Expression of Cytolytic Lymphocyte-Associated Antigens in Pulmonary Lymphomatoid Granulomatosis

William G. Morice, MD, PhD, Paul J. Kurtin, MD, and Jeffrey L. Myers, MD

Key Words: Adult; Lung diseases; Lymphomatoid granulomatosis; Pathology; Biology; Lymphoproliferative disorders; Etiology; Immunophenotyping

Abstract

Paraffin-embedded lung wedge biopsy specimens from 14 patients with pulmonary lymphomatoid granulomatosis (LYG) were analyzed using immunoperoxidase stains specific for T cell– and natural killer cell–associated antigens. Nine cases had a minor population of CD20+ large B-cells (B-cell LYG) amidst a background of CD3- and betaF1-immunoreactive T cells. In 8 of the 9 B-cell LYG cases, the majority of the background T lymphocytes had a cytotoxic phenotype as defined by the expression of CD8 and the cytotoxic granule proteins TIA-1 (granule membrane protein 17) and granzyme B. Five cases lacked CD20+ large cells and, instead, showed predominantly CD3+ and betaF1+ T cells (T-cell LYG). Whereas the small, medium, and large atypical lymphocytes were all positive for CD3 and betaF1 in the T-cell LYG cases, immunoreactivity for CD8, TIA-1, and granzyme B was limited to the small lymphocytes, with a distribution indistinguishable from that seen in B-cell LYG. These findings indicate that LYG is composed of a heterogeneous group of lymphoproliferative disorders that share, as unifying features, a relative paucity of neoplastic cells and a prominent reactive infiltrate rich in cytolytic lymphocytes.

Pulmonary lymphomatoid granulomatosis (LYG) was described by Liebow and colleagues in 1972 as a discrete histopathologic entity characterized by a tumefactive polymorphous lymphoid infiltrate with prominent vascular infiltration (“angiitis”) and variable amounts of necrosis (“granulomatosis”). With the advent of immunohistochemical analysis, it became clear that the histologic features of LYG could be seen in a variety of lymphoproliferative diseases. Most examples of LYG are now known to be large B-cell lymphomas with an exuberant host T-cell response, histologically and phenotypically analogous to T cell–rich B-cell lymphomas described in other sites. In almost all such cases, the large B cells are positive for Epstein-Barr virus (EBV). A smaller subset of LYG cases contain cytologically atypical CD3+ T cells and lack atypical CD20+ B cells. These cases are negative for EBV. The disease in patients with this latter uncommon phenotype may have an aggressive clinical course, suggesting that some cases indeed may represent peripheral T-cell lymphomas. In addition, posttransplant lymphoproliferative disorders (PTLDs) and nasal-type extranodal T/natural killer (NK)–cell lymphoma may be morphologically indistinguishable from classic LYG when they involve the lung.

Although we have gained insight into the diversity of the entities that constitute the lesion described by Liebow et al 30 years ago, our understanding of these disease processes remains incomplete. Recent studies have demonstrated that cytotoxic T cells and NK cells, collectively referred to as cytolytic lymphocytes, represent a major component of the host response in many lymphoid and nonlymphoid neoplasms. Furthermore, primary extranodal T-cell lymphomas from a variety of sites frequently have a cytotoxic...
These observations have been made, in large part, through the use of recently available antibodies to cytolytic lymphocyte-associated antigens that are reactive in paraffin-embedded tissue. Table II. The polyclonal antibody to CD3 used in paraffin-embedded tissue is directed against a subunit of this multiprotein complex expressed both by T and NK cells and, therefore, will stain both cell types. In contrast, the antibody betaF1 is specific for the T-cell antigen receptor beta chain and, therefore, will stain only T cells bearing the alpha-beta antigen receptor heterodimer. CD8 is a cell-surface-expressed glycoprotein found on cytotoxic T cells and a subset of NK cells that bind major histocompatibility complex class I molecules on potential target cells. TIA-1 (granule membrane protein 17) and granzyme B are cytotoxic granule proteins involved in the lysis of target cells. TIA-1 is expressed constitutively in the cytoplasmic granules of both cytotoxic T cells and NK cells. In contrast, granzyme B is expressed constitutively in NK cells and is found in cytotoxic T cells only after cellular activation.

We examined a group of previously described LYG cases, primarily with immunohistochemical studies using antibodies to these antigens expressed by cytolytic lymphocytes. These studies were performed in an effort to further characterize the host response in cases with large, atypical B cells and to determine the immunophenotypic characteristics of the cases containing atypical T cells in which there were no large B cells.

Materials and Methods

Pathologic and clinical features of 17 cases of pulmonary LYG from the files of the Mayo Clinic, Rochester, MN, and the consultation files of one of the case contributors (Dr Katzenstein) have been described previously. Of these cases, 14 had sufficient tissue for further analysis.

Immunohistochemical analysis was performed on sections of paraffin-embedded tissue using the antibodies and dilutions summarized in Table I. Deparaffinization and rehydration were performed before all treatments. For citrate buffer (1-mmol/L concentration of citrate, pH 6.0) and EDTA buffer (1 mmol/L-concentration of EDTA, pH 8.0) pretreatments, slides were steamed in the buffer in Heddahl jars for 30 minutes (effective temperature 95°C) and then allowed to cool to room temperature. Protease pretreatment was performed using a protease-2 endopeptidase solution (an alkaline serine protease family endopeptidase) supplied by the manufacturer (2.5 U/25 mL).

The pretreated slides were rinsed and treated with methanolic peroxide to block endogenous peroxide activity, rinsed again, and loaded onto a Ventana ES Autostainer (Ventana Medical Systems, Tucson, AZ) or a BioTek instrument (BioTek Solutions, Santa Barbara, CA) or were stained manually. On the Ventana instrument incubations were performed at 42°C, and a standard labeled streptavidin-biotin peroxidase detection method was used. Manual incubations and those done on the on the BioTek instrument were performed at room temperature and used the avidin-biotin complex antibody detection method. For all of the primary antibodies except betaF1, aminoethylcarbazole (AEC) was used as the chromogen; for betaF1, diaminobenzidine (DAB) was used as the chromogen. Double-stained slides were first treated and stained with antibodies to granzyme B, followed by exposure to AEC chromogen with subsequent protease treatment and staining with antibodies to betaF1, followed by exposure to DAB chromogen. Granzyme B staining and betaF1 staining were distinguished both by the distribution of staining (cytoplasmic for granzyme B vs membranous for betaF1) and the tinctorial differences between AEC and DAB. Hematoxylin counterstain was used in all cases.

In situ hybridization for EBV-specific RNA was performed using probes to EBV-encoded small RNAs (EBER-1 and EBER-2) according to a previously published method.

Table II
Antibodies and Methods Used for Immunohistochemical Analysis of Paraffin Sections of Pulmonary Lymphomatoid Granulomatosis Biopsy Tissue

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Specificity</th>
<th>Clone</th>
<th>Source</th>
<th>Pretreatment</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3</td>
<td>T cell, NK cell</td>
<td>Polyclonal</td>
<td>DAKO, Carpinteria, CA</td>
<td>EDTA</td>
<td>Ventana</td>
</tr>
<tr>
<td>CD8</td>
<td>Cytotoxic T cell, NK-cell subset</td>
<td>C8/144B</td>
<td>DAKO</td>
<td>EDTA</td>
<td>Ventana</td>
</tr>
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<td>CD20</td>
<td>B cell</td>
<td>L26</td>
<td>DAKO</td>
<td>EDTA</td>
<td>Ventana</td>
</tr>
<tr>
<td>CD57</td>
<td>NK cell, T-cell subset</td>
<td>HNK-1</td>
<td>Becton Dickinson, San Jose, CA</td>
<td>Citrate</td>
<td>Ventana</td>
</tr>
<tr>
<td>CD68</td>
<td>Macrophage</td>
<td>KP-1</td>
<td>Coulter, San Francisco, CA</td>
<td>Citrate</td>
<td>Ventana</td>
</tr>
<tr>
<td>betaF1</td>
<td>T-cell subset</td>
<td>8A3</td>
<td>Coulter, Woburn, MA</td>
<td>Protease-2</td>
<td>Ventana</td>
</tr>
<tr>
<td>TIA-1</td>
<td>Cytotoxic T cell, NK cell</td>
<td>266A10FS</td>
<td>Coulter, Westbrook, ME</td>
<td>EDTA</td>
<td>Manual</td>
</tr>
<tr>
<td>Granzyme B</td>
<td>Cytotoxic T-cell subset, NK cell</td>
<td>GRB-7</td>
<td>Caltag</td>
<td>EDTA</td>
<td>BioTek</td>
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<tr>
<td>CD56</td>
<td>NK cell, T-cell subset</td>
<td>123C3</td>
<td>Caltag</td>
<td>EDTA</td>
<td>Ventana</td>
</tr>
</tbody>
</table>

NK, natural killer.

* Ventana, Ventana Medical Systems, Tucson, AZ; BioTek, BioTek Solutions, Santa Barbara, CA.
Each case was reviewed independently by each of us and by multiple authors at a multihed scope. To determine the percentage of antigen-positive cells in each case, one of us (W.G.M.) counted a total of 500 cells from 10 randomly selected ×400 high-power fields, and the number of antigen-positive cells was tabulated. The percentage of positive cells was calculated and rounded to the nearest 5%. The calculated percentage of antigen-positive cases then was confirmed by two of us (W.G.M. and J.L.M.) at a multihed scope.

Results

Eight of 11 cases described previously as containing large, atypical B cells had sufficient tissue for further review. Large atypical cells positive for CD20 and EBV also were identified in additional material studied from a single case that originally was thought to lack these cells, resulting in a total of 9 B-cell LYG cases. All had variable numbers of CD20+ large cells in a background of small CD3+ T-lymphocytes. Image 1. The staining pattern for these 9 cases is shown in Table 2. In each B-cell LYG case, CD3+, betaF1+ alpha-beta T cells constituted the majority of the lymphocytic infiltrate. In all cases except case 7, about half or more of the CD3+ cells also were TIA-1+, indicating a predominance of cytotoxic T cells. Granzyme B stained fewer cells than did TIA-1, consistent with the expression of this protein in a subset of activated cytotoxic T cells (Table 1, Image 2). A minority of cells were CD8+ in all cases except one. The single exception (case 9) also was distinctive in that a high percentage of cells that expressed both TIA-1 and granzyme B and the CD20+ large cells did not show evidence of EBV positivity by in situ hybridization analysis. Antibodies to CD56 and CD57 stained few cells (<10%) in all cases.

The staining pattern observed in our 5 cases lacking CD20+ large cells is shown in Table 3. These lesions were composed predominantly of CD3+, betaF1+ mononuclear cells. Image 2. In each case, the majority of cells that failed to express CD3 were CD68+ (KP-1+) macrophages. The frequency of reactivity with antibodies to TIA-1, granzyme B, and CD8 was similar to that observed in biopsy specimens containing CD20+ large B cells. In all of these cases, CD3 and betaF1 both stained small lymphocytes as well as cells with medium and large irregular nuclei. In contrast, antibodies to TIA-1 and granzyme B seemed to exclusively stain small lymphocytes (Image 2). To confirm that the large cells failed to express the cytotoxic granule protein granzyme B, single slides were stained with both betaF1 and antibodies to granzyme B. These studies demonstrated granzyme B immunoreactivity exclusively in the small lymphocytes, whereas the large cells stained only with betaF1 antibody. In all of the T-cell LYG cases, a minority of cells was found to express CD56 and CD57, similar to the B-cell LYG cases.

Follow-up data were available for 6 patients with B-cell LYG. Three of these patients died of disease, two of whom were treated with systemic multiagent chemotherapy (cases 3 and 5), and one of whom received no therapeutic intervention (case 2). Three patients with B-cell LYG were alive with no evidence of disease after a minimum follow-up of 4.9 years, 2 (cases 1 and 7) were treated with systemic multiagent chemotherapy, and 1 (case 9) received no therapeutic intervention. Follow-up information was available for 3 patients with T-cell LYG, all of whom received systemic multiagent chemotherapy. One patient with T-cell LYG (case 11) died of disease 0.5 year after diagnosis; the other two for whom follow-up was available (cases 10 and 13) were alive with no evidence of disease after a minimum follow-up interval of 4.2 years.

Discussion

Classically defined LYG is a morphologic final common pathway for a heterogeneous group of lymphoproliferative disorders. In most instances, LYG is a lymphoma of clonal EBV-infected B cells with a prominent host T-cell response.21 This disorder is accepted as a distinct subtype of diffuse large B-cell lymphoma in the recent World Health Organization classification of hematologic and lymphoid malignant neoplasms.22 In the present study, it was demonstrated that cytolytic lymphocytes represent a major component of the host response in B-cell LYG, a finding similar to studies of other lymphoid malignant neoplasms with a preponderance of reactive T cells, such as Hodgkin lymphoma and T cell–rich B-cell lymphoma.7,9 In these disorders, the neoplastic lymphoid cells have been shown to have the phenotypic attributes of activated antigen-presenting cells.23-25 They are positive for HLA-DR, CD80/CD86, and CD58. Thus, they potentially can interact with and activate T cells through interactions with the T-cell antigen-receptor complex, CD28, and CD2, resulting in the elaboration of cytokines and chemokines that can recruit the cytolytic lymphocytes and macrophages that characterize the host response to these tumors. Because of the similarities in the cell composition of B-cell LYG and these other lymphoproliferative disorders, it is likely that the neoplastic cells of B-cell LYG use similar immunologic mechanisms to induce the host response. Furthermore, analogous to the examples of primary EBV infection and Kikuchi necrotizing histiocytic lymphadenitis, the presence of broad zones of necrosis associated with TIA-1+ and granzyme B+
The character of the host response to B-cell lymphomas potentially could be of prognostic significance. In T cell–rich B-cell lymphoma, as well as Hodgkin lymphoma, the presence of large numbers of granzyme B+ cells associated with the neoplastic cells has been associated with an aggressive disease course. Although the present study contains too few cases to make definitive statements about clinical behavior, we observed a similar trend (data not shown) suggesting that B-cell LYG may have behavior and biology similar to other lymphoid neoplasms with a prominent host cytolytic lymphocyte response.

Among LYG cases with classic histologic features and CD20+ large, atypical B cells, we observed phenotypic and biologic heterogeneity. Although most of our B-cell LYG cases demonstrated similar histologic and immunophenotypic characteristics, a single case served to highlight the diversity that occurs even within this narrowly defined subset. This case differed in that the large, atypical B cells were negative for EBV, and the reactive T cells demonstrated an unusually high frequency of CD8 and granzyme B expression.

Our findings serve to further emphasize the relationship between B-cell LYG and PTLDs. PTLDs and other immunosuppression-related B-cell lymphoproliferative disorders in...
the lung can have morphologic and immunophenotypic features identical to B-cell LYG.5,30 Furthermore, studies of PTLDs in other sites have shown that cytolytic lymphocytes represent a major component of the host response.6 None of our patients were known to be immunosuppressed or immunodeficient, but subtle defects in immune function may have escaped detection.1,21,31 Given the similarity of B-cell LYG to pulmonary involvement by PTLD, the immune status of any patient with B-cell LYG should be investigated vigorously.

A minority of the LYG cases in the present study lacked CD20+ large cells and probably represent T-cell lymphomas (T-cell LYG) that are accompanied by an exuberant host response.

### Table 2
Expression of T Cell–Associated Antigens* and Epstein-Barr Virus (EBV) In Situ Hybridization (ISH) Results in Pulmonary Lymphomatoid Granulomatosis Cases With CD20+ Large B Cells

<table>
<thead>
<tr>
<th>Case No.</th>
<th>CD3</th>
<th>betaF1</th>
<th>CD8</th>
<th>TIA-1</th>
<th>Granzyme B</th>
<th>EBV-ISH</th>
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<tbody>
<tr>
<td>1</td>
<td>75</td>
<td>65</td>
<td>10</td>
<td>40</td>
<td>20</td>
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<tr>
<td>2</td>
<td>80</td>
<td>50</td>
<td>15</td>
<td>80</td>
<td>20</td>
<td>Positive</td>
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<tr>
<td>3</td>
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<td>90</td>
<td>15</td>
<td>70</td>
<td>25</td>
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</tr>
<tr>
<td>4</td>
<td>90</td>
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<td>10</td>
<td>40</td>
<td>0</td>
<td>Positive</td>
</tr>
<tr>
<td>5</td>
<td>95</td>
<td>90</td>
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<td>60</td>
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<tr>
<td>6</td>
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<td>90</td>
<td>20</td>
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<td>10</td>
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<tr>
<td>7</td>
<td>90</td>
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<td>20</td>
<td>50</td>
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<td>Positive</td>
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<tr>
<td>8</td>
<td>90</td>
<td>70</td>
<td>85</td>
<td>90</td>
<td>70</td>
<td>Negative</td>
</tr>
</tbody>
</table>

* Expressed as a percentage of all positive lesional cells.

### Table 3
Expression of T Cell–Associated Antigens* and Epstein-Barr Virus (EBV) In Situ Hybridization (ISH) Results in Pulmonary Lymphomatoid Granulomatosis Cases Lacking CD20+ Large B Cells

<table>
<thead>
<tr>
<th>Case No.</th>
<th>CD3</th>
<th>betaF1</th>
<th>CD8</th>
<th>TIA-1</th>
<th>Granzyme B</th>
<th>EBV-ISH</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>75</td>
<td>50</td>
<td>15</td>
<td>50</td>
<td>20</td>
<td>Negative</td>
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<tr>
<td>11</td>
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<td>90</td>
<td>10</td>
<td>40</td>
<td>15</td>
<td>Negative</td>
</tr>
<tr>
<td>12</td>
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<td>85</td>
<td>20</td>
<td>15</td>
<td>5</td>
<td>Negative</td>
</tr>
<tr>
<td>13</td>
<td>90</td>
<td>90</td>
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<td>75</td>
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<tr>
<td>14</td>
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<td>90</td>
<td>15</td>
<td>70</td>
<td>50</td>
<td>Negative</td>
</tr>
</tbody>
</table>

* Expressed as a percentage of all positive lesional cells.
The cytolytic lymphocyte response. The latter was recognized in the double-labeling studies that demonstrated that the nonneoplastic-appearing small lymphocytes had a cytotoxic phenotype distinct from that of the cytologically abnormal lymphocytes. Presumably, the atypical cells were able to spontaneously elaborate cytokines that recruited the host response. Although there was insufficient tissue in our cases to perform molecular genetic analyses and prove T-cell clonality in the T-cell LYG cases, many of the features of the cases suggested that these truly were T-cell malignant neoplasms. Pathologically, these lesions formed masses, destroying the underlying pulmonary architecture, and contained atypical medium- and large-sized neoplastic cells, similar to other more established types of T-cell lymphomas. The abnormal cells expressed betaF1, an antigen with very high specificity for T cells, and they lacked staining for the cytolytic lymphocyte antigens CD8, CD56, TIA-1, and granzyme B. They also were negative for EBV. These features distinguish them from other extranodal T-cell malignant neoplasms with which they might be confused, particularly NK/T-cell lymphoma of nasal type. Nasal-type NK/T-cell lymphomas often are characterized by
an angioinvasive, angiodestructive growth pattern, and they contain large areas of necrosis. However, in most instances they are composed of EBV+ cells that express CD56, granzyme B, and/or TIA-1.

This study provides evidence that a heterogeneous group of lymphoproliferative disorders comprises LYG, a morphologically defined entity. Most cases of LYG contain large, atypical B cells that are frequently, but not uniformly, EBV+. In these cases, there is a prominent, reactive lymphoid infiltrate primarily composed of cytotoxic T cells. A smaller subset of LYG cases lacks large B cells and, rather, is characterized by the presence of cytologically atypical, EBV– T cells. Like the cases of LYG containing large B cells, in this latter group of cases, there are numerous reactive cytotoxic T cells. However, the atypical T cells in these LYG cases do not have a cytotoxic phenotype in contrast with other extranodal T/NK-cell lymphomas that involve the upper aerodigestive tract. Further studies of LYG are necessary to better understand the relationship of B-cell LYG to large B-cell lymphomas with a predominance of reactive T cells occurring in other sites and to establish how T-cell LYG relates to B-cell LYG, as well as other extranodal T-cell lymphoproliferative disorders.

From the Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN.

Address reprint requests to Dr Morice: Dept of Laboratory Medicine and Pathology, Division of Hematopathology, Hilton 1020, Mayo Clinic, 200 First Street SW, Rochester, MN 55905.

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References


