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The Frequency of Immunoglobulin Heavy Chain Gene and T-Cell Receptor γ-Chain Gene Rearrangements and Epstein-Barr Virus in ALK$^+$ and ALK$^-$ Anaplastic Large Cell Lymphoma and Other Peripheral T-Cell Lymphomas

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We previously identified a relatively high frequency of B-cell proliferations along with simultaneous T-cell receptor γ-chain gene (*TRG*) and immunoglobulin heavy chain gene (*IGH*) rearrangements in a series of angioimmunoblastic T-cell lymphoma and peripheral T-cell lymphoma, unspecified. Here, we report on a series of 74 peripheral T-cell lymphoma (PTCL) cases composed entirely of specific PTCL subtypes, including 28 cases of ALK$^+$ anaplastic large-cell lymphoma (ALCL), 35 cases of ALK$^-$ ALCL, and 11 cases that represent other specific PTCL subtypes. We performed *IGH* and *TRG* gene rearrangement studies and *in situ* hybridization for Epstein-Barr virus (EBV) to determine the frequency of *IGH* clonality and to investigate the relationship between EBV, clonality, and associated B-cell proliferations. Using BIOMED-2 PCR assays, we detected *TRG* clones in 64 of 74 (86%) cases and *IGH* clones in 6 of 74 (8%) cases, with all *IGH*-positive cases exhibiting a concurrent *TRG* clone. Despite the detection of occasional *IGH* clones, there was no correlation between *IGH* clonality and EBV, and B-cell proliferations were not identified in any of the cases. These findings suggest that other factors contribute to *IGH* clonality and demonstrate that, in the absence of an associated B-cell proliferation, *IGH* clonality occurs infrequently (8%) in specific PTCL subtypes. ***(J Mol Diagn 2008, 10:502–512; DOI: 10.2353/jmoldx.2008.080054)***

Peripheral T-cell lymphoma (PTCL) is an uncommon malignancy that accounts for less than 10% of non-Hodgkin lymphomas worldwide. By current World Health Organization criteria, the diagnosis and classification of PTCL is based on the combination of clinical, histologic, immunophenotypic, and genetic findings.[1] However, the diagnosis of PTCL is difficult even for experienced pathologists, given the infrequency of cases, the often unusual histologic features, and perhaps most importantly, the lack of good immunophenotypic markers to assess for clonality in T-lineage neoplasms.

In cases of suspected PTCL, pathologists often evaluate for clonality and assess for lineage by performing PCR-based assays for clonal rearrangement in both the T-cell receptor γ-chain gene (*TRG*) and the immunoglobulin heavy chain gene (*IGH*). Although PCR-based assays for *TRG* typically detect clones in 80 to 90% of PTCL cases, several studies have shown that *IGH* clones are also detected relatively frequently (9 to 16%).[2–6] The detection of an *IGH* clone, with or without a concurrent *TRG* clone, complicates an already challenging diagnosis.

Recently, we investigated the etiology of *IGH* clones in PTCL by studying a large series of two of the most common subtypes of PTCL, angioimmunoblastic T-cell lymphoma (AILT) and PTCL-unspecified (PTCL-U).[7] A subset of cases were complicated by associated B-cell proliferations, a finding that we and others have described as an atypical infiltrate of B cells that is often associated with Epstein-Barr virus (EBV).[8–11] Using multiplex PCRs developed in a European collaborative study (BIOMED-2), we detected *TRG* clones in approximately 80% of cases and *IGH* clones in approximately 35% of cases, with the majority of *IGH* clones detected along with simultaneous *TRG* clones.

Interestingly, a positive *IGH* clone correlated, at least in part, with the presence of a B-cell proliferation, suggesting that B-cell proliferations contribute to *IGH* clonality. However, alternate explanations for the detection of simultaneous *TRG* and *IGH* clones include those that are technical in nature and so-called lineage infidelity. In this context, lineage infidelity refers to recombination of both *TRG* and *IGH* in the same clone. However, this phenomenon is more common in immature hematolymphoid neoplasms and occurs only rarely (<5%) in mature B- and T-cell non-Hodgkin lymphomas.[12–15]

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Regardless of the etiology, our findings raised several important questions. First, it remains unclear whether *IGH* clonality can occur in all PTCL subtypes or whether it is limited to AILT and PTCL-U, which represent the only two subtypes in which B-cell proliferations have been reported. If *IGH* clonality were limited to AILT and PTCL-U, this would suggest that B-cell proliferations are responsible for *IGH* clonality. Alternatively, although B-cell proliferations have not been described in other subtypes, this possibility has not been thoroughly investigated using the combination of immunohistochemical stains and assays for clonality and EBV. If *IGH* clones occur in other PTCL subtypes, they may be the manifestation of subtle or incipient B-cell proliferations that have been overlooked thus far.

To address these issues, we collected a series of 74 PTCL cases composed of subtypes other than AILT and PTCL-U, which corresponds to specific subtypes in which B-cell proliferations have not been reported. This series includes 28 cases of ALK^+ anaplastic large-cell lymphoma (ALCL), 35 cases of ALK^- ALCL, and 11 additional cases representing other specific PTCL subtypes. We assayed all cases for *IGH* and *TRG* clonality, and we performed the experiments under "blinded" conditions to minimize experimental and interpretive bias. In addition, we evaluated cases for the presence of B-cell proliferations using immunohistochemical stains, and we performed *in situ* hybridization for EBV to investigate the relationship between EBV, *IGH* clonality, and possible B-cell proliferations.

Materials and Methods

Cases

Twenty-eight cases of ALK^+ ALCL, 35 cases of ALK^- ALCL, 3 cases of subcutaneous panniculitis-like T-cell lymphoma, 3 cases of enteropathy-type T-cell lymphoma, 1 case of hepatosplenic T-cell lymphoma, 4 cases of T-cell large granular lymphocytic leukemia (T-LGL), and 24 cases of precursor B-cell acute lymphoblastic leukemia/lymphoma (pre-B-ALL) were selected from the Laboratory of Hematopathology, Stanford University Medical Center (Stanford, CA). Cases were received between January 1, 1995 and December 31, 2006, and diagnostic classification for all cases in this study was based on current World Health Organization criteria.[1] Cases of $CD30^+$, ALK^- PTCL that lacked the morphologic features of ALCL as defined by the World Health Organization criteria were classified as PTCL-unspecified and excluded from the study. Cases were selected based on the availability of archived paraffin-embedded tissue or frozen bone marrow cells (decalcified specimens were excluded). The selected materials represented cases in which no previous DNA-based clonality study had been performed, and these cases have not been included in any previously published report, including our recent study on AILT and PTCL-U.[7] All cases showed greater than 10% involvement, and the majority showed >50% involvement. Use of tissue for this study was approved by the Stanford University Panel on Medical Human Subjects [Protocol ID 79034; Institutional Review Board number 348 (Panel 1)].

Histology and Immunohistochemistry

Histologic sections were prepared from formalin-fixed, paraffin-embedded tissue by cutting 3- to 4-μm-thick sections and staining with H&E. Sections were stained for immunohistochemistry on either a BenchMark instrument (Ventana Medical Systems, Tucson, AZ) or Autostainer (Dako, Carpinteria, CA) using the biotin-avidin technique with diaminobenzidine as the chromogen.[16] All cases were stained with at least one B- and one T-cell marker. The following mouse monoclonal antibodies were used: AB75 (anti-CD2; Vision BioSystem, Norwell, MA), IF6 (anti-CD4; Vision BioSystem), 4C7 (anti-CD5; Vision BioSystem), CD7-272 (anti-CD7; Vision BioSystem), C8/144B (anti-CD8; Dako), 56C6 (anti-CD10; Vision BioSystem), MMA (anti-CD15; Ventana), L26 (anti-CD20; Dako), Ber-H2 (anti-CD30; Dako), MY10 (anti-CD34; Becton Dickinson, San Jose, CA), L60 (anti-CD43; Becton Dickinson), ROA6 (anti-CD45RO; Zymed, South San Francisco, CA), JCV117 (anti-CD79a; Dako), ALK1 (anti-ALK; Dako), E029 (anti-EMA; Dako), 24 (anti-PAX5; BD Transduction, Lexington, KY), GrB-7 (anti-granzyme; Dako), and 2G9 (anti-T-cell intracellular antigen-1; Immunotech, Quebec, Canada). The following rabbit polyclonal antibodies were used: anti-CD3 (Cell Marque, Hot Springs, AR) and anti-TdT (Supertechs, Bethesda, MD). Antigen retrieval was performed by using automated heat pretreatment (Ventana) for AB75, IF6, 4C7, CD7-272, C8/144B, 56C6, L26, Ber-H2, MY10, L60, JCV117, ALK1, and anti-CD3 or by using manual heat-induced epitope retrieval (Dako) with an ethylene diamine tetraacetic acid buffer for E029 or citrate buffer for GrB-7, 2G9, 24, and anti-TdT. For MMA and ROA6, staining was performed without antigen retrieval using the BenchMark instrument (Ventana).

Flow Cytometry

Bone marrow aspirate specimens were diluted with 1 volume of Dulbecco's PBS (Sigma, St. Louis, MO), vortexed for 15 seconds, and incubated at 37°C for 20 minutes. After incubation, the mixture was centrifuged at 500 $\times$ *g* for 5 minutes, and the supernatant was discarded. The cells were resuspended in PBS with 20% fetal calf serum (HyClone, Logan, UT), washed three times in PBS, and diluted in PBS to 10^4 cells/μL for staining. An aliquot of cells was resuspended in PBS with 20% fetal calf serum and 20% dimethyl sulfoxide (Sigma) and archived at −70°C for subsequent clonality assays.

For detection of surface antigens, the following four-color antibody combinations were used (fluorescein isothiocyanate/phycoerythrin/peridinin-chlorophyll-protein/allophycocyanin): CD5/CD38/CD45/CD19, lambda/kappa/CD45/CD19, CD20/CD10/CD45/CD19, CD8/CD3/CD45/CD4, CD16/CD3/CD45/CD56, CD57/CD8/CD45/CD3, CD7/CD3/