Granzyme B-Expressing Peripheral T-Cell Lymphomas: Neoplastic Equivalents of Activated Cytotoxic T Cells With Preference for Mucosa-Associated Lymphoid Tissue Localization

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T-cell non-Hodgkin’s lymphomas can be considered the neoplastic equivalents of immunologically functional, site-restricted T lymphocytes. Little is known about the occurrence and clinical behavior of T-cell lymphomas that are the neoplastic equivalents of different functional T-cell subsets. Here, we investigated the prevalence, preferential site, immunophenotype, and clinical behavior of the neoplastic equivalents of activated cytotoxic T cells (CTLs) in a group of 140 nodal and extranodal T-cell lymphomas. Activated CTLs were shown immunohistochemically with a monoclonal antibody against granzyme B, a major constituent of the cytotoxic granules of activated T cells. Granzyme B-positive T-cell lymphomas were mainly found in mucosa-associated lymphoid tissue (MALT; nose, 63% of the cases; gastrointestinal tract, 46%; and lung, 33%). Granzyme B-positive cases with primary localization in MALT were more often associated with angioinvasion (P = .005), necrosis (P = .002), and histologic characteristics of celiac disease in adjacent mucosa not involved with lymphoma. Eosinophilia was more often observed in granzyme B-negative cases (P = .03). Most cases belonged to the pleomorphic medium- and large-cell group of the Kiel classification. CD30 expression was more often found in granzyme B-positive lymphomas of MALT (P = .04), whereas CD56 expression was exclusively found in nasal granzyme B-negative lymphomas. Immunophenotypically, most of the cases should be considered as neoplastic equivalents of activated CTLs based on the presence of T-cell markers on tumor cells. In two cases of nasal lymphoma, tumor cells probably were the neoplastic counterparts of natural killer cells. The prognosis of the granzyme B-positive gastrointestinal T-cell lymphomas was poor but did not differ from granzyme B-negative gastrointestinal T-cell lymphomas. This indicates that, in peripheral T-cell lymphomas, site of origin is more important as a prognostic parameter than derivation of activated CTLs.

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T-cell malignancies are rare and constitute about 15% to 20% of the non-Hodgkin’s lymphomas in the United States and Western Europe. The only classification with a detailed subdivision of T-cell lymphomas is the updated Kiel classification. In this classification, T-cell lymphomas are classified based on morphologic criteria of nodal T-cell lymphomas. However, its reproducibility has been questioned. From homing studies with T cells, the existence of tissue-restricted T-cell subsets that have their own biologic behavior became apparent. Thus, mucosal T cells, skin T cells, and nodal T cells show organ-specific homing mediated by organ-specific homing receptors that bind with tissue-specific position markers on endothelial cells, termed vascular addressins. Also, T-cell non-Hodgkin’s lymphomas derived from these different tissue-restricted T-lymphocyte subsets not only show differences in adhesion molecule expression, but also show a different clinical behavior and prognosis. Therefore, we and others proposed to include site of origin in a future classification of T-cell lymphomas.

Until recently, it was impossible to differentiate most functional subsets, eg, suppressor T cells and cytotoxic T cells (CTLs) by membrane characteristics only. Therefore, little is known about the occurrence, presentation, and clinical behavior of T-cell lymphomas that are the neoplastic counterparts of different functional T-cell subsets. The production of monoclonal antibodies (MoAbs) against human perforin and serine esterases, major components of the cytotoxic granules found in the cytoplasm of CTLs on activation, have made the identification of CTLs by immunohistochemistry possible. In humans, three serine proteases have been identified in the cytoplasmic granules of activated CTLs and natural killer (NK) cells at the protein level, granzyme A, granzyme B, and granzyme 3. In mice, it was shown that, with few exceptions, expression of granzyme genes is restricted to NK cells and T cells with their thymic precursors. In mature T cells, granzymes are expressed only on activation. In addition, granzyme B transcripts have been detected in mast cell and macrophage cell lines. Although the precise role of granzymes in cell-mediated cytotoxicity is poorly understood and more functions than just DNA fragmentation can probably be attributed to granzymes, the presence of these proteins can be used to identify activated CTLs and, consequently, T-cell lymphomas derived from activated CTLs.

Here, we investigated the prevalence, preferential site, phenotype, and clinical behavior of T-cell lymphomas derived from activated CTLs using an MoAb against the serine esterase granzyme B in a large series of T-cell lymphomas from nodal and extranodal sites to see if differences could be found in relation to granzyme B-negative T-cell lymphomas. The results show that granzyme B-positive lymphomas are preferentially localized in mucosa-associated lymphoid tissue (MALT), have a poor clinical behavior, and show some typical histologic characteristics.
Granzyme B expression in T-Cell Non-Hodgkin’s Lymphoma

<table>
<thead>
<tr>
<th>Subtype (n)</th>
<th>GI</th>
<th>Nose</th>
<th>Lung</th>
<th>Rect</th>
<th>Skin</th>
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<tbody>
<tr>
<td>MF (2)</td>
<td>—</td>
<td>—</td>
<td>0/2</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Le (4)</td>
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<tr>
<td>AILD (2)</td>
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<td>—</td>
<td>0/2</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>PSC (7)</td>
<td>1/3</td>
<td>3/4</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>PMLC (84)</td>
<td>15/37</td>
<td>2/4</td>
<td>1/1</td>
<td>0/33</td>
<td>0/9</td>
</tr>
<tr>
<td>IBL (8)</td>
<td>3/4</td>
<td>—</td>
<td>—</td>
<td>0/1</td>
<td>0/3</td>
</tr>
<tr>
<td>ALCL (25)</td>
<td>1/2</td>
<td>—</td>
<td>0/1</td>
<td>2/16</td>
<td>1/6</td>
</tr>
<tr>
<td>LBL (5)</td>
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<td>—</td>
<td>—</td>
<td>—</td>
<td>0/5</td>
</tr>
<tr>
<td>Unclass (3)</td>
<td>1/2</td>
<td>0/1</td>
<td>—</td>
<td>0/2</td>
<td>—</td>
</tr>
</tbody>
</table>

Abbreviations: GI, gastrointestinal; MF, mycosis fungoides; Le, Lentner’s lymphoma; AILD, angioimmunoblastic lymphohenopathy with dysproteinemia; PSC, pleomorphic small cell lymphoma; PMLC, pleomorphic medium and large cell lymphoma; IBL, immunoblastic lymphoma; ALCL, anaplastic large cell lymphoma; LBL, lymphoblastic lymphoma; Unclass, unclassifiable.

Table 1: Relation Between Histological Subtype, Site of Origin and Granzyme B Expression in T-Cell Non-Hodgkin’s Lymphoma
GRANZYME B EXPRESSION IN T-CELL LYMPHOMAS

Fig 1. Primary gastrointestinal T-cell lymphoma, anaplastic large cell lymphoma subtype is shown. Tumor cells are granzyme-B positive (brown, diffuse granular, cytoplasmic staining). (Hematoxylin counterstain; original magnification × 400.)

or dot-like perinuclear staining. Overall, 29 of 140 (21%) T-cell lymphomas were found to be granzyme B-positive (ie, showed more than 50% staining of the tumor cell population; see Table 1). In an additional 2 primary gastrointestinal cases, 11% to 20% of the tumor cells were GB9-positive. No cases were found with 0% to 10% or 21% to 50% granzyme B-positive tumor cells. A total of 27 of 77 (38%) primary extranodal T-cell lymphomas and 2 of 63 (3%) primary nodal T-cell lymphomas were granzyme B-positive in more than 50% of the tumor cells. The extranodal granzyme B-positive cases were predominantly found in the nose and nasal cavities (5 of 8; 63%), gastrointestinal tract (20 of 46; 43%; see Fig 1), and lung (1 of 3; 33%; see Fig 2). The remaining granzyme B-positive extranodal case was localized in the skin (1 of 20; 5%). Mitotic cells were often GB9-positive in granzyme B-positive cases (Fig 3). In 1 gastrointestinal lymphoma case, interpretation was difficult because CD8-positive tumor cells were surrounded by numerous granzyme B-positive small-to-intermediate sized, CD8-positive, possibly reactive, cells.

The relationship between lymphoma subtype and granzyme B expression is depicted in Table 1. Most cases belonged to the pleomorphic medium- and large-cell subtype category. Pleomorphic small-cell, immunoblastic and, less frequently, large-cell anaplastic granzyme B-positive T-cell lymphomas were also observed.

Necrosis and angioinvasion were more prominent in the group of granzyme B-positive T-cell lymphomas, whereas eosinophilia was slightly more prominent in granzyme B-negative cases. Epitheliotropism in the overlying mucosa of neoplastic T cells did not differ between the granzyme B-positive or granzyme B-negative groups (Table 2). According to the criteria described by Chott and associates, the primary gastrointestinal lymphomas were separated in enteropathy-associated T-cell lymphomas (EATCLs; n = 13), EATCL-like lymphoma without enteropathy (EATCL-LLWE; n = 7) and T-cell lymphoma without enteropathy and without features of EATCL (non-EATCL; n = 14). Granzyme B-positive neoplastic cells were mainly associated with EATCL. A total of 77% (n = 10) of the EATCL cases versus 14% (n = 2) of the non-EATCL cases were granzyme B-positive. In 57% (n = 4) of EATCL-LLWE cases, granzyme B expression was detected.

In the granzyme B-positive cases of which Giemsa-stained imprint preparations were available (n = 4), numerous azurophilic granules were found in the cytoplasm of the neoplastic

Table 2. Histopathologic Findings in Gastrointestinal and Nasal T-Cell Lymphomas in Relation to Granzyme B Expression

<table>
<thead>
<tr>
<th></th>
<th>Granzyme B Positive (%)</th>
<th>Granzyme B Negative (%)</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Necrosis</td>
<td>16/23 (70%)</td>
<td>7/27 (26%)</td>
<td>.002</td>
</tr>
<tr>
<td>Epitheliotropism</td>
<td>5/15 (33%)</td>
<td>8/21 (38%)</td>
<td>NS</td>
</tr>
<tr>
<td>Angioinvasion</td>
<td>13/22 (59%)</td>
<td>5/26 (19%)</td>
<td>.005</td>
</tr>
<tr>
<td>Eosinophilia</td>
<td>1/24 (4%)</td>
<td>7/27 (26%)</td>
<td>.03</td>
</tr>
</tbody>
</table>

* Abbreviation: NS, not significant. * x^2 test.
cells. In a primary nasal granzyme B-positive case, the characteristic secondary lysosomes could be found using electron microscopy (Fig 4).

**Immunophenotypic analysis.** The phenotypes of T-cell lymphomas originating in gastrointestinal tract, nose, and lung are listed in Table 3. CD30 expression was found significantly more often in granzyme B-positive cases than in granzyme B negative cases. Moreover, there was a trend for the CD4+/CD8+ phenotype to be present in granzyme B-positive cases. Other markers demonstrable on paraffin wax-embedded tissue were not associated with granzyme B expression. From the markers that can only be used on frozen material (ie, TCRβ1, HML-1, and CD56), an association was found between CD56 expression and granzyme B-positive nasal T-cell lymphoma. However, the number of cases investigated was rather low (n = 12). Interestingly, no association was found between the presence of TCRβ1 and granzyme B expression in T-cell lymphomas. Granzyme B-positive cases were always naphthol-AS-D-chloroacetate esterase-negative. Both granzyme B-positive nodal cases were CD3-, CD4-, CD8-, CD56-negative. The granzyme B-positive cutaneous case was CD3-positive, but CD4- and CD8-negative.

**Granzyme B expression in control lymphoid tissues.** The results were the same as recently described. Granzyme B-positive cells were detected in all lymphoid tissues tested. In nonneoplastic lymph nodes and tonsillar specimens, granzyme B-positive cells were scarce. When found, they were mostly confined to the sinuses or medullary cords. In the jejunum, with normal mucosa, only sporadic small granzyme B-positive cells were present in the lamina propria. Granzyme B-positive intraepithelial lymphocytes (IELs) were not observed. A similar pattern was observed for normal mucosa adjacent to or overlying the tumor tissue in gastrointestinal lymphomas. Preliminary findings indicate that granzyme B-positive IELs are more often found in patients with EATCL or EATCL-LLWE in the nonlymphoma-involved, adjacent mucosa (this study). In the control tissues tested, hardly any of the granzyme B-positive cells stained with MoAb against CD3. Most granzyme B-positive cells stained with MoAbs against CD16 or CD56, indicating that the majority of granzyme B-positive cells were NK cells in these control lymphoid tissues. The control-irrelevant primary antibody did not stain lymphoid cells.

**Clinical features.** Patients were treated in different institutes in different countries, and therapy was, therefore, heterogeneous. The male:female ratio of patients with granzyme B-positive tumors was 1.25. The patients’ ages ranged from 6 to 82 years (median, 54 years).

Patients with primary nasal T-cell lymphoma presented with localized disease (stage IE according to Ann Arbor) in all but 1 case (stage IV according to Ann Arbor with cervical lymph node and liver involvement). None were leukemic. Of 5 patients with granzyme B-positive nasal tumors, 4 died of disease between 1 and 27 months (median, 4 months) after initial presentation; 1 patient was alive with disease with ongoing chemotherapy. Of 3 patients with granzyme B-negative nasal T-cell lymphomas, 1 died of disease after 4 months (stage IV disease), 1 died of pneumonia without evidence of disease after 14 months, and 1 is still alive after 53 months with disease in CR.

Patients with primary gastrointestinal T-cell lymphomas most often presented with tumors of the small intestine. Most

<table>
<thead>
<tr>
<th>Table 3. Phenotypes of T-Cell Lymphomas of MALT</th>
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</thead>
<tbody>
<tr>
<td>GI Tract T-NHL</td>
</tr>
<tr>
<td>GrB+</td>
</tr>
<tr>
<td>CD3+</td>
</tr>
<tr>
<td>CD4+</td>
</tr>
<tr>
<td>CD8+</td>
</tr>
<tr>
<td>CD4+/CD8-</td>
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<tr>
<td>CD30+</td>
</tr>
<tr>
<td>CD45RO+</td>
</tr>
<tr>
<td>CD43+</td>
</tr>
<tr>
<td>TCRβ1+</td>
</tr>
<tr>
<td>HML-1+</td>
</tr>
<tr>
<td>CD56+</td>
</tr>
</tbody>
</table>

Abbreviations: GI, gastrointestinal; NS, not significant; GrB, granzyme B; T-NHL, T-cell non-Hodgkin’s lymphoma; ND, not done.

* χ² test.
patients presented with stage I or II disease (n = 31). When more generalized disease was present (n = 8), spread was often apart from lymph nodes to other mucosal sites (lung, stomach, and palatum). No apparent differences were noted with regard to clinical presentation between granzyme B-positive or granzyme B-negative cases. Median survival was 5 and 3.5 months for granzyme B-positive and granzyme B-negative tumors, respectively, (P = .5). No significant differences in survival were noted between patients with CD4-positive and CD8-positive or CD4-negative and CD8-negative gastrointestinal T-cell lymphomas (P > .1). This also accounted for CD30-negative gastrointestinal T-cell lymphomas when compared with CD30-negative gastrointestinal T-cell lymphomas (P = .15).

Both patients with nodal granzyme B-positive lymphomas were young (6 and 17 years), with stage I and III disease and with disease in CR of long duration (25 and 70 months, respectively) after aggressive chemotherapy. The remaining granzyme B-positive primary cutaneous lymphomas had a favorable course (alive with disease in CR after 104 months).

**DISCUSSION**

We have shown that a substantial percentage (21%) of our T-cell non-Hodgkin’s lymphomas are granzyme B-positive, indicating that they probably are the neoplastic equivalents of activated CTLs. Primary extranodal peripheral T-cell lymphomas that made up a significant proportion of our study group almost exclusively accounted for this substantial percentage of granzyme B-positive T-cell lymphomas that included 10 of 13 EATCL cases. Of the 29 granzyme B-positive cases, 26 were localized in mucosal sites, ie, nose, gastrointestinal tract, and lung. Only few were localized in lymph nodes (n = 2) or skin (n = 1). Therefore, these lymphomas have a preference for localization in MALT. Histologically granzyme B-positive cases showed significantly more angiogenesis and necrosis and less infiltration with eosinophils than granzyme B-negative lymphomas.

Most granzyme B-positive cases belonged to the pleomorphic subtype categories of the updated Kiel classification. Moreover, granzyme B-positive lymphomas of the gut were associated with histologic characteristics of celiac disease, ie, villous atrophy and intraepithelial lymphocytosis in the adjacent normal mucosa. The association between clinical and/or histologic evidence of celiac disease and the occurrence of primary gastrointestinal T-cell lymphoma has been well-established.31-33 Interestingly, most cases of T-cell lymphoma with eosinophilia of the gastrointestinal tract have not been associated with celiac disease.34

Activation of resting CTLs induces morphologic changes with the occurrence of azurophilic granules.35 In granzyme B-positive cases of which Giemsa-stained cytologic slides were available, azurophilic granules were found. Reports of peripheral T-cell lymphomas with azurophilic granules are scarce, and cases reported most often had extranodal disease and often mucosal involvement.36-41 In general, cytotoxic granules are enveloped by a lipid bilayer and contain small internal vesicles and an electron-dense core surrounded by a membrane.42-44 Granules with identical morphology were also found, by electron microscopy, in the cytoplasm of the granzyme B-positive tumor cells of the nasal T-cell lymphoma investigated.

Low levels of granzyme B are constitutively expressed by NK cells but not by unstimulated CD3⁺/CD8⁺ or CD3⁺/CD4⁺ unstimulated peripheral blood lymphocytes.45-47 This might be the reason for the low number of CD3-positive granzyme B-positive cells in control lymphoid tissue. NK cells are CD3-negative, CD56-positive, and do not rearrange TCR genes, but are otherwise remarkably similar to T cells with respect to the expression of other membrane antigens.48 The T-cell origin of our granzyme B-positive gastrointestinal and cutaneous and of part of the nasal cases was confirmed by the presence of CD3. Two nasal granzyme B-positive cases were CD56-positive and CD3-negative. These might represent lymphomas of NK cell origin. In accordance, non-major histocompatibility complex-restricted cytotoxicity by tumor cells has been described recently for CD3-negative, CD56-positive nasal T-cell lymphoma with TCR genes in germline configuration.49 Several studies have addressed the relationship between CD56 expression and T-cell lymphomas.49,50,51 CD56-positive T-cell lymphomas were reported to have a propensity for extranodal sites, in particular the upper aerodigestive tract,49 which is in agreement with our findings. Moreover, CD56-positive lymphomas were reported to share a very aggressive clinical behavior and resistance to conventional chemotherapy.49

Although our results do not provide conclusive evidence as to whether granzyme B-positive lymphomas are neoplastic counterparts from IELs or activated CTLs in the lamina propria of the small intestine,35-37 we slightly favor the first option. In mice, IELs have been shown to express granzymes50 and are predominantly γδ TCR-positive. In humans, IELs are mostly aβ TCR-positive51 and probably also express granzyme proteins after activation. IELs are increased in celiac disease, and granzyme B-positive IELs seemed to be more prominent in the adjacent nonlymphoma involved mucosa in cases classified as EATCL or EATCL-like lymphoma without enteropathy. In addition, normal human small intestine CD3⁺CD8⁻/CD4⁺ T lymphocytes comprise a minor fraction of IELs (6%) that are increased in celiac disease.52 This phenotype proved to be the dominant phenotype in granzyme B-positive gastrointestinal T-cell lymphomas.

We can only speculate on the association between signs of celiac disease and the occurrence of tumors derived from activated CTLs. It has been assumed that the mucosal permeability for luminal antigens is increased in celiac disease because of local and systemic IgG responses to gluten and other dietary proteins.53 This might exert a cytotoxic T-cell response. The continuous antigenic stimulation might contribute to malignant transformation as has been proposed for gastric B-cell lymphomas in association with the presence of Helicobacter pylori.54,55

In conclusion, granzyme B-positive T-cell lymphomas are predominantly localized in MALT and differ from granzyme B-negative T-cell lymphomas in increased frequency of angiogenesis and necrosis but less infiltration with eosinophils. Skin and lymph nodes are occasionally involved. Although in some nasal cases an NK cell nature can not be excluded, most cases should be considered as neoplastic...
equivalents of activated CTLs. The prognosis of the granzyme B-positive gastrointestinal T-cell lymphomas is as equally poor as the prognosis of granzyme B-negative gastrointestinal T-cell lymphomas, indicating that site of origin rather than derivation of activated CTLs predicts clinical outcome of peripheral T-cell lymphomas.

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