Expression of Master Regulators of Helper T-Cell Differentiation in Peripheral T-Cell Lymphoma, Not Otherwise Specified, by Immunohistochemical Analysis

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Key Words: Peripheral T-cell lymphoma, not otherwise specified; Th-POK; T-Bet; GATA3; Central memory T cells; Follicular helper T cells

Abstract

The normal counterparts of peripheral T-cell lymphoma, not otherwise specified (PTCL-NOS) have not been accurately identified. We immunohistochemically analyzed 10 PTCL-NOS cases to examine the expression of the master regulators of T-cell differentiation and of surface antigens, including chemokine receptors. All cases were positive for the master regulator of helper T cells (Th-POK) and the marker of effector T cells (CD45RO). Three cases each were positive for T-Bet and GATA3, which are master regulators of helper T cells (TH) type 1 (TH1) and TH2, respectively. Two cases were positive for the surface antigens of central memory (TCM) (CCR7 and CD62L), and 1 case was positive for follicular helper T-cell (TFH) phenotype (BCL6, CXCL13, and PD-1). The remaining case was negative for all markers of effector TH subtypes. These results suggest the postulated normal counterparts of PTCL-NOS identified in 9 of the 10 cases consist of TH1, TH2, TCM, and TFH.

Peripheral T-cell lymphoma, not otherwise specified (PTCL-NOS), which accounts for approximately 6% to 8% of all lymphomas in Japan, comprises nodal and extranodal mature T-cell lymphomas, which do not correspond to any of the specifically defined entities of mature T-cell lymphoma in the current classification. During T-cell maturation, naive T cells include 2 distinct cell types, CD4+ helper T cells (TH) and CD8+ cytotoxic T cells. Following antigen stimulation, naive TH cells differentiate into several subtypes of effector TH cells, including TH1 (T\textsubscript{H1}), TH2 (T\textsubscript{H2}), follicular helper T cells (TFH), regulatory T cells (Treg), interleukin-17 producing T cells (Th17), central memory T cells (TCM), and effector memory T cells.

Previously, these subtypes of effector TH cells have been classified according to their pattern of cytokine secretion and expression of surface antigens, including chemokine receptors. More recent immunologic research has identified several transcription factors responsible for T-cell differentiation. The master regulators associated with TH1, TH2, Th17, and Treg differentiation are reported to be Th-POK, T-Bet, GATA3, ROR\textgreek{\gamma}, and FOXP3, respectively.

Recently, the normal counterparts of some T-cell lymphomas were identified through detection of the expression of specific surface antigens and transcriptional factors. For example, angioimmunoblastic T-cell lymphoma (AITL) cells were found to express BCL6, CXCL13, and PD-1, and their normal counterpart was identified to be TFH. In addition, the normal counterpart of some adult T-cell leukemia cases was reported to be Treg because of the detection of FOXP3 expression.

The origin of the neoplastic cells of PTCL-NOS is thought to be effector TH cells because the tumor cells usually...
express specific surface antigens such as pan–T-cell markers (CD2 and CD3), an effector T-cell marker (CD45RO), and a T<sub>n</sub> marker (CD4). However, the normal cellular derivation of PTCL-NOS remains unclear because the immunophenotypic profiles of PTCL-NOS are heterogeneous and not entirely disease-specific.

To identify the normal counterparts of PTCL-NOS, we used immunohistochemical analysis to examine the expression of the master regulators of T-cell differentiation and surface antigens, including chemokine receptors associated with the subtypes of T<sub>n</sub> cells.

Materials and Methods

Patients

The study population included 10 Japanese patients who were diagnosed histopathologically with PTCL-NOS at the hospital of Kyoto Prefectural University of Medicine, Kyoto, Japan, from 1993 to 2008. There were 6 men and 4 women who ranged in age from 26 to 78 years (mean, 56 years) at diagnosis. Informed consent was obtained from each patient according to our institutional guidelines. Each case in the study was reviewed by 2 pathologists for confirmation of PTCL-NOS. For histopathologic subclassification, we used the World Health Organization classification of neoplastic diseases of the lymphoid tissues.

The relevant clinical and histologic information, including expression of CD4, CD8, and cytotoxic molecules (perforin, granzyme B, and T-cell intracytoplasmic antigen 1) using immunohistochemical analysis and/or flow cytometry analysis; T-cell receptor Cβ rearrangement using Southern blotting (Table 1); and karyotype using G-banding were recorded.

Immunohistochemical Staining

Immunohistochemical staining was applied to 4-μm-thick paraffin-embedded sections of diagnostic lymph node, skin, or liver tumor biopsy specimens (EnVision, DAKO, Carpinteria, CA; and Cell & Tissue Staining Kit; R&D Systems, Minneapolis, MN). In brief, tissue sections were deparaffinized, rehydrated, and then submitted to antigen retrieval by heating in 10 mmol/L citrate buffer at pH 6.0 or 1 mmol/L EDTA buffer at pH 8.0. After cooling, endogenous peroxidase was blocked by means of a peroxidase blocking reagent. Sections were incubated at room temperature with the primary antibodies, stained using a standard indirect avidin-biotin horseradish peroxidase method and diaminobenzidine color development, and counterstained with hematoxylin. Cases were considered positive if ≥25% or more of the tumor cells were stained with an antibody, based on a previous report. For negative and positive staining control samples, we used paraffin-embedded tissue sections of tonsillitis, nonspecific lymphadenitis, AILT, follicular lymphoma, and diffuse large B-cell lymphoma.

Primary antibodies against the following transcription factors were introduced: Th-POK (dilution 1:50; Abgent, San Diego, CA), T-Bet (4B10, dilution 1:100; Santa Cruz Biotechnology, Santa Cruz, CA), GATA3

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**Table 1**

Characteristics of 10 Cases With Peripheral T-Cell Lymphoma, Not Otherwise Specified

<table>
<thead>
<tr>
<th>Case No./Age (y)/Sex</th>
<th>Ann Arbor Biopsy</th>
<th>EBER-ISH</th>
<th>TCR Cβ Rearrangement</th>
<th>Cytotoxic Molecules</th>
<th>Survival (mo)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/29/M</td>
<td>IA</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>ND*</td>
</tr>
<tr>
<td>2/75/F</td>
<td>IVA</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>ND*†</td>
</tr>
<tr>
<td>3/46/M</td>
<td>IVB</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>ND*†</td>
</tr>
<tr>
<td>4/88/F</td>
<td>IIB</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>ND*†</td>
</tr>
<tr>
<td>5/78/F</td>
<td>IIB</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>ND*†</td>
</tr>
<tr>
<td>6/55/M</td>
<td>IVB</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>ND*†</td>
</tr>
<tr>
<td>7/66/M</td>
<td>IVA</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>ND*†</td>
</tr>
<tr>
<td>8/50/M</td>
<td>IVA</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>ND*†</td>
</tr>
<tr>
<td>9/75/F</td>
<td>IVA</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>ND*†</td>
</tr>
<tr>
<td>10/26/M</td>
<td>IVA</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>ND*†</td>
</tr>
</tbody>
</table>

EBER-ISH, Epstein-Barr virus (EBV)-encoded RNA in situ hybridization; LN, lymph node; ND, not done; NE, not evaluable; TCR, T-cell receptor; TIA, T-cell intracytoplasmic antigen.

* Anti-EBV viral capsid antigen (VCA) IgG antibody, <10×; IgM antibody, <10×; anti-EBV nuclear antigen (EBNA) antibody, <10×.
† EBV DNA, 1.6 × 10<sup>6</sup> copies/10<sup>6</sup> WBCs.
‡ EBV DNA, 1.6 × 10<sup>6</sup> copies/10<sup>6</sup> WBCs.
§ Anti-EBV VCA IgM antibody, 640×; IgG antibody, <10×; anti-EBNA antibody, 40×.
§ Anti-EBV VCA IgG antibody, <10×; IgM antibody, <10×; Anti-EBNA antibody, <10×.
(HG3-31, dilution 1:80; Santa Cruz Biotechnology), FOXP3 (mAbcam 22510, dilution 1:200; Abcam, Cambridge, MA; and 259D/C7, dilution 1:50; BD Pharmingen, San Diego, CA), RORγt (dilution 1:100; MBL International, Woburn, MA), and BCL6 (PG-B6p, dilution 1:15; DAKO). Primary antibodies against the following chemokines, chemokine receptors, and surface antigens were used: CD45RA (4KB5, dilution 1:70; DAKO), CD45RO (UCHL-1, dilution 1:70; DAKO), CXCR3 (IC6/CXR3, dilution 1:75; BD Pharmingen), CCR3 (Y31, dilution 1:100; Abcam), CCR4 (dilution 1:100; Lifespan Biosciences, Seattle, WA), CXCL13 (dilution 15 μg/mL; R&D Systems), PD-1 (dilution 15 μg/mL; R&D Systems), CCR7 (Y59, dilution 1:200; Abcam), and CD62L (9H6, dilution 1:30; Abcam). All antibodies were treated and used according to the manufacturers’ instructions.

Results

Immunohistochemical results are shown in Table 3.

Expression of the Master Regulator of Th (Th-POK), Naive T-Cell Antigen (CD45RA), and Effector T-Cell Antigen (CD45RO)

All PTCL-NOS cases were immunohistochemically positive for Th-POK and CD45RO, and all cases except case 9 were negative for CD45RA. The tissue section of case 9 contained CD45RA+ cells and CD45RO+ cells in nearly equal proportions (47% vs 45%).

Expression of the Master Regulator and the Chemokine Receptor of Tn 1 (T-Bet and CXCR3)

Cases 1, 2, and 3 were positive for T-Bet, whereas cases 1 and 2 were positive for CXCR3 Image 1, but case 3 was negative. No T-Bet– cases expressed CXCR3.
**Image 1** (Case 1)  
A, H&E, ×1,000. B, CD45RA, ×1,000. C, CD45RO, ×1,000. D, Th-POK, ×1,000. E, T-Bet, ×1,000. F, CXCR3, ×1,000.
Expression of the Master Regulator and the Chemokine Receptors of T_{h2} (GATA3, CCR3, and CCR4)

Cases 4, 5, and 6 were positive for GATA3, whereas case 4 was positive for CCR3 and CCR4 Image 2, but case 5 was negative. Expression of these chemokine receptors could not be evaluated in case 6 owing to insufficiency of the required sample. GATA3- cases expressed neither CCR3 nor CCR4.

Expression of the Master Regulators of Treg and Th17 (FOXP3 and ROR_{γt})

All PTCL-NOS cases were negative for FOXP3 and ROR_{γt} by definition. The percentage ranges of FOXP3+ and ROR_{γt}+ cells were 0% to 5.1% and 0% to 6.7%, respectively.

Expression of the Chemokine Receptor and the Surface Antigen of Tcm (CCR7 and CD62L)

Cases 7 and 8 were positive for CCR7 and CD62L. CCR7 was also detected in case 1 and CD62L in cases 2 and 9 Image 3.

Expression of Transcription Factor, Chemokine, and Surface Antigen of TfH (BCL6, CXCL13, and PD-1)

Case 9 was positive for BCL6, CXCL13, and PD-1. While CXCL13 and PD-1 were negative in all cases except case 9, BCL6 was also detected in cases 3 and 5. Conventional chromosomal analysis detected t(5;9)(q33;q22) in case 9 Image 4.

Discussion

Immunohistochemical examination of expression of the master regulators of T-cell differentiation and surface antigens, including chemokine receptors associated with the subtypes of T_{n} cells, enabled us to identify the normal counterparts of PTCL-NOS.

First, we examined the expression of Th-POK, CD45RA, and CD45RO. Th-POK is a master regulator of differentiation of T_{n},^{2} and CD45RA and CD45RO are surface markers of
naive and effector T cells, respectively. Although a nearly equal number of CD45RA+ cells and CD45RO+ cells were detected in the section of case 9, expression of CD45RO indicated that the tumor cells were derived not from naive but from effector T cells. Furthermore, the results of our study showed that all 10 PTCL-NOS cases were positive for Th-POK. While these results suggest that these 10 cases were derived from effector T+ cases 3 and 6 did not exhibit a set of surface antigens compatible with T+ (CD4+ and CD8−), and cases 6 and 10 exhibited cytotoxic molecules.

Next, we examined the expression of the master regulators of differentiation to subpopulations within T+ cells (T+, T+, T+, T+, and Th17). Three cases each were positive for T-Bet and GATA3, but none of the cases were positive for FOXP3 or RORγt. Dorfman et al10 reported that 21 (51%) of 41 PTCL unspecified cases were immunoreactive for T-Bet, while Bonzheim et al12 reported that only 1 among 14 PTCL-NOS cases expressed FOXP3. To the best of our knowledge, there have been no previous reports concerning GATA3 and RORγt expression. Our results suggest a significant number of PTCL-NOS cases were derived from T+, and T+ cells and no cases were derived from Treg or Th17. Although not all T-Bet cases concomitantly expressed T+ nor all GATA3+ cases expressed T+2-associated chemokine receptors, none of the T-Bet− cases expressed the T+1 chemokine receptor (CXCR3), and none of the GATA3− cases expressed the T+2 chemokine receptors (CCR3 and CCR4). Because our study could analyze only a limited number of cases, further investigation of more cases is needed to clarify the association between master regulators and chemokine receptors.

Among the 4 cases not expressing these master regulators (T-Bet, GATA3, RORγt, and FOXP3), 2 (cases 7 and 8)
percentages of PTCL-NOS cases expressing it have differed (10%-33%) depending on the report.7,18 Tumor cells of case 10 were found to have been derived from effector TH because of positivity for CD45RO and Th-POK but were negative for all markers of effector TH subtypes.

All 10 PTCL-NOS cases exhibited CD45RO and Th-POK, which characterized effector TH cells. Furthermore, the lymphoma cells of 9 of the 10 cases exhibited the expression of master regulators or surface antigens, which characterized TH1, TH2, TCM, and TFH. However, at least 3 cases were not compatible with TH (positive for CD4 and negative for CD8 and cytotoxic molecules) in this study. Recently, gene expression profiling studies identified normal counterparts of certain lymphomas, such as AITL19,20 and chronic lymphocytic leukemia.21,22 Clarification of the origins of PTCL-NOS based on gene expression profile remains an issue for future studies.

One case (case 9) showed the TH phenotype, which was positive for CD4 and negative for CD8 and cytotoxic molecules.13 Geissinger et al14 reported that 5 of 8 PTCL-NOS cases exhibited a CD4+ TCM phenotype.

Exhibited the TCM phenotype, which was identified as negative for CD45RA and positive for CD45RO, CCR7, and CD62L. Memory T cells have been divided into 2 main subsets based on their expression of CD62L and CCR7: TCM expressing CD62L and CCR7 and effector memory T cells lacking CD62L and CCR7 cell surface expression.13 Geissinger et al14 reported that 5 of 8 PTCL-NOS cases exhibited a CD4+ TCM phenotype.

One case (case 9) showed the TH phenotype, which was positive for CD4 and negative for CD8 and cytotoxic molecules.13 Geissinger et al14 reported that 5 of 8 PTCL-NOS cases exhibited a CD4+ TCM phenotype.
Image 3 A-D (Case 7), A, CD45RA, ×1,000. B, CD45RO, ×1,000. C, CCR7, ×1,000. D, CD62L, ×1,000. Tumor cells were positive for CD45RO, CCR7, and CD62L and negative for CD45RA. E-H (Case 9), E, BCL6, ×1,000. F, CXCL13, ×1,000.


11. Heyd F, ten Dam G, Möröy T. Auxiliary splice factor 6 (Case 9) Conventional chromosomal analysis detected t(5;9)(q33;q22).

References


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