laboratory. Lymphocytes were disaggregated and released from solid tissue by RPMI injection. Cells were stained with various combinations of the following fluorescein isothiocyanate– or phycoerythrin-labeled monoclonal antibodies against the following antigens: CD1, CD2, CD3, CD4, CD5, CD7, CD8, CD10, CD11c, CD19, CD20, CD23, HLA-DR, IgG, IgM, IgD, IgA heavy chains, and kappa and lambda light chains. In selected cases, monoclonal antibodies against other antigens also were run, including CD13, CD14, CD33, CD34, CD41a, and terminal deoxynucleotidyl transferase. Two-color flow cytometric immunophenotyping was performed on a FACScan (Becton-Dickinson, San Jose, CA) or a Coulter XL cytometer (Coulter, Miami, FL) by collecting 10,000 ungated list mode events, selecting an appropriate lymphocyte gate on the combination of forward and side scatter, and analyzing cells with the most appropriate lymphocyte gate. In the cases of more than one lymphocyte gate, the gate with the largest number of B cells and/or showing aberrant coexpression of antigens and closely corresponding to the cell size of the lymphoma in question was chosen for analysis. However, with few exceptions, similar data were obtained from additional gates when present.

An antigen was considered positively expressed when at least 25% of the gated lymphocytes expressed that antigen. Clonality was determined when the immunoglobulin kappa light chain/lambda light chain ratio was more than 4:1 or less than 1:2 in a substantial population of B lymphocytes. Only cases with unequivocal aberrant expression of the T-cell–associated antigens (other than CD5) were included. This definition of “unequivocal aberrant expression” included the following: (1) cases in which aberrant expression was demonstrated by dual expression of the B-cell antigens (CD19 and/or CD20) and T-cell–associated antigens (2 cases); (2) cases in which expression of 1 T-cell antigen was grossly (>50 percentage points or more) in excess of CD3 expression (7 cases). CD3 was chosen as the reference T-cell antigen because of its specificity for T cells, and in none of our cases was CD3 aberrantly coexpressed on CD20+ B lymphocytes. This strict definition was chosen to exclude the possibility of contamination by monocytes or macrophages that normally express CD2 and CD4 antigens and may lead to slightly excessive readings for CD2 and/or CD4 antigens.

Results

Nine (4.3%) of 210 cases showed aberrant expression of T–cell–associated antigens: Table 1 and Table 2. The distribution of nonoverlapping cases was as follows: 4 of 68 cases of PLL (LN, 1/22; EN, 0/5; PB, 2/27; BM, 1/14), 1 of 32 cases of mantle cell lymphoma (LN, 1/19; EN, 0/3; PB, 0/4; BM, 0/6), 2 of 50 cases of follicle center cell lymphoma (LN, 2/30; EN, 0/4; PB, 0/5; BM, 0/11), 2 of 30 cases of diffuse large B-cell lymphoma (LN, 1/14; EN, 0/10; BM, 1/6), 0 of 9 cases of marginal zone lymphoma, 0 of 9 cases of lymphoplasmacytoid lymphoma, 0 of 7 cases of Burkitt lymphoma, and 0 of 5 cases of hairy cell leukemia.

None of the 9 cases showed unusual morphologic features. CD2 was the most common aberrantly expressed T–cell–associated antigen seen in 2 cases of PLL/SLL (Figure 1 and Image 1), 2 cases of follicle center cell lymphoma (FCCL) (Figure 2 and Image 2), and 1 case of diffuse large B-cell lymphoma. Viciana et al8 described 1 case of PLL with CD2 expression that showed unusually aggressive clinical behavior. We did not observe, however, unusually aggressive behavior in cases 2 and 3, which were Rai stages II and I, respectively. Similarly, a report9 of CD8+ PLL/SLL describes unusual disease progression, but no unusual disease progression was noted in our cases 1 and 4 or in cases in several other reports.6,7,17,20,21 Table 3 and Figure 3. Both patients with CD2+ FCCL had advanced stage disease; however, this cannot be attributed unequivocally to aberrant CD2 expression in the absence of statistical comparisons. One of the two cases of FCCL showed nodular and diffuse morphologic features with small and large cleaved and noncleaved cells and, thus, did not have low-grade morphologic features. Table 2.

Discussion

We have described 9 well-characterized cases of B-cell NHLs that showed aberrant coexpression of 1 or 2
T-cell–associated antigens. This phenomenon has been reported previously but only rarely documented with adequate clinicopathologic details. Most of the reported cases implicated CLL as the most common B-cell malignant neoplasm with aberrant expression of T-cell–associated antigens. It is unclear, however, whether this reflects the sheer number of CLL cases identified and analyzed or whether this phenomenon is truly more common with CLL.

We found CD2 as the most common aberrantly expressed T-cell–associated antigen. CD2 is a transmembrane glycoprotein that is implicated in signal transduction and serves as a receptor for various ligands, mainly CD58, but also CD48, CD59, and CD15. The CD2 molecule, also known as SRBC receptor, is responsible for rosette formation of T lymphocytes with SRBC and, as such, has been used widely to identify T cells in earlier studies.12-14 Most thymocytes, including double negative (CD4–CD8–), double positive (CD4+CD8+), and single positive (CD4+CD8– or CD4–CD8+) cells; peripheral CD4+ and CD8+ T cells; and NK cells, express CD2 receptor.22 Earlier studies also revealed that a small proportion of mature surface immunoglobulin–expressing B cells express CD2, as indicated by rosette formation with SRBC and a positive reaction with anti–T-cell serum.23,24 The fact that CD2 is expressed

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**Table 1**

Clinicopathologic Findings

<table>
<thead>
<tr>
<th>Case No./ Sex Age (y)</th>
<th>Adenopathy</th>
<th>Organomegaly</th>
<th>Bone Marrow</th>
<th>WBC Count, µL (× 10^9/L)</th>
<th>Hemoglobin, g/dL (g/L)</th>
<th>Platelet Count, µL (× 10^9/L)</th>
<th>“B Symptoms”</th>
<th>Clinical Stage</th>
<th>Clinical Course/Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/F/63</td>
<td>Extensive</td>
<td>Spleen</td>
<td>Involved</td>
<td>70,500 (70.5)</td>
<td>16.3 (163)205 (205)</td>
<td>Absent</td>
<td>II (Rai)</td>
<td>Lost to follow-up after 6 y with persistent disease</td>
<td></td>
</tr>
<tr>
<td>2/F/62</td>
<td>Moderate</td>
<td>Liver, spleen</td>
<td>Involved</td>
<td>17,100 (17.1)</td>
<td>13 (130)/330 (330)</td>
<td>Present</td>
<td>II (Rai)</td>
<td>AWD after 8 y; follow-up continues</td>
<td></td>
</tr>
<tr>
<td>3/F/56</td>
<td>Moderate</td>
<td>None</td>
<td>Involved</td>
<td>13,900 (13.9)</td>
<td>12 (120)/249 (249)</td>
<td>Absent</td>
<td>I (Rai)</td>
<td>Lost to follow-up after 2.5 y; no evidence of aggressive disease</td>
<td></td>
</tr>
<tr>
<td>4/M/50</td>
<td>Extensive</td>
<td>None</td>
<td>Involved</td>
<td>12,900 (12.9)</td>
<td>14.4 (144)/141 (141)</td>
<td>Absent</td>
<td>I (Rai)</td>
<td>Died with disease</td>
<td></td>
</tr>
<tr>
<td>5/M/71</td>
<td>Moderate</td>
<td>None</td>
<td>Involved</td>
<td>7,700 (7.7)</td>
<td>8.4 (84)/159 (159)</td>
<td>Absent</td>
<td>IV</td>
<td>DWD after 46 mo; also had bilateral lung cancer</td>
<td></td>
</tr>
<tr>
<td>6/M/77</td>
<td>Extensive</td>
<td>None</td>
<td>Not done</td>
<td>8,900 (8.9)</td>
<td>14 (140)/173 (173)</td>
<td>Absent Not staged</td>
<td>Not DWD in 2 wk with complications of abdominal surgery</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7/F/64</td>
<td>Moderate</td>
<td>Spleen</td>
<td>Not involved</td>
<td>5,900 (5.9)</td>
<td>12 (120)/166 (166)</td>
<td>Absent</td>
<td>IV</td>
<td>DWD after 21 mo</td>
<td></td>
</tr>
<tr>
<td>8/F/81</td>
<td>Moderate</td>
<td>None</td>
<td>Not involved</td>
<td>4,300 (4.3)</td>
<td>12 (120)/237 (237)</td>
<td>Absent</td>
<td>III</td>
<td>Lost to follow-up after 13 mo of stable disease</td>
<td></td>
</tr>
<tr>
<td>9/F/61</td>
<td>Minimal</td>
<td>Liver, spleen</td>
<td>Involved</td>
<td>Unavailable</td>
<td>Unavailable</td>
<td>Present</td>
<td>IV</td>
<td>Lost to follow-up after 9 mo of treatment and stable disease</td>
<td></td>
</tr>
</tbody>
</table>

AWD, alive with disease; DWD, died with disease.

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**Table 2**

Surface Expression of Various B- and T-Cell Antigens and Immunoglobulin kappa and lambda Light Chains

<table>
<thead>
<tr>
<th>Case No.</th>
<th>kappa/ lambda</th>
<th>CD19+</th>
<th>CD3+</th>
<th>CD1+</th>
<th>CD2+</th>
<th>CD4+</th>
<th>CD7+</th>
<th>CD8+</th>
<th>CD19–CD5+</th>
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<tbody>
<tr>
<td>Chronic lymphocytic leukemia/small lymphocytic lymphoma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>1</td>
<td>44/1</td>
<td>90</td>
<td>2</td>
<td>ND</td>
<td>0</td>
<td>1</td>
<td>ND</td>
<td>98</td>
<td>8</td>
</tr>
<tr>
<td>2</td>
<td>1/48</td>
<td>98</td>
<td>1</td>
<td>0</td>
<td>82</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
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<td>3</td>
<td>80/1</td>
<td>98</td>
<td>1</td>
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<td>98</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>4/1</td>
<td>87</td>
<td>11</td>
<td>0</td>
<td>11</td>
<td>7</td>
<td>12</td>
<td>85</td>
<td>11</td>
</tr>
<tr>
<td>Follicle center cell lymphoma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>38/1</td>
<td>97</td>
<td>1</td>
<td>0</td>
<td>85</td>
<td>5</td>
<td>11</td>
<td>12</td>
<td>4</td>
</tr>
<tr>
<td>6</td>
<td>85/1</td>
<td>88</td>
<td>9</td>
<td>0</td>
<td>79</td>
<td>13</td>
<td>11</td>
<td>8</td>
<td>11</td>
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<tr>
<td>Mantle cell lymphoma</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>1/84</td>
<td>78</td>
<td>13</td>
<td>ND</td>
<td>ND</td>
<td>7</td>
<td>89</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Diffuse large B-cell lymphoma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>37/1</td>
<td>86</td>
<td>8</td>
<td>0</td>
<td>12</td>
<td>69</td>
<td>70</td>
<td>5</td>
<td>15</td>
</tr>
<tr>
<td>9</td>
<td>1/26</td>
<td>85</td>
<td>7</td>
<td>0</td>
<td>91</td>
<td>1</td>
<td>0</td>
<td>3</td>
<td>6</td>
</tr>
</tbody>
</table>

ND, not done.
by a very small proportion of normal B cells raises the possibility that CD2+ B-cell NHL may represent clonal expansion of a CD2+ subpopulation of normal B lymphocytes. Although the exact role of CD2 in B lymphocytes is unknown, the widespread distribution of its ligands CD58 and CD59 suggests that CD2+ B lymphocytes might have a distinct role in cell-cell interaction with a variety of inflammatory and other cells, including endothelial cells, fibroblasts, epithelial cells, and, possibly, other B cells.

The expression of CD8, both on the cell surface and in soluble form in the serum of patients with B-cell NHL, is likely the next most common aberrantly expressed T-cell-associated antigen after CD2. Although CLL is the most cited B-cell malignant neoplasm, rare cases of mantle cell lymphomas with aberrant expression of CD8 also are described. CD8 is a 2-chain cell surface glycoprotein that is expressed on a subset of thymocytes, NK cells, and peripheral T cells and functions as a coreceptor molecule that binds with a major histocompatibility complex class I molecule and, as such, is involved in T-cell receptor ligand binding and T-cell activation. Because of more than occasional identification of CD8+ B-CLL, it can be speculated that these lymphomas may represent clonal expansion of an as yet unidentified subset of normal B lymphocytes that express CD8 or, alternatively, genetic aberrations that are unique to the pathogenesis of some B-CLL that may lead to activation of the CD8 receptor gene in some cases. The latter hypothesis is supported by the demonstration of anomalous configuration of the CD8alpha gene owing to alterations in the cis-acting regulatory elements upstream of the coding sequence in a case of CD8+ B-CLL expressing only CD8 alpha/alpha homodimers.

In contrast with a few reports, we did not identify unequivocal aberrant expression of CD3 in any of our B-cell lymphoma cases.

The expression of CD5 by CLL and mantle cell lymphoma does not constitute aberrant expression, because these malignant neoplasms are thought to represent clonal expansion of a CD5+ subpopulation of B lymphocytes that...
normally is present in the mantle zone cells of secondary follicles. A few cases of diffuse large B-cell lymphoma also express CD5 that might result from large cell transformation of a CD5+ subpopulation of small B lymphocytes.

The expression of CD7 by mantle cell lymphoma is previously unreported. Although CD7 frequently is identified in cases of minimally differentiated acute myelogenous leukemia (AML-M0 in the French-American-British classification), it is rarely observed in B-cell lymphomas.

**Figure 2** (Case 5) Follicle center cell lymphoma showing significant expression of CD2 (85%) (A) in a population of cells that is mainly composed of CD20+ B cells and negligible numbers of CD3+ T cells (B).

**Figure 3** (Case 6) Follicle center cell lymphoma showing significant expression of CD2 (79%) (A) in a population of cells that is mainly composed of CD20+ B cells and small numbers of CD3+ T cells (B).
British classification), its expression in mature B-cell lymphomas is rare.

The expression of both CD4 and CD7 by a diffuse large B-cell lymphoma in our series is quite intriguing and the least understood. The CD4 coreceptor normally is present on a subset of thymocytes, peripheral T cells, and monocytes or macrophages, whereas CD7 is present on all T lymphocytes, NK cells, and pluripotent hematopoietic stem cells. However, unlike CD2 and CD5, no B-cell fractions are known to express CD4 and CD7 normally. One plausible explanation is the deregulated control of gene expression in malignant B cells, which leads to activation of some silent or repressed genes of T-cell differentiation.

It has been shown that the PAX5-negative pro-B cells that express the genes associated with B-cell differentiation (E2A, EBF, VpreB, lambda5, Igalpha, and Igbeta) can be directed to T-cell differentiation on appropriate stimulation.30,31 However, in the presence of PAX5, pro B-cells differentiated only along B-cell lineage, leading to the hypothesis that the transcription factor PAX5 has a dual function in B-lineage commitment by activating B-lymphoid genes and simultaneously repressing lineage-inappropriate genes. It can, therefore, be speculated that deregulation or inactivation of PAX5 in malignant B cells leads to unopposed activation of certain lineage-inappropriate genes. Consistent with the hypothesis of inappropriate activation of T-cell–associated antigens was the finding of appearance of CD8 in long-term cultures of B-CLL cells.32 Formal proof of this hypothesis is yet to be provided, however.

The expression of T-cell–associated antigens in B-cell NHL has diagnostic and pathogenetic significance. Normal B cells do not express T-cell–associated antigens in substantial amounts. This observation was verified in our group of

<table>
<thead>
<tr>
<th>Reference</th>
<th>Sex/ Age (y)</th>
<th>Signs and Symptoms</th>
<th>WBC Count, /µL (× 10⁹/L)</th>
<th>Rai Stage</th>
<th>Other Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perl et al21</td>
<td>F/66</td>
<td>Fatigue, night sweats, rash, cervical lymphadenopathy, and splenomegaly</td>
<td>22,100 (22.1)</td>
<td>II*</td>
<td>Combination chemotherapy resulted in marked reduction in spleen size, lymph nodes, and lymphocytosis</td>
</tr>
<tr>
<td>Porwit et al7</td>
<td>F/74</td>
<td>Lymphocytosis; no lymph node or spleen enlargement at initial examination but developed 1 y later; no symptoms except night sweats</td>
<td>53,000 (53.0)</td>
<td>III*</td>
<td>During 12-mo follow-up, no chemotherapy given</td>
</tr>
<tr>
<td>Koelliker et al20</td>
<td>M/71</td>
<td>Asymptomatic with lymphocytosis; found during routine CBC count; no lymphadenopathy or hepatosplenomegaly</td>
<td>31,600 (31.6)</td>
<td>0</td>
<td>No evidence of aggressive disease; follow-up data not provided</td>
</tr>
<tr>
<td>Koelliker et al20</td>
<td>F/49</td>
<td>Asymptomatic with lymphocytosis, found during routine physical examination; no lymphadenopathy or hepatosplenomegaly</td>
<td>26,900 (26.9)</td>
<td>0</td>
<td>No evidence of aggressive disease; follow-up data not provided</td>
</tr>
<tr>
<td>Ghosh et al19</td>
<td>M/68</td>
<td>Dyspnea; examination showed axillary lymphadenopathy and lymphocytosis; no organomegaly</td>
<td>99,200 (99.2)</td>
<td>1</td>
<td>Initial response to chemotherapy, then recurrence; died with disease 18 mo after diagnosis</td>
</tr>
<tr>
<td>Attadia et al17</td>
<td>M/49</td>
<td>Asymptomatic; evaluated for lymphadenopathy; splenomegaly but not hepatomegaly present</td>
<td>13,500 (13.5)</td>
<td>II*</td>
<td>Stable clinical and hematologic findings 3.5 y after diagnosis without treatment</td>
</tr>
</tbody>
</table>

*Rai staging was assigned according to the clinical information provided; otherwise it was given in the article.
59 cases of morphologically and immunophenotypically benign lymph nodes, none of which expressed T-cell–associated antigens. Thus, expression of substantial amounts of T-cell–associated antigens in B-cell proliferative processes signifies the malignant nature of the lymphoproliferative lesion in question. However, technical factors, including inappropriate lymphocyte gating, must be excluded before concluding aberrant expression of T-cell–associated antigens in B-cell lymphomas. Admixture of benign T cells with the B-lymphoma cells also may incorrectly suggest aberrant expression of T-cell antigens on B-lymphoma cells, but multicolor labeling eliminates that misinterpretation. The rare, true examples of these lymphomas may prove invaluable tools to understand the mechanisms causing expression of silent or repressed genes and functions, if any, of these T-cell–associated antigens on B cells.

No substantial controversy exists as to the clinical behavior of these lymphomas. Only single case reports project aggressive behavior of CD2- and CD8-expressing B-CLL. Neither in our cases nor in most reported cases have we found conclusive evidence of unusually aggressive clinical behavior. We emphasize that otherwise typical cases of B-cell
NHL should not be treated aggressively solely on the basis of aberrant expression of T-cell–associated antigens unless aggressive behavior is consistently noted in future reports.

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References


