Differential Expression of T-bet, a T-box Transcription Factor Required for Th1 T-Cell Development, in Peripheral T-Cell Lymphomas

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Abstract

We studied T-bet expression in 91 cases of peripheral T-cell lymphoma (PTCL) by immunostaining and found expression in 42 cases (46%), including all 5 lymphoepithelioid lymphoma cases and 12 (86%) of 14 angioimmunoblastic lymphoma cases, but only 9 (25%) of 36 anaplastic large cell lymphoma cases. Expression of T-bet in PTCL correlates with expression of other markers of Th1 T-cell differentiation, including CXCR3 (P < .0001), CD69 (P = .0013), LEF-1 (P = .0007), and OX40/CD134 (P = .005), and absence of expression of markers of Th2 T-cell differentiation, including CD30 (P = .0001) and CXCR4 (P = .0144). Of 22 cases of PTCL immunoreactive for all Th1-associated markers previously studied and nonreactive for Th2-associated markers, 20 (91%) were immunoreactive for T-bet. Of 22 PTCL cases immunoreactive for Th2-associated markers studied and nonreactive for all Th1-associated markers studied, 4 (18%) were immunoreactive for T-bet. The remaining 47 PTCL cases (52%) exhibited incomplete or mixed staining for Th1- and Th2-associated markers, with 18 (38%) of 47 immunoreactive for T-bet. T-bet is a new marker that may contribute to the diagnosis and subtyping of PTCLs. T-bet expression in these neoplasms provides further support for a model of PTCL in which tumor subsets express markers of, and may be derived from, Th1- or Th2-committed T cells.

Naïve CD4+ T-helper precursor (Thp) cells differentiate into 2 distinct cell types, the Th1 cell, which is involved in delayed hypersensitivity and immune protection against intracellular pathogens and viruses, and the Th2 cell, which is involved in B cell–directed antibody production and immune response to extracellular pathogens such as helminths.1,2 T-bet, a recently discovered Th1 T cell–specific T-box transcription factor, directs Th1 development in naïve Thp cells and can redirect Th2 T cells to Th1 development.3 CD4+ T cells fail to differentiate into Th1 cells and default to a Th2 state in mice with T-bet gene deletion, indicating that T-bet expression is required for Th1 T cell development.4 T-bet is a 62-kd protein expressed in the Th1 subset of CD4+ T cells in lymphoid tissues.3 There are decreased numbers of T-bet+ airway T cells in patients with asthma, and mice with targeted T-bet gene deletion spontaneously develop features of asthma, including spontaneous airway hyperresponsiveness, airway inflammation, and remodeling, suggesting that decreased T-bet expression might be a major factor in the development of inflammatory airway disease.5 Expression of T-bet might have a role in autoimmune disease, eg, absence of T-bet is protective in a mouse inflammatory bowel disease model, and overexpression of T-bet promotes Th1-mediated colitis.6

Previously we reported that a number of T-cell activation markers and chemokine receptors that are specific for Th1 or Th2 T cells are expressed in generally nonoverlapping subsets of peripheral T-cell lymphoma (PTCL).7-11 This included chemokine receptor CXCR3, which is expressed in Th1 T cells, and chemokine receptors CCR4 and CXCR4, which are expressed in Th2 T cells. Cases of
anaplastic large cell lymphoma (ALCL), which express Th2 T-cell–associated activation marker CD30, generally also were immunoreactive for CCR4 and CXCR4 and not for CXCR3. In contrast, a number of cases of PTCL, unspecified, including cases of lymphoepithelioid (Lennert) lymphoma, as well as most cases of angioimmunoblastic lymphoma (AIL), were immunoreactive for Th1 T-cell–associated CCR3 and not for Th2 T-cell–associated CCR4, CXCR4, or CD30. Expression of Th1 T-cell–associated activation marker CD69 in PTCL correlated with that of other Th1 T-cell markers and was not present in PTCL expressing Th2 T-cell markers. These results suggest a bipartite model of PTCL development, in which at least a subset of cases of PTCL may arise through neoplastic transformation of peripheral T cells activated to a Th1 or Th2 state.

We studied a variety of cases of PTCL for T-bet expression, to determine whether expression of this Th1 T-cell transcription factor correlates with that of other markers of Th1 but not Th2 T-cell differentiation. We found that a subset of PTCL cases express T-bet, that its expression correlates with that of other Th1 T-cell markers, and that it may contribute to the diagnosis and subtyping of PTCL.

Materials and Methods

Case material was obtained from the Brigham and Women’s Hospital, Boston, MA, in accordance with institutional policies. All diagnoses were based on the features described in the World Health Organization lymphoma classification system, except that cases of lymphoepithelioid lymphoma were separated from the category of PTCL, unspecified. Cases previously were characterized immunophenotypically with antibodies directed against the B-cell marker CD20 (L26) and the T-cell markers CD3, CD45RO, CD43 (Leu22), CD8, CD30, ALK-1, CXCR3, CD134/OX40, CD69, LEF-1, TCF-1, and CXCR4, using formalin-fixed, paraffin-embedded tissue sections. In cases in which frozen tissue was available, immunophenotypic analysis also was performed using antibodies directed against CD4 and CCR4, as previously described. Cases of PTCL were included if CD4 immunoreactivity was demonstrated by immunohistochemical staining of frozen sections and/or absence of CD8 immunoreactivity in immunohistochemical staining of paraffin-embedded sections.

Immunostaining for T-bet was performed on formalin-fixed, paraffin-embedded tissue sections following microwave antigen retrieval in a 1-mmol/L concentration of EDTA, pH 8.0, with a previously described antihuman T-bet monoclonal antibody (4B10), using a standard indirect avidin-biotin horseradish peroxidase method and diaminobenzidine color development, as previously described. Cases were regarded as immunoreactive for T-bet if at least 25% of neoplastic cells exhibited positive staining. T-bet staining was compared with that of mouse IgG isotype control antibody diluted to identical protein concentration for all cases studied, to confirm staining specificity. Statistical analysis of T-bet staining with other T-cell markers was performed using the Fisher exact test.

Results

T-bet Immunostaining in Reactive Lymphoid Tissue

Monoclonal antibody 4B10 for T-bet was used to stain formalin-fixed, paraffin-embedded specimens of reactive lymphoid tissue, thymus, and a range of cases of T-cell lymphoproliferative disorders. In specimens of reactive lymphoid tissue with follicular hyperplasia, scattered small lymphocytes in the interfollicular T-cell zone exhibited nuclear staining for T-bet. Virtually no T-bet staining was observed in germinal centers. Similar results were seen in histologic sections of reactive lymph node and spleen (data not shown).

In adult thymus, scattered small lymphocytes in the medulla, adjacent to Hassall corpuscles, exhibited nuclear staining for T-bet, with few T-bet+ cells observed in the thymic cortex where immature T cells reside. Thymic epithelial cells did not stain for T-bet. Similar results were seen in histologic sections of fetal thymus (data not shown).

T-bet Immunostaining in Paraffin-Embedded Tissue Sections of T-Cell Lymphoproliferative Disorders

All 8 cases of precursor T-lymphoblastic leukemia/lymphoma failed to exhibit immunostaining for T-bet (data not shown). These results are consistent with the finding that T-bet is a marker of mature T cells and is expressed at very low levels in Thp cells.

We then studied a series of PTCL cases that previously were characterized for the expression of a number of chemokine receptors and other markers that correlate with Th1 and Th2 T-cell differentiation. Of 91 PTCL cases, 42 (46%) were immunoreactive for T-bet. The results are summarized in Table 1. This included 21 cases of PTCL, unspecified, including 5 (100%) of 5 cases of lymphoepithelioid lymphoma, 12 (86%) of 14 cases of AIL, and 9 (25%) of 36 cases of ALCL. A typical T-bet+ case of PTCL is shown in Image 3, in which virtually all neoplastic cells exhibited nuclear staining for T-bet. In cases of lymphoepithelioid lymphoma Image 4 and AIL...
the vast majority of neoplastic T cells also exhibited nuclear staining for T-bet. In contrast, in the majority of cases of ALCL, neoplastic cells were nonreactive for T-bet, although scattered, small, nonneoplastic cells were present that were T-bet+. In a minority of cases of ALCL, neoplastic cells were immunoreactive for T-bet.

Because T-bet expression is associated with Th1 T-cell differentiation, we compared T-bet expression in cases of PTCL with that of other Th1-associated and Th2-associated markers that were studied previously. The results are summarized in Table 2. Expression of T-bet in T-cell lymphomas correlates with expression of other markers of Th1 T-cell differentiation, including CXCR3 (P < .0001), OX40/CD134 (P = .005), CD69 (P = .0013), and LEF-1, a T-cell transcription factor expressed in thymocytes and mature T cells (P = .0007). Expression of T-bet correlates with absence of expression of markers of Th2 T-cell differentiation, including CD30 (P = .0001) and CXCR4 (P = .0144).

We also studied T-bet expression in subsets of PTCL that were uniformly reactive for previously studied Th1-
associated and Th2-associated markers. The results are summarized in Table 1. Of 91 PTCL cases, 22 (24%) were immunoreactive for all Th1-associated markers previously studied and were nonreactive for Th2-associated markers. Of these Th1-restricted cases, 20 (91%) of 22 were immunoreactive for T-bet. This group of Th1 marker–positive and T-bet+ cases included 5 of 5 cases of lymphoepithelioid lymphoma and 8 of 14 cases of AIL. A separate set of 22 (24%) of 91 PTCL cases were immunoreactive for Th2-associated markers studied and were nonreactive for all Th1-associated markers studied. All of the cases in this group were diagnosed as ALCL. Of these Th2-restricted cases, only 4 (18%) of 22 were immunoreactive for T-bet. T-bet expression correlated with expression of other Th1-associated markers and the absence of expression of Th2-associated markers in these 2 subsets of PTCL ($P < .0001$).

The remaining 47 (52%) of 91 PTCL cases exhibited overall staining for Th1- or Th2-associated markers, with absence of staining for one or more markers (14 cases and 2 cases, respectively) or mixed staining for Th1- and Th2-associated markers (31 cases). Of these, 18 (38%) of 47 cases were immunoreactive for T-bet. There were 27 cases of PTCL, unspecified, 9 of which were immunoreactive for T-bet; 6 cases of AIL, 4 of which were immunoreactive for T-bet; and 14 cases of ALCL, 5 of which were immunoreactive for T-bet.

**Discussion**

We report that T-bet, the T-box transcription factor expressed in Th1-committed CD4+ T cells, is expressed by lymphocytes in T-cell zones of reactive lymphoid tissue, by medullary thymocytes, and by a subset of PTCLs. The expression pattern of T-bet in reactive lymphoid tissues and thymus, as well as the absence of T-bet expression in neoplastic T-cell lymphoblasts, is consistent with previous data showing that T-bet is expressed in mature CD4+ T cells.

Consistent with the restriction of T-bet expression to Th1-differentiated cells, T-bet is expressed in neoplastic T cells from cases of PTCL with evidence for Th1 differentiation, based on the presence of a number of markers

<table>
<thead>
<tr>
<th><strong>Table 1</strong></th>
<th>T-bet Immunostaining in T-Cell Lymphoproliferative Disorders*</th>
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<tbody>
<tr>
<td><strong>T-bet Immunostaining</strong></td>
<td></td>
</tr>
<tr>
<td>Precursor T-LL/L</td>
<td>0/8 (0)</td>
</tr>
<tr>
<td>Peripheral T cell NHL</td>
<td>42/91 (46)</td>
</tr>
<tr>
<td>AIL</td>
<td>12/14 (86)</td>
</tr>
<tr>
<td>PTCL, unspecified</td>
<td>21/41 (51)</td>
</tr>
<tr>
<td>LEL</td>
<td>5/5 (100)</td>
</tr>
<tr>
<td>ALCL</td>
<td>9/36 (25)</td>
</tr>
<tr>
<td>Th1 T-cell–like NHL</td>
<td>20/22 (91)</td>
</tr>
<tr>
<td>Th2 T-cell–like NHL</td>
<td>4/22 (19)</td>
</tr>
<tr>
<td>Mixed type</td>
<td>18/47 (38)</td>
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associated with Th1 differentiation and absence of staining for markers of Th2 differentiation. This includes chemokine receptor CXCR3, which is expressed in Th1 T cells; chemokine receptors CCR4 and CXCR4, which are expressed in Th2 T cells; activation marker CD69, expressed in Th1 T cells; and transcription factors LEF-1 and TCF-1, which are expressed in cases of PTCL that express Th1 T-cell markers but not Th2 T-cell markers. Cases of ALCL, which usually exhibit a Th2 T-cell expression pattern based on our previous studies, generally are negative for T-bet expression. This finding also is consistent with previous studies demonstrating that CD30, a characteristic marker of ALCL, is a Th2 T-cell–associated activation marker and that the murine anaplastic lymphoid cell line TS1G6 (an animal model for ALCL) and L82 (a human ALCL cell line) both exhibit a Th2 T-cell cytokine profile. In contrast, a number of cases of PTCL, unspecified, including cases of lymphoepithelioid (Lennert) lymphoma, as well as most cases of AIL, are immunoreactive for Th1 T-cell–associated markers and not Th2 T-cell–associated markers. These results suggest a bipartite model of PTCL development, in which at least a subset of cases and subtypes of PTCL may arise from neoplastic transformation of peripheral T cells activated to a

**Image 4** Lymphoepithelioid lymphoma (A, H&E, ×1,000), in which frequent, scattered, intermediate-sized lymphoid cells are immunoreactive for T-bet (B, T-bet, ×1,000).

**Image 5** Angioimmunoblastic T-cell lymphoma (A, H&E, ×400), in which frequent, intermediate- to large-sized lymphoid cells are immunoreactive for T-bet (B, T-bet, ×400).
Th1 or Th2 state. The present finding that T-bet expression correlates with that of other markers of Th1 differentiation in PTCL and inversely correlates with markers of Th2 differentiation in PTCL supports this bipartite model of T cell lymphomagenesis.

A subset of cases of PTCL, 31 (34%) of 91 cases, has a mixed Th1-Th2 expression pattern for T-bet and other markers of Th1 and Th2 T-cell differentiation noted in the preceding text. A possible explanation may be found in recent findings that suggest that naive CD4+ T cells differentiate into Th1 or Th2 cells by a sequential process, with lineage commitment dependent on the expression of lineage-specific transcription factors T-bet and GATA-3.18 It is well established that B-cell lymphomagenesis can be mapped to the process of B-cell ontogeny, with specific subtypes of B-cell lymphomas that correspond to the normal stages of B-cell development.15 It might be the case that a similar process occurs in T-cell lymphomagenesis, with subtypes of PTCL corresponding to various stages in T-cell development and differentiation, including fully committed Th1 and Th2 T cells, as well as T cells in a less committed state.

Recently, Cousins and coworkers19 found that in human as opposed to murine T cells, Th subset–specific transcription

![Image 6](https://example.com/image6.png)

**Image 6**  Anaplastic large cell lymphoma (A, H&E, ×1,000), in which large neoplastic cells are uniformly negative for T-bet (B, T-bet, ×1,000), although scattered small nonneoplastic lymphocytes exhibit staining for T-bet.

![Image 7](https://example.com/image7.png)

**Image 7**  A second case of anaplastic large cell lymphoma (A, H&E, ×1,000), in which neoplastic cells are uniformly positive for T-bet (B, T-bet, ×1,000).
Comparison of T-bet Immunostaining With Other T-Cell Markers in T-Cell Non-Hodgkin Lymphoma*

<table>
<thead>
<tr>
<th></th>
<th>CXCR3+</th>
<th>CXCR3−</th>
<th>OX40+</th>
<th>OX40−</th>
<th>CD69+</th>
<th>CD69−</th>
<th>LEF+</th>
<th>LEF−</th>
<th>CD30+</th>
<th>CD30−</th>
<th>CXCR4+</th>
<th>CXCR4−</th>
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<tr>
<td>T-bet+</td>
<td>34</td>
<td>8</td>
<td>29</td>
<td>11</td>
<td>33</td>
<td>9</td>
<td>29</td>
<td>13</td>
<td>12</td>
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<td>14</td>
<td>28</td>
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<tr>
<td>T-bet−</td>
<td>15</td>
<td>34</td>
<td>20</td>
<td>29</td>
<td>22</td>
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<td>16</td>
<td>33</td>
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* Data are given as the number of cases.

Transcription factors expressed in murine Th2 cells that regulate Th2 cytokine expression, were detectable in human naïve T cells and in early differentiated Th1 cells, although they were expressed at higher levels in Th2 T cells. Similarly, T-bet was expressed in differentiating Th2 T cells, albeit at lower levels than in Th1 T cells. The authors concluded that relative levels rather than on-off expression of Th1 and Th2 T-cell–associated transcription factors might regulate a balance between commitment to a Th1 or Th2 phenotype, a conclusion supported by the existence of dual Th1-Th2 “Th0” cells in humans. In a similar manner, at least a subset of cases of PTCL may be derived from T cells that express Th1- and Th2-associated markers or from activated, T-bet+ Th2 cells, for example. In preliminary immunohistochemical staining studies, we found that c-Maf expression was present in a significant subset of PTCL cases with an overall Th1 immunophenotype and in cases with an overall Th2 immunophenotype (D.M.D., A.S., L.H.G., unpublished data, 2003), which might be explained by the findings of Cousins and coworkers.19

The prognostic implications of T-bet expression in PTCL remain to be directly determined. However, because T-bet expression is correlated closely with the expression of Th1 T-cell–associated markers and certain subtypes of PTCL such as AIL and inversely correlated with the expression of Th2 T-cell–associated markers and ALC, some preliminary conclusions are possible. Previous studies have shown that ALC generally has a better prognosis than other T-cell lymphomas, including those with a Th1 T-cell–like immunophenotype typically, such as AIL.20-22 It is of interest to determine the prognostic significance of T-bet expression and overall Th1 or Th2 T-cell marker expression in PTCL, as well as the possible significance of mixed Th1-Th2 marker expression in PTCL. Studies are in progress to assess the prognostic significance of mixed Th1-Th2 marker expression vs exclusive Th2 marker expression in cases of ALC.

Transcription factors expressed in the B-cell lineage and in B-cell lymphomas, such as Oct1, Oct2, B-cell–specific activator protein, and PU.1 seem to be expressed widely in all subtypes of B-cell lymphoma.23-25 In contrast, T-bet is expressed in mature T cells and at very low levels in Thp cells and is absent in precursor T-lymphoblastic leukemia/lymphoma cells. On the other hand, in a previous study, we found that LEF-1 and TCF-1, transcription factors that are expressed in immature and in mature T cells, are expressed in precursor T-lymphoblastic leukemia/lymphoma cells but in only a subset of cases of PTCL that correspond to a specific subtype of activated T cell.11 The genetic program for transcription regulation in T cells is complex, and understanding of the signaling mechanisms for activation and commitment of T cells to Th1 or Th2 differentiation is far from complete.26 Our results suggest that as additional steps in the Th1 and Th2 T-cell commitment process are elucidated, it may be possible to map specific cases and subtypes of PTCL to discrete stages in T-cell development and to specific T-cell gene and protein expression signatures.

References


