**BCL-6 Protein Expression in Human Peripheral T-Cell Neoplasms Is Restricted to CD30⁺ Anaplastic Large-Cell Lymphomas**

By Antonino Carbone, Annunziata Gloghini, Gianluca Gaidano, Riccardo Dalla-Favera, and Brunangelo Falini

The expression pattern of the *BCL-6* transcription factor has been assessed in normal and neoplastic B-cell populations and in Hodgkin’s disease. However, little is known about *BCL-6* expression and its biological significance in T-cell neoplasms. In this study, a series of 59 lymphoma samples, including 27 CD30⁺ anaplastic large-cell lymphomas (ALCLs), 24 other peripheral T-cell neoplasms, and 8 T-cell lymphoblastic lymphomas (T-LLs), as well as a panel of (t(2;5))-positive lymphoma-derived human cell lines, were evaluated for *BCL-6* protein expression by immunohistochemistry on frozen sections and cell smears. To define the relationship between *BCL-6* protein and CD30 antigen in CD30⁺ ALCLs and in non-neoplastic lymph nodes, serial section immunohistochemistry and two-color staining were used in selected CD30⁺ ALCLs as well as in reactive lymph nodes with non-neoplastic T-cell proliferations. *BCL-6* protein was expressed in 12 of 27 (45%) CD30⁺ ALCL cases, irrespective of their antigenic phenotypes (T-cell or null-cell type), and in the (t(2;5))-positive cell lines. In contrast, the remaining 24 peripheral T-cell neoplasms as well as the 8 T-LLs were considered negative for *BCL-6* expression. Coexpression of CD30 and *BCL-6*, as detected in CD30⁺ ALCLs, was also found in a subset of non-neoplastic lymphoid elements, namely the large lymphoid cells scattered in the interfollicular areas of reactive lymph nodes. These findings suggest that CD30⁺ ALCLs may represent the neoplastic transformation of extrafollicular CD30⁺ cells and that *BCL-6* may provide an additional marker for characterizing CD30⁺ ALCLs.

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Table 1. BCL-6 Expression in Human T-cell Neoplasms

<table>
<thead>
<tr>
<th>Diagnosis*</th>
<th>No. of Positive/ Tested Cases²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Precursor T-cell neoplasm</td>
<td></td>
</tr>
<tr>
<td>Precursor T-lymphoblastic lymphoma</td>
<td>0/8</td>
</tr>
<tr>
<td>Peripheral T-cell neoplasms</td>
<td></td>
</tr>
<tr>
<td>T-cell chronic lymphocytic leukemia</td>
<td>0/1</td>
</tr>
<tr>
<td>Mycosis fungoides</td>
<td>0/7</td>
</tr>
<tr>
<td>Peripheral T-cell lymphomas</td>
<td></td>
</tr>
<tr>
<td>Unspecified</td>
<td>0/11</td>
</tr>
<tr>
<td>AILD-like</td>
<td>0/4</td>
</tr>
<tr>
<td>Angiocentric</td>
<td>0/1</td>
</tr>
<tr>
<td>Anaplastic large-cell CD30+ lymphomas</td>
<td></td>
</tr>
<tr>
<td>T cell</td>
<td>8/19</td>
</tr>
<tr>
<td>null cell§</td>
<td>4/8</td>
</tr>
</tbody>
</table>

Abbreviation: AILD, angioimmunoblastic T-cell lymphoma.

* According to the revised European-American lymphoma classification.¹⁴

† The percentage of positive neoplastic cells ranged from 10% to 60%.

§ Null cell group included two cases classified as anaplastic large-cell lymphoma Hodgkin's-like. They were BCL-6 negative.

CD30+ ALCLs. CD30+ tumor cells were consistently absent in the peripheral T-cell neoplasms other than CD30+ ALCLs. Frozen tissue from nine clinical samples with non-neoplastic T-cell proliferations (seven reactive lymph nodes without obvious cause and with a high degree of interfollicular/paracortical expansion and two reactive lymph nodes in infectious mononucleosis) were also included in the study. The expanded interfollicular/paracortical areas of all non-neoplastic (reactive) lymph nodes were predominantly populated by CD4+ T lymphocytes.

Cell lines. The t(2; 5)-positive Karpas 299, SU-DHL1, and DEL human-derived cell lines¹⁶ were also included in the study.

Immunohistochemistry. Deparaffinized and cryostat sections were used for immunophenotyping and lineage assignment of lymphoma cases with monoclonal antibodies (MoAbs; ßF1, CD3, CD4, CD5, CD8, CD9, CD10, CD15, CD19, CD20, CD21, CD22, CD24, CD30, CD43, CD45, CD45RA, CD45RO, CD68, CD74, CDw75, LN3, MB2, DDBB42, DBA44, OPD4, DRC-1, Leu8, anti-TdT, anti-k and ñ immunoglobulin [Ig] light chains, epithelial membrane antigen [EMA], vimentin, and cytokeratin [MNF116]).¹⁵,¹⁷ Immunohistochemistry was performed with the avidin-biotin-peroxidase complex (ABC-px)¹⁸ or alkaline phosphatase antialkaline phosphatase (APAAP)¹⁹ methods, as previously described.¹⁸,¹⁹

Staining with BCL-6. The BCL-6 protein was immunostained using the MoAb PG-B6⁵,⁷ on frozen sections by the APAAP method.¹⁹

Cases were considered positive when ≥10% of neoplastic cells showed nuclear staining for the MoAb PG-B6. A threshold of 10% positive cells was arbitrarily chosen because T-cell neoplasms are almost always either infiltrated by a background population of small, non-neoplastic T cells, or admixed with residual small and large B cells. However, to formally clarify the nature of occasional BCL-6+...
cells in samples containing less than 10% of the stained cells, double label studies were performed. In mycosis fungoides lesions, \(BCL-6\) staining was assessed on the cytologically abnormal lymphoid cells in the epidermis, thus minimizing the potential interference from dermal reactive lymphocytes.

Cytospin smears of human-derived cell lines were fixed in acetone-chloroform at room temperature for 10 minutes and immunostained with the MoAb PG-B6 by the APAAP method.

Two-color staining. Multiple immunohistochemical staining was performed to detect \(BCL-6\) protein plus CD30 in selected CD30\(^+\) ALCL cases and reactive lymph nodes, as previously reported. Double label studies\(^1\) (\(BCL-6\) plus CD19; \(BCL-6\) plus CD3) were also performed in CD30\(^+\) ALCL cases and other peripheral T-cell neoplasms.

### RESULTS

Expression of \(BCL-6\) in T-cell neoplasms. Results of \(BCL-6\) expression in the total series of T-cell neoplasms studied is listed in Table 1. \(BCL-6\) protein expression was restricted to the CD30\(^+\) ALCL group, in which 12 of 27 (45%) cases were positive (Fig 1A). In positive cases, 10% to 60% of the tumor cells were labeled with \(BCL-6\). In five cases more than 50% of the tumor cells were stained, whereas in the remaining positive cases 10% to 25% (3 cases) and 25% to 50% (4 cases) of the tumor cells were stained with \(BCL-6\). Anti-\(BCL-6\) MoAb stained the nuclei of large tumor cells. The staining pattern on frozen tissue sections was of moderate to strong intensity. Nuclear positivity was diffuse/microgranular (Fig 1B). Coexpression of \(BCL-6\) protein and CD30 was observed in the tumor cells of all CD30\(^+\) ALCL cases tested (7/7; Fig 1C). Both T-cell (8 cases) and null-cell (4 cases) immunophenotypes were found among the \(BCL-6^+\) CD30\(^+\) ALCL cases (Table 1).

Among the 15 CD30\(^+\) ALCL cases that were considered negative (<10%) for \(BCL-6\) protein expression, 11 cases did not show any staining. Conversely, four samples had scattered large cells that stained for \(BCL-6\)-protein and accounted for less than 1% to 1% to 2% of the cellularity. Two-color staining with \(BCL-6\)-protein plus CD30 and \(BCL-6\)-protein plus CD19 showed that a fraction of these scattered \(BCL-6\)-expressing cells were also CD30\(^+\) and presumably were tumor cells. The remaining \(BCL-6\)-protein expressing cells were host CD19\(^+\) B-cells.

The 24 peripheral T-cell neoplasms other than CD30\(^+\) ALCLs as well as the eight T-cell lymphoblastic lymphomas were considered negative for \(BCL-6\) expression. Four cases of peripheral T-cell neoplasms, all lacking CD30\(^+\) cells, contained a minor component (<1%) of scattered \(BCL-6\)-expressing small and large cells. Two-color staining with \(BCL-6\) plus CD19 and \(BCL-6\) plus CD3 confirmed that in two cases the \(BCL-6\)-expressing cells were host CD19\(^+\) B-cells. In the other two cases, the \(BCL-6\)-expressing cells were predominantly B cells, although occasional \(BCL-6^-\) CD19\(^-\) cells could be observed. The lineage of these rare cells remains presently undefined.

Expression of \(BCL-6\) in human lymphoma-derived cell lines. On cytospin preparations cultured Karpas 299 ALCLs, SU-DHL1, and DEL cells were characterized by a strong granular nuclear staining with anti-\(BCL-6\) MoAb (not shown).

Expression of \(BCL-6\) and CD30 in non-neoplastic lymph nodes. Serial sections immunohistochemistry was used to analyze the expression of \(BCL-6\) and CD30 in expansions of activated T cells because of chronic and acute inflammatory conditions. \(BCL-6^+\) cells as well as CD30\(^+\) cells were consistently present in reactive lymph nodes, but varied markedly in number from case to case and area to area.

As expected, numerous B cells within the germinal centers consistently stained for \(BCL-6\), whereas cells positive for CD30 were usually absent (Fig 2A and B). On the other hand, \(BCL-6^+\) and CD30\(^+\) cells were localized around B-cell follicles, although they were also seen at the margins of germinal centers (Fig 2A and B). Coexpression of CD30 and \(BCL-6\) was detected by two-color staining in large cells outside normal germinal centers (Fig 2C) in which, as seen in this and previous studies,\(^1,22\) CD30\(^+\) cells, larger than most centroblasts, are all of B-cell phenotype.

In the interfollicular areas principally populated by CD3\(^+\)/CD4\(^+\) T cells (Fig 3A), CD30\(^+\) cells were consistently present, but varied markedly in number from case to case (Fig 3B). Serial sections provided immunarchitectural evidence of a similar immunostaining with both CD30 and \(BCL-6\) (not shown). Coexpression of \(BCL-6\)-protein was observed in a fraction of CD30\(^+\) large cells (Fig 3C). These cells were localized in the interfollicular areas, in which B cells were relatively sparse, thus suggesting that most of the cells were, in fact, of T-cell origin.

### DISCUSSION

The present study was aimed at defining the expression of \(BCL-6\) protein in distinct pathological categories of T-cell neoplasms. Our data indicate that \(BCL-6\) is expressed by a substantial fraction (45%) of CD30\(^+\) ALCLs, whereas it is consistently absent in all other peripheral and precursor T-cell neoplasms tested. Notably, coexpression of CD30 and \(BCL-6\), as detected in CD30\(^+\) ALCLs, may also occur in a specific subset of non-neoplastic lymphoid elements, namely the large lymphoid cells scattered in the interfollicular areas of reactive lymph nodes.

The relevance of these data is twofold and concerns the histogenesis of CD30\(^+\) ALCLs and the potential diagnostic use of \(BCL-6\) expression in differentiating CD30\(^+\) ALCLs from other categories of peripheral T-cell neoplasms.

Regarding CD30\(^+\) ALCL histogenesis, the frequent and selective occurrence of \(BCL-6\) expression in CD30\(^+\) ALCLs, combined with the evidence that large lymphoid cells localized in the interfollicular areas of non-neoplastic lymph nodes also coexpress CD30 and \(BCL-6\) protein, further corroborate the concept that CD30\(^+\) ALCLs, or at least a proportion of these lymphomas, represent the neoplastic transformation of extrafollicular CD30\(^+\) cells. As recently shown, \(BCL-6\) expression is detectable also in Reed-Sternberg (RS) CD30\(^+\) cells of classical HD\(^2\), another disorder putatively related to extrafollicular CD30\(^+\) lymphoid cells.\(^3\) However, the frequency of \(BCL-6^+\) HD cases (about 30%) and the
percentage of BCL-6+ RS cells (about 10%) are different from the findings in CD30+ ALCLs (present study).

The biological basis accounting for the expression of BCL-6 protein in human CD30+ ALCLs is currently unknown. In physiological conditions, BCL-6 protein is expressed by a small fraction of normal resting CD4+ T lymphocytes. After activation by 12-O-tetradecanoylphorbol-13-acetate (TPA) and ionomycin, the proportion of BCL-6+ T cells increases significantly, returning to baseline levels by 24 to 48 hours. In this respect, our detection of BCL-6 protein in a fraction of interfollicular CD30+ cells in non-neoplastic lymph nodes may reflect the fact that these lymphoid cells are activated by the network of inflammatory cytokines mounted by the underlying disease. It may be postulated that BCL-6 expression in normal and reactive CD30+ cells localized in the interfollicular areas is a scheduled event occurring as a consequence of time-limited cellular activation, whereas the tumor cells of CD30+, BCL-6+ ALCL would constitute maintain the activated state associated with expression of CD30 and BCL-6. Alternatively, it may be hypothesized that BCL-6 expression in CD30+ ALCLs occurs as a consequence of a presently unknown genetic lesion causing either directly or indirectly the deregulated expression of the BCL-6 protein. Because BCL-6 expression is detectable in primary CD30+ ALCLs and ALCL cell lines expressing NPM/ALK chimeric protein (this study and Falini et al., unpublished observation, October 1996), it is likely that BCL-6 expression does not represent a second pathogenetic mechanism for CD30+ ALCLs mutually exclusive with t(2;5) (p23;q35) translocation.

Independent of histogenetic and pathogenetic implications, the selectivity of the association between BCL-6 expression and CD30+ ALCLs provides a new tool that may prove potentially relevant toward the refinement of the phenotypic criteria separating this category from other types of peripheral T-cell neoplasms.

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