

# Pitfalls in Immunohistochemistry in Hematopathology: CD20 and CD3 Can Let Me Down?!

Judith A. Ferry, MD  
Massachusetts General Hospital

This discussion focuses on diagnostic pitfalls related to immunohistochemistry in Hematopathology, focusing on CD20 and CD3, and specifically on diagnostic challenges that arise when (1) CD20 is not expressed in B-cell lymphomas, when (2) CD20 is expressed in plasma cell neoplasms and T-cell lymphomas, and when (3) CD3 is expressed in B-cell lymphomas and Hodgkin lymphoma.

## **CD20 Negativity in B-Cell Lymphomas**

The single most commonly used marker to identify B-cell lymphomas is CD20. Certain types of B-lineage lymphomas are characteristically negative for CD20, however. These include plasmablastic lymphoma, HHV8+ primary effusion lymphoma (and its extracavitary or solid variant), B lymphoblastic lymphoma/leukemia and ALK+ diffuse large B-cell lymphoma. On encountering one of these high grade lymphomas, the lack of CD20 may lead the pathologist to consider a diagnosis other than lymphoma. In addition, some CD20+ B-cell lymphomas in patients who have been treated with rituximab become CD20-negative.

### **Plasmablastic Lymphoma**

Plasmablastic lymphoma (PBL) is a rare high-grade large B-cell lymphoma with distinctive clinical and pathological features including strong association with immunodeficiency, plasma cell immunophenotype and frequent association with MYC rearrangement and EBV. PBL was first described in 1997 in a study that included 16 patients, 15 of whom were HIV+, with high-grade, CD20-negative lymphomas in the oral cavity.<sup>1</sup> Since the initial publication, PBL has been recognized in other anatomic sites and in many patients who are not HIV+.

PBL is defined as a diffuse proliferation of large neoplastic cells, most with the morphology of immunoblasts, but with the immunophenotype of plasma cells.<sup>2</sup> Although PBL was first identified in the oral cavity, it has since been found in other extranodal sites, including paranasal sinuses, gastrointestinal tract, bone, liver and spleen, testes, skin and soft tissue, and less often in lymph nodes.<sup>3-11</sup> PBL affects patients from early childhood to advanced age; however, most patients with this type of lymphoma are HIV+ adults with a median age in the fifth decade and a striking male preponderance.<sup>6-8, 12</sup> Children with PBL are almost all HIV+.<sup>8, 13</sup>

Even among HIV+ individuals this type of lymphoma is uncommon; in one study only 6% of AIDS-related lymphomas were plasmablastic lymphomas.<sup>12</sup> PBL has been even less common in other studies.<sup>14</sup> In a small minority of cases (only 7% in one series)<sup>8</sup> the development of PBL is the first sign that the patient is HIV+.<sup>6</sup> In most cases patients have been HIV+ for years before the development of PBL, and some of

them have received anti-retroviral therapy (ART), so that treatment with ART does not serve as definitive protection against PBL.<sup>6</sup>

Cases of PBL are also described in iatrogenically immunosuppressed patients and in HIV-negative, apparently immunocompetent individuals, mostly older adults.<sup>3, 5, 9, 11, 14, 15</sup> Compared to HIV+ patients with PBL, HIV-negative PBL patients are on average older, with a less striking male preponderance and are less likely to present with disease in the oral cavity.<sup>5, 8, 16</sup> Iatrogenically immunosuppressed posttransplant PBL patients are more likely to have involvement of lymph nodes or skin than HIV+ PBL patients.<sup>8</sup> Among transplant recipients with PBL, the most common allograft is cardiac (accounting for 38% of cases), followed by renal allograft and hematopoietic stem cell transplant, with PBL being uncommonly associated with other types of transplants.<sup>8</sup>

A few cases of plasmablastic transformation of low-grade B-cell lymphoma have been described. The transformed high-grade lymphomas have histologic and immunophenotypic features similar to those of PBL arising *de novo*, although EBV is less frequently identified.<sup>17</sup> Presentation of these high-grade lymphomas with extranodal disease is common, similar to *de novo* PBL. Common cytogenetic or genetic alterations between the low-grade and the high-grade lymphomas have been demonstrated in a number of cases, supporting the concept that the high-grade lymphomas do indeed represent transformation of the low-grade lymphomas rather than new, unrelated primary lymphomas.<sup>17</sup>

PBL is typically composed of a monotonous proliferation of large cells with the appearance of immunoblasts, plasmacytoid immunoblasts or plasmablasts, with vesicular nuclei and prominent nucleoli. In other cases there is plasmacytic differentiation, with a subset of cells showing maturation toward plasma cells.<sup>18</sup> These neoplasms have a high mitotic rate, frequent single cell or zonal necrosis and scattered tingible body macrophages. The immunophenotype is closer to that of a plasma cell than to a normal B cell. Neoplastic cells are typically positive for CD138, CD38, Blimp1 and MUM1/IRF4; negative for CD20, Pax5, BCL6, ALK, and EBV-LMP; and with variable expression of CD45, CD79a, CD56, CD10 and CD30. MYC and cytoplasmic immunoglobulin are expressed in most cases. Aberrant expression of keratin has been reported.<sup>1, 3-6, 8, 9, 11, 12, 15, 16, 19</sup> There is a high proliferation fraction among the neoplastic cells.<sup>2-4</sup> The proliferation index is greater than 80% in approximately 80% of cases.<sup>6, 12</sup> In situ hybridization for EBER demonstrates EBV in approximately two-thirds of all cases and in nearly all HIV+ PBLs. HIV-negative patients appear somewhat more likely to have EBV-negative PBL.<sup>5, 8, 9, 14, 18</sup> EBV latency pattern has varied among studies.<sup>1, 8</sup> HHV8 is absent, by definition.<sup>2</sup>

Evaluation of cytogenetic and genetic features typically reveals clonal rearrangement of IGH and polyclonal TCR. There is a translocation involving *MYC* in approximately 50% of cases, with the partner in most cases being *IGH* [t(8;14)]<sup>6, 8, 10, 15, 20, 21</sup> or *IGL*.<sup>7</sup> The *MYC* rearrangement is typically present in the setting of a complex karyotype, in contrast to Burkitt lymphoma. In a subset of the remaining cases, there is *MYC* amplification. *MYC* aberrations appear to be more common in HIV+ patients.<sup>14</sup> There is no rearrangement of *BCL6* or *BCL2* genes; thus plasmablastic lymphoma is not part of the spectrum of so-called "double-hit" lymphomas.<sup>8, 21</sup> Comparative genomic hybridization studies suggest features more like other DLBCL than like myeloma.<sup>22</sup> In one small series, complex cytogenetic abnormalities were identified which were considered to be more like those encountered in plasma cell myeloma than in

lymphoma.<sup>7</sup> Also common in PBL is recurrent somatic mutation of *PRDM1* (which codes for Blimp1). *PRDM1* normally plays a role in regulation of such targets as *MYC*, and also drives terminal B-cell differentiation. Mutation of *PRDM1* in the setting of PBL may contribute to dysregulation of *MYC*, while apparently retaining the capacity to induce terminal B-cell differentiation.<sup>11</sup>

Gene expression analysis of PBL identifies some variability. In general there is downregulation of the B-cell receptor signaling program (including *CD79A*, *CD79B*, *BLK*, *LYN*, *SYK* and others) and upregulation of genes associated with plasma cell differentiation (such as *XBP1*, *CD138*, *BLIMP1* and others) compared to DLBCL.<sup>18</sup> Gene expression profiling of post-transplant PBLs has shown upregulation of *DNMT3B* (DNA methylation, expressed in myeloma), *PTP4A3* (role in migration of malignant plasma cells) and *CD320* (plasma cell differentiation).<sup>14</sup> Also found were upregulation of genes involved in a positive feedback loop regulating *C-MYC* expression, as well as high overexpression of cancer-related genes from X chromosome (such as *MAGEA1*, *SSX1* and *SSX4*) belonging to the category of cancer/testis antigens which are highly immunogenic proteins almost only expressed in normal testis, or in certain cancers. These markers were recently found to be highly expressed in myeloma.<sup>14</sup> The significant heterogeneity at both the immunophenotypic and also the genetic levels suggests different pathogenetic mechanisms among PBLs.<sup>14</sup>

The differential diagnosis includes plasmablastic myeloma, EBV+ DLBCL, NOS (formerly, EBV+ DLBCL of the elderly), extracavitary (“solid”) primary effusion lymphoma (PEL), HHV8+ DLBCL, NOS (arising in most cases from Castleman disease), ALK+ DLBCL and the recently described EBV+ plasmacytoma in immunocompetent patients (“EPIC”).<sup>23</sup>

EPIC is rare. It has been found in the nasal cavity, esophagus, and mediastinum. It is characterized by a diffuse proliferation of mature plasma cells intermixed with reactive CD8+ TIA-1+ cytotoxic T cells. Bi- and multinucleated plasma cells are common. Russell bodies and Dutcher bodies can be found. Occasional mitoses are found, although tingible body macrophages and necrosis are not seen. Immunohistochemistry shows plasma cells that are CD138+, cIg+, MUM1+, CD56+/-, CD45 +/-, Ki67 5-40% (median 25%), CD20-, MYC-, ALK-. EPIC is diffusely + for EBER. *MYC* rearrangement has not been found in the rare cases tested.<sup>23</sup> Treatment has varied. Patients have been well on follow-up.

In the majority of cases patients present with advanced stage disease. PBL is an aggressive lymphoma with a poor prognosis (median survival < 1 year in multiple studies). HIV-associated PBL appears to have a worse outcome than HIV-associated DLBCL and Burkitt lymphoma.<sup>12</sup> Factors suggested as predicting a poor prognosis include ECOG performance status of 2 or more, advanced stage disease, failure to achieve a complete remission, and *MYC* rearrangement.<sup>6, 8-10, 14</sup> Aggressive therapy, including autologous stem cell therapy may improve the outcome. Some investigators suggest that treatment with agents useful in plasma cell myeloma may be efficacious in plasmablastic lymphoma.<sup>6, 7, 12, 16</sup> The optimal therapy remains to be determined.

### **HHV8+ Primary Effusion Lymphoma**

Primary effusion lymphoma (PEL), previously called body cavity-based lymphoma, is a rare, distinctive type of HHV8+ (human herpes virus 8, also known as Kaposi's sarcoma-associated herpes virus [KSHV]) large B-cell lymphoma characterized by lymphomatous effusions involving pleural, pericardial or peritoneal cavities unaccompanied by a solid mass.<sup>24, 25</sup> PEL affects young and middle-aged adults with males much more often affected than females. Nearly all patients are HIV-positive; they present late in the course of HIV infection and are often profoundly immunodeficient at the time they present with lymphoma. Only occasional patients are HIV-negative; they are often older adults from HHV8-endemic areas.<sup>26, 27</sup> PEL has a very poor prognosis, although among HIV+ patients, the outcome may be better for those receiving antiretroviral therapy (ART).<sup>28</sup> Death is due to lymphoma, sometimes complicated by opportunistic infection or Kaposi's sarcoma.<sup>29</sup>

The neoplastic cells are uniform and immunoblast-like, or pleomorphic and very large. Binucleated or multinucleated forms may be present. Some neoplastic cells may resemble Reed Sternberg cells. Neoplastic cells express CD45 and MUM1/IRF4, and typically lack CD20, Pax5, CD79a, BCL6 and immunoglobulin. CD138 and CD30 are sometimes expressed. Aberrant expression of T-cell associated antigens is relatively common.<sup>30</sup> Immunoglobulin heavy and light chains are clonally rearranged. The HHV8+ tumor cells are usually co-infected by EBV.<sup>15, 24, 31, 32</sup>

Cases of HHV8+ lymphoma with morphologic, immunophenotypic and genetic features similar to those of PEL but producing mass lesions in lymph nodes or in extranodal sites have also been described.<sup>32-34</sup> These have been called HHV8+ or KSHV+ solid PEL or extracavitary PEL,<sup>32, 34, 35</sup> or KSHV+ solid immunoblastic/plasmablastic diffuse large B-cell lymphoma.<sup>15, 36</sup> Compared to classic PEL, extracavitary HHV8-associated large B-cell lymphoma appears slightly more likely to express pan B-cell antigens such as CD20 and CD79a and also monoclonal immunoglobulin.<sup>37</sup> In one study 29% of solid PELs showed aberrant expression of CD3, potentially leading to misinterpretation as T-cell lymphoma, especially anaplastic large cell lymphoma, particularly as B-cell antigens are not well expressed.<sup>37</sup>

### **ALK+ Diffuse Large B-Cell Lymphoma**

ALK+ diffuse large B-cell lymphoma (DLBCL) is a rare, aggressive large cell lymphoma with unusual immunophenotypic features including expression of ALK protein as well as lack of CD20.<sup>38</sup>

ALK+ DLBCL affects patients over a broad age range but patients are mostly young to middle-aged, immunocompetent adults (median, approximately 35 years), with a male preponderance (M:F ratio of about 3.5:1).<sup>39</sup> Constitutional symptoms are common. Disease is often widespread, usually involving lymph nodes but also often affecting one or more of a wide variety of extranodal sites (soft tissue, bone, GI tract and others). The most common pattern of involvement is a diffuse proliferation of neoplastic cells, although in lymph nodes there is often a striking sinusoidal pattern, or a combination of sinusoidal and diffuse involvement.<sup>39</sup>

Neoplastic cells have the morphology of immunoblasts and/or plasmablasts, sometimes with maturation to plasmacytic cells.<sup>39</sup> Infrequently, tumor cells are pleomorphic and may be multinucleated. The usual immunophenotype is ALK+ (granular cytoplasmic staining), CD45+, CD20-, CD79a-/+, Pax5-/+, CD3-

CD4+/-, OCT2+, BOB1+, CD138+, Ig light chain+ (kappa or lambda), MUM1+, CD30-, EMA+/-, HHV8-. The immunoglobulin most often expressed is IgA. EBER is negative.

Clonal rearrangement of IGH can typically be demonstrated. The cytogenetic abnormality most characteristic of ALK+ DLBCL is t(2;17)(p23;q23), resulting in a translocation involving the *ALK* gene on chromosome 2 and *clathrin* gene on chromosome 17. Clathrin protein normally coats cytoplasmic vesicles involved in endocytosis. The presence of the t(2;17) leads to a fusion protein that shows a granular cytoplasmic pattern of staining with antibody to ALK. Uncommonly ALK has a different gene partner in the translocation, such as *NPM1* (the usual gene partner for *ALK* in cases of ALK+ anaplastic large cell lymphoma, a T-lineage lymphoma), *SEC31A* and rarely, others. The pattern of immunostaining is different in such cases; when *NPM1* is the gene partner for example, ALK staining is nuclear and cytoplasmic. The cytogenetic changes are believed to result in activation of ligand-independent ALK tyrosine kinase, and subsequently of STAT3 and other pathways, leading to a plasmablastic phenotype and to lymphomagenesis.<sup>39</sup>

ALK+ DLBCL is associated with a poor prognosis in patients treated with CHOP or similar therapeutic regimens, with a five-year overall survival of 34% and a median survival of 1.8 years in a recent large series.<sup>39</sup> A worse prognosis is associated with advanced stage disease and with age over 35. Better therapeutic strategies are required for ALK+ DLBCL. Crizotinib, an inhibitor of ALK and c-Met receptor tyrosine kinases, has shown therapeutic efficacy in some ALK+ neoplasms; whether it will prove to be efficacious in ALK+ DLBCL remains to be demonstrated.

### **B-Cell Lymphomas Treated with Rituximab**

Many patients with CD20+ B-cell lymphoma treated with rituximab, usually in conjunction with combination chemotherapy, achieve a sustained complete remission. If relapses occur or if the lymphomas persist, they are usually also CD20+ B-cell lymphomas, but occasionally, recurrent or persistent disease shows loss or diminution of CD20.<sup>40-45</sup> In most cases, the CD20-negative lymphoma is of the same histologic type as the original lymphoma.<sup>45</sup> In some instances however, a CD20+ low-grade B-cell lymphoma treated with rituximab can progress to a CD20-negative diffuse large B-cell lymphoma. CD20 expression can also become heterogeneous.<sup>41</sup> Occasionally CD20 expression is restored.<sup>41</sup> The mechanism for lack of CD20 is not known in all cases. For cases treated a short time before, it is theoretically possible that recurrent or persistent CD20-negative lymphoma is due to masking or removal of CD20 molecules by rituximab. In other cases, when studied, CD20 negativity has been attributed to deletion of the CD20 gene,<sup>43</sup> mutations within the CD20 coding region or to epigenetic mechanisms.<sup>40,41</sup> In one case report, a recurrence of follicular lymphoma regained partial CD20 expression after 5-azacytidine was administered as treatment for myelodysplasia,<sup>44</sup> further suggesting that gene methylation can play a role in CD20 downregulation.

The lack of CD20 may lead to consideration that the patient has developed a second, unrelated neoplasm. Bone marrow involvement by a CD10+ follicular lymphoma that has lost CD20 can be mistaken for B lymphoblastic leukemia.<sup>42</sup> Familiarity with this phenomenon, along with staining for

other markers known to be expressed by the lymphoma, including other pan-B cell markers, is useful in establishing a diagnosis.

### **CD20 Positivity in Plasma Cell Neoplasms and T-Cell Lymphomas**

#### **Expression of CD20 in Plasma Cell Neoplasms**

A minority of plasma cell myelomas express cyclin D1 and harbor a t(11;14), involving CCND1 and IGH.<sup>46</sup> These myelomas are more likely to have lymphoplasmacytic morphology. Expression of CD20 is uncommon in plasma cell myeloma in general, but cyclin D1+ myeloma is often CD20+. IgM plasma cell myeloma is rare, accounting for less than 1% of all cases of plasma cell myeloma. Despite the unusual immunoglobulin expressed, IgM myelomas have clinical and laboratory features similar to those of other cases of plasma cell myeloma; they typically have an IgM M-component and often have lytic bone lesions.<sup>47, 48</sup> Lymphoplasmacytic morphology is common. Like other myelomas, IgM myeloma is CD138+; however, its immunophenotypic features are otherwise unusual. The majority of IgM myelomas are cyclin D1+ (73% in one series)<sup>47, 48</sup> and often express B-cell associated antigens that are typically negative in plasma cell neoplasms.<sup>47, 48</sup> In one series, CD19, CD20 and Pax5 were expressed in neoplastic plasma cells in 67%, 40% and 73% of cases, respectively.<sup>47</sup>

The cytomorphology, CD20 expression and cyclin D1 expression, together with the rarity of IgM myeloma lead to problems in differential diagnosis. The lymphoplasmacytic morphology and CD20 expression in a patient with a serum IgM M-component may lead to a mistaken diagnosis of lymphoplasmacytic lymphoma. The small cell size, with plasmacytic differentiation that may be subtle, together with the expression of B-cell markers and cyclin D1 and t(11;14) by cytogenetics may lead to a misdiagnosis of mantle cell lymphoma. Lymphoplasmacytic lymphoma and mantle cell lymphoma both have clonal B-cell populations expressing monotypic surface immunoglobulin by flow cytometry, in contrast to myeloma. Lymphoplasmacytic lymphoma is not associated with lytic bone lesions (commonly present in IgM myeloma) and is in more than 90% of cases associated with the MYD88 L265P mutation. IgM plasma cell myeloma lacks the MYD88 L265P mutation characteristic of lymphoplasmacytic lymphoma.<sup>49</sup> Mantle cell lymphoma often presents with multifocal lymphadenopathy, sometimes with splenomegaly and involvement of extranodal sites such as GI tract (multiple lymphomatous polyposis) and Waldeyer's ring, a distribution of disease that would be against myeloma. CD5 expression which is characteristic of mantle cell lymphoma is also against a diagnosis of plasma cell myeloma.

#### **Expression of CD20 in T-Cell Lymphomas**

Expression of B-lineage antigens by T-cell lymphomas is a rare but well described phenomenon.<sup>50, 51</sup> As B-cell lymphomas are so much more common than T-cell lymphomas, seeing expression of CD20 in a lymphoma can easily lead the pathologist to make an erroneous diagnosis of B-cell lymphoma, unless a panel of immunostains is performed, and the pattern of staining with T-cell markers is also carefully noted. Molecular genetic studies to evaluate B and T-cell clonality may be required for a definitive diagnosis.<sup>50, 51</sup>

CD20 expression has been observed sporadically in T-cell lymphomas of a variety of types; it can be detected by immunohistochemistry or flow cytometry.<sup>50, 52</sup> Patients with such lymphomas are mostly older adults with a male predominance.<sup>50, 51</sup> CD20 expression is most commonly encountered in peripheral T-cell lymphoma, NOS that have involved lymph nodes and extranodal sites;<sup>50, 51</sup> it is also described in a few cases of monomorphic epitheliotropic intestinal T-cell lymphoma,<sup>53</sup> mycosis fungoides, T lymphoblastic lymphoma/leukemia, adult T-cell leukemia/lymphoma and others.<sup>50</sup> CD20+ T-cell lymphomas often behave in an aggressive manner, frequently resulting in the death of the patient. The pattern of staining with CD20 varies among cases. CD20 may be strongly and diffusely expressed, or it can be weakly and variably expressed in a given specimen. CD20 can be positive in T-cell lymphoma in one anatomic site or at one point in time, and absent in other sites or at other times.<sup>50, 53, 54</sup> Increasing expression of CD20 over time has been described in mycosis fungoides, in the course of histologic progression to large cell transformation.<sup>50</sup> Loss of CD20 has been described in a few CD20+ T-cell lymphoma patients who were treated with rituximab.<sup>50</sup>

CD20+ T-cell lymphomas are usually negative for other B-cell antigens, but in rare cases, other B-cell markers, including CD19 and CD79a, are also positive.<sup>50, 51</sup> Gene rearrangement studies in these cases typically show clonal TCR and polyclonal IGH rearrangement.<sup>50, 52-54</sup>

The cause of CD20 expression in T-cell lymphomas is uncertain. CD20 is a transmembrane protein that functions as a calcium channel; it may have a role in promoting progression through the cell cycle.<sup>55</sup> There is a small normal subset of T cells that co-expresses CD20 (dim), and some CD20+ T-cell lymphomas may originate from this type of T cell.<sup>50, 56</sup> Alternatively, CD20 expression may represent an activation marker; cases such as those mycosis fungoides that acquire CD20 with large cell transformation lend support to this hypothesis.<sup>50</sup> It has also been suggested that, for those cases in which CD20 is only weakly or variably expressed, it is not an integral part of the immunophenotype of the tumor and is unlikely to be clinically relevant.<sup>54</sup>

### **CD3 Positivity in B-Cell Lymphomas and Hodgkin Lymphoma**

#### **B-Cell Non-Hodgkin Lymphomas and Classical Hodgkin Lymphoma Expressing CD3**

Expression of certain T-cell associated markers, specifically CD5 and CD43, is commonly encountered in B-cell lymphomas. CD3 expression, in contrast, is seen infrequently in B-lineage neoplasms, and when it occurs, it can lead to problems in diagnosis. Staining for CD3 has been described in B-lineage non-Hodgkin lymphomas,<sup>57, 58</sup> classical Hodgkin lymphoma<sup>59</sup> and plasma cell neoplasms.<sup>57</sup> The non-Hodgkin lymphomas expressing CD3 have mostly been large B-cell neoplasms (including EBV+ DLBCL, NOS; pyothorax-associated lymphoma, plasmablastic lymphoma, primary effusion lymphoma and primary mediastinal large B-cell lymphoma),<sup>57, 58</sup> but CD3 expression has also been reported in Burkitt lymphoma and follicular lymphoma. CD3 expression is relatively frequent in HHV8+ lymphomas (see above).<sup>37</sup> CD3 expression in B-cell lymphomas appears to be more frequent in EBV+ lymphomas and/or lymphomas occurring in immunodeficient patients. Other T-cell associated markers may also be expressed in these cases. Immunoglobulin genes are typically clonally rearranged while analysis of TCR genes usually shows no clonal rearrangement.<sup>58</sup> In a few cases IG genes and TCR genes have both been clonally rearranged,<sup>60</sup>

raising the question of true biphenotypic lymphomas; however, these lymphomas were EBV+, and oligoclonal and even clonal TCR gene rearrangement are well described in EBV+ B-cell lymphomas,<sup>61</sup> possibly reflecting selected T cells with a focused immune response to EBV.

In the largest series on this topic,<sup>57</sup> the authors found two groups of B-lineage neoplasms expressing CD3:

1. 7 DLBCLs with plasmacytic differentiation, 4 plasma cell neoplasms, 2 plasmablastic lymphomas; these neoplasms were characterized by extranodal presentation, cytoplasmic CD3 and absence of other T-cell markers in most cases.
2. 5 DLBCLs with anaplastic features and 1 follicular lymphoma. These presented with lymph nodal involvement, expressed CD3 in a membranous pattern and expressed T-cell markers in addition to CD3.

In a small proportion of cases of classical Hodgkin lymphoma (5% in one analysis)<sup>62</sup> there is expression of one or more T-cell antigens by neoplastic cells. T-antigen+ cases of classical Hodgkin's lymphoma were most often nodular sclerosis type in one large series;<sup>59</sup> of these most had an abundance of tumor cells, consistent with NS2 morphology. Among T antigens investigated, CD2 and CD4 were those most commonly expressed by neoplastic cells. Less commonly expressed were CD3, CD5, CD7 and CD8.<sup>59</sup> Expression of more than one T-cell associated antigen on tumor cells was common. Only a minority of neoplastic cells expressed any one T-cell antigen (ranging from 10 to 35% of cells in one study). Staining was usually in a membrane pattern; "blob-like" staining in a Golgi pattern was found less commonly. T-antigen+ Hodgkin's lymphoma is typically negative for EBV. Clonal T-cell receptor gene rearrangement has been documented rarely, but in general TCR genes are in the germline configuration.<sup>59</sup> T-antigen expression has been associated with shorter overall and event-free survival.<sup>59</sup>

The explanation for CD3 expression on neoplastic B cells is not known. One proposal is that inactivation of PAX5, a gene which is required for B-cell differentiation, could permit expression of CD3.<sup>57, 63</sup> Inactivation of PAX5, for example by methylation, can lead to repression of B-cell differentiation and permit transdifferentiation into other types of cells, including T/NK cells, histiocytes and dendritic cells.<sup>63</sup> However, PAX5 is expressed in many CD3+ B-lineage tumors, so this effect could not account for all such cases. Other considerations are derepression of genetic activity during neoplastic transformation, neoplastic transformation of a lymphoid cell before full commitment to B lineage, or neoplastic transformation of a B-cell subset normally expressing T-cell antigen(s).<sup>58</sup> The latter explanation could apply to B-cell lymphomas expressing certain markers such as CD5, but a normal CD3+ B-cell population has not been recognized. The frequent association with EBV, at least among non-Hodgkin lymphomas, suggests that EBV may promote lineage infidelity.<sup>60</sup>

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