**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-LBLs), 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-LBLs 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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Submitted January 6, 1997; accepted May 15, 1997.

Supported in part by the Associazione Italiana per la Ricerca sul Cancro, Milan, Italy; by the Ministero della Sanità, Rome, Italy; and by National Institutes of Health Grant No. CA-44029.

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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 Cases†</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 tr(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; βF1, 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 λ 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.16,19

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⁺

![Fig 1. (A and B) Immunohistochemical demonstration of BCL-6 protein expression in a CD30⁺ anaplastic large-cell lymphoma employing the APAAP method on frozen tissue sections. (A) In this field several tumor cells show a nuclear staining pattern with anti–BCL-6 MoAb PG-B6. (B) Anaplastic large cells show strong nuclear immunoreactivity with the anti–BCL-6 MoAb PG-B6. The positivity is microgranular. (C) Coexpression of BCL-6 protein (nuclear, blue) is observed in a proportion of CD30⁺ (cytoplasmic and membranous, reddish) anaplastic large tumor cells, as detected by two-color staining (see Materials and Methods). Original magnification (A) ×250, (B) ×400, and (C) ×400.](image-url)
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.20

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.19

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.21

Double label studies21 (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+ 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.

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 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 node 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, another disorder putatively related to extrafollicular CD30+ lymphoid cells. However, the frequency of BCL-6+ HD cases (about 30%) and the
Fig 2.

Fig 3.
Fig 2. (A and B) Serial sections from a reactive lymph node with non-neoplastic T-cell proliferation. Several CD30+ (A) and BCL-6+ (B) large lymphocytes are present at the margins of a germinal center (GC), although they are also seen in the perifollicular (PF) zone. Within the GC numerous B cells stain for BCL-6 (B), whereas cells positive for CD30 are absent (A). (C) A large cell near to a GC coexpresses CD30 antigen (reddish) and BCL-6 protein (blue), as detected by two-color staining (see Materials and Methods). No coexpression by the BCL-6+ GC cells is detectable. (A and B) APAAP immunostaining, hematoxylin counterstain, original magnification ×250; (C) frozen section, original magnification ×400.

Fig 3. (A, B, and C) Serial sections from a reactive lymph node with non-neoplastic T-cell proliferation. In the interfollicular area, where almost all cells, including large cells (arrow), are positive for CD3 antigen (A), some CD30+ (B) large lymphocytes are present. (C) Coexpression of BCL-6 protein (nuclear, blue) is observed in a large CD30+ (cytoplasmic and membranous, reddish) cell, as detected by two-color staining (see Materials and Methods). Individual cells expressing either BCL-6 or CD30 are also seen. (A and B) APAAP immunostaining, hematoxylin counterstain, original magnification ×400; (C) frozen section, original magnification ×400.

percentage of BCL-6+ RS cells (about 10%)7 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.7 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.23,24 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 constitutively 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 B 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.14

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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