Hematopathology / CXCL13 and PD-1 Expression in AITL

Germinal-Center T-Helper-Cell Markers PD-1 and CXCL13 Are Both Expressed by Neoplastic Cells in Angioimmunoblastic T-Cell Lymphoma

Hongbo Yu, MD, PhD, Aliakbar Shahsafaei, MS, and David M. Dorfman, MD, PhD

Key Words: Non-Hodgkin lymphoma; CXCL13; PD-1; Angioimmunoblastic lymphoma; T-cell lymphoma

Abstract

Gene expression profiling identified genes uniquely expressed by human germinal-center T-helper (GCTH) cells, including programmed death-1 (PD-1) and CXCL13. Recently, we demonstrated that PD-1 is an immunophenotypic marker of GCTH cells and angioimmunoblastic T-cell lymphoma (AITL). The goal of this study was to investigate the expression pattern of CXCL13 in comparison with PD-1. We studied 63 cases of T-cell lymphoproliferative disorders, including 22 cases of AITL. In cases of AITL, PD-1+ and CXCL13+ neoplastic cells were seen at foci of expanded CD21+ follicular dendritic cell networks. CXCL13 expression was limited in other peripheral T-cell lymphomas. PD-1 and CXCL13 identified germinal-center T-helper cells, showed a similar pattern of expression in AITL, and should serve as useful new markers for AITL. The similar pattern of expression of CXCL13 and PD-1 in AITL provides further evidence that AITL is a neoplasm derived from germinal-center T-helper cells.

Angioimmunoblastic T-cell lymphoma (AITL) is a peripheral T-cell lymphoma characterized by systemic disease, a polymorphous infiltrate involving lymph node, with a prominent proliferation of high endothelial venules and follicular dendritic cells (FDCs). The architectural changes in AITL fall into 3 overlapping patterns: I, preservation of the lymph node architecture with hyperplastic follicles with poorly developed mantle zones and ill-defined borders; II, loss of architecture with occasional depleted follicles with concentrically arranged FDCs; and III, completely effaced architecture with no B-cell follicles. The differential diagnosis of AITL is broad and includes atypical reactive processes, Hodgkin lymphoma, B-cell lymphomas, and other peripheral T-cell lymphomas. Because the morphologic features and immunophenotypic profile of AITL can overlap with a variety of neoplastic and reactive processes, the diagnosis of AITL may be delayed in a significant number of cases until more definitive morphologic features develop or molecular studies for T-cell clonality are pursued owing to aggressive clinical manifestations. Thus, further characterization of the immunophenotypic features of AITL may help to improve recognition of this important diagnosis.

Gene expression profiling identified genes uniquely expressed by human germinal-center T-helper (GCTH) cells compared with other T-cell subsets, including programmed death-1 (PD-1), a member of the CD28 receptor family that regulates the cellular immune response, and CXCL13, a critical chemokine for B-cell entry to lymphoid follicles. Recently, we demonstrated that PD-1 is a marker of GCTH cells and AITL, but not other peripheral T-cell lymphomas. The goal of the present study was to investigate the expression pattern of CXCL13 in comparison with PD-1 in AITL and other peripheral T-cell lymphomas.
Materials and Methods

Cases were retrieved from the surgical pathology files of Brigham and Women’s Hospital, Boston, MA. We studied 63 cases of T-cell lymphoma/leukemia, including 22 AITL, 12 anaplastic large cell lymphoma (ALCL), 10 peripheral T-cell lymphoma, unspecified (TCL), 7 NK/T-cell lymphoma, 7 precursor T-lymphoblastic lymphoma/leukemia, and 5 T-cell prolymphocytic leukemia (T-PLL). All diagnoses were based on the histologic and immunophenotypic features described in the World Health Organization classification system, and, in all cases, diagnostic material was reviewed by 2 hematopathologists (H.Y. and D.M.D.). Some of the cases were used in our previous study. A tonsil specimen showing reactive lymphoid hyperplasia was also included in this study as a control sample.

CXCL13 monoclonal antibody 53610 was obtained from R&D, Minneapolis, MN. PD-1 antibody (EH12) was generated by immunization of mice with recombinant human PD-1 fusion protein. Spleen cells were fused with SP2/0 myeloma cells, cloned, and hybridoma supernatants screened by cell surface staining of PD-1 transfected 300.19, Jurkat, and CHO cells and for lack of reactivity with vector alone transfected cells. Clone EH12 (mouse IgG1) was chosen for further analysis based on its capacity to stain paraffin-embedded tissue. Antibodies for CD3 and CD20 (L26) were obtained from DakoCytomation, Carpinteria, CA. CD4 antibody (4B12) was obtained from Vector Laboratories, Burlingame, CA, and CD21 (BU32) was obtained from The Binding Site, Birmingham, England.

Immunohistochemical studies were performed on formalin-fixed, paraffin-embedded tissue sections following microwave heat-induced epitope retrieval in 10 mmol/L of citrate buffer, pH 6.0, for PD-1 monoclonal antibody EH12, CD3, CD21, and CD4 or 1 mmol/L of EDTA buffer, pH 8.0, for CXCL13 monoclonal antibody 53610, using a standard indirect avidin-biotin horseradish peroxidase method and diaminobenzidine color development. Immunostaining for CD20 was performed as above, except no antigen retrieval was used. The pattern and intensity of immunoreactivity were evaluated for both markers. All cases, including the ones reported previously, were stained with PD-1 and CXCL13 for this study. Cases were regarded as immunoreactive for CXCL13 and PD-1 if at least 20% of neoplastic cells exhibited positive staining.

For 1 representative AITL case and 1 specimen of tonsillar lymphoid tissue, 2-color immunostaining was performed using Permanent Red (DAKO, Carpinteria, CA) as an additional reagent for color development, in addition to diaminobenzidine.

This study was approved by the Brigham and Women’s Hospital Institutional Review Board (Boston, MA).

Results

CXCL13 and PD-1 Immunostaining in Reactive Lymphoid Tissue

In a tonsil specimen exhibiting reactive changes [Image 1A], including follicular hyperplasia, the germinal centers were mainly composed of CD20+ B cells [Image 1B], and the interfollicular areas were mainly composed of CD3+ T cells [Image 1C]. CD4+ T cells were seen in the germinal centers and in the interfollicular areas [Image 1D]. The intact follicular dendritic meshwork was highlighted by stain for CD21 [Image 1E]. As previously demonstrated, a subset of T cells in the germinal centers was reactive for PD-1 with cell surface and cytoplasmic staining patterns [Image 1F]. Here, we demonstrated that CXCL13 also highlighted a subset of small lymphocytes in the germinal centers with cytoplasmic staining with a perinuclear dot pattern [Image 1G]. Double immunostaining for PD-1 and CD21 showed similar distributions of these 2 markers but separate populations of positively staining cells [Image 1H]. However, double immunostaining for PD-1 and CD4 [Image 1I] and double immunostaining for CXCL13 and CD4 [Image 1J] showed a subpopulation of dually stained cells. The CXCL13 staining pattern within germinal centers was virtually identical to that seen with PD-1, as demonstrated by double staining for PD-1 and CXCL13 showing a population of dually stained cells [Image 1L].

CXCL13 and PD-1 Immunostaining in T-Cell Lymphoproliferative Disorders

We studied a range of T-cell lymphoproliferative disorders for CXCL13 and PD-1 expression. The results are summarized in Table 1.

All 22 cases of AITL were immunoreactive for pan-T-cell markers such as CD3 and CD4, and all contained foci of CXCL13+ cells, which represented more that 20% of CD3+ T cells in lesional tissue. In contrast, of 41 cases of other T-cell lymphoproliferative disorders, only 1 case (1/12 [8%]) of ALCL and 1 case (1/7 [14%]) of NK/T cell lymphoma showed focal reactivity for CXCL13. All cases of TCL, precursor T-lymphoblastic lymphoma/leukemia, and T-PLL were negative for CXCL13. In some cases, nonneoplastic reactive lymphoid tissue was present and showed a CXCL13 staining pattern as seen in tonsil and other reactive lymphoid tissue noted previously (data not shown). We also performed PD-1 immunostaining simultaneously on the aforementioned cases, and the results are also summarized in Table 1. All 22 cases of AITL showed reactivity for PD-1; only 1 case (1/10 [10%]) of TCL and 1 case (1/7 [14%]) of precursor T-lymphoblastic lymphoma/leukemia showed focal reactivity for PD-1. All cases of ALCL, NK/T cell lymphoma, and T-PLL were negative for PD-1.
Image 1. PD-1 and CXCL13 immunostaining in tonsil. A, Tonsil with follicular hyperplasia and germinal center formation. B, CD20 highlights numerous CD20+ B cells in germinal centers. C, CD3+ T cells are located mainly in the interfollicular area. D, CD4 highlights interfollicular T cells and some cells in the germinal centers. E, CD21 highlights intact follicular dendritic meshwork. F and G, Stains for PD-1 (F) and CXCL13 (G) highlight the vast majority of germinal center–associated T cells, but also few T cells in the interfollicular T-cell zone.
Image II (cont) H, Double immunostaining for PD-1 (brown) and CD21 (red) shows similar distributions of these 2 markers but separately stained cells. I, Double immunostaining for CXCL13 (brown) and CD21 (red) shows similar distributions of these 2 markers but separately stained cells. J, Double immunostaining for PD-1 (brown) and CD4 (red) shows dually stained cells. K, Double immunostaining for CXCL13 (brown) and CD4 (red) shows dually stained cells. L, Double immunostaining for CXCL13 (brown) and PD-1 (red) shows dually stained cells (A-K, ×200; L, ×400).
In general, in AITL, CXCL13+ and PD-1+ cells were consistently found at foci of expanded CD21+ FDC networks, a characteristic feature of AITL. Image 2A shows a representative case of AITL showing vascular proliferation and numerous, atypical, intermediate-sized lymphoid cells with clear cytoplasm (Image 2A). CD20 highlighted rare B cells (Image 2B), whereas CD3 highlighted numerous T cells (Image 2C). CD4 also was reactive in the majority of T cells (Image 2D). An expanded CD21+ FDC meshwork was associated with the neoplastic T-cell proliferation (Image 2E). A significant subset of T cells expressed PD-1 (Image 2F) and CXCL13 (Image 2G). The staining pattern of CXCL13 was mainly cytoplasmic with a perinuclear dot distribution (Image 2G, inset). Two-color immunostaining was performed on a representative case of AITL to further characterize CXCL13+ and PD-1+ cells. As shown in Images 2H and 2I, CXCL13+ cells and PD-1+ cells showed similar distributions when compared with the follicular dendritic meshwork highlighted by CD21, but no dual-positive CXCL13/CD21 or PD-1/CD21 cells were seen. However, double staining for CD4/PD-1 and CD4/CXCL13 revealed subsets of dually stained cells in both cases (Images 2J and 2K). Double staining for CXCL13 and PD-1 also showed that essentially the same subset of T cells was highlighted by both markers (Image 2L).

In the staining of AITL in general, anti-CXCL13 antibody stained neoplastic cells in a pattern similar to that observed for PD-1. However, the staining intensity varied between CXCL13 and PD-1, with 5 cases showing similar intensity for both markers, 9 cases showing stronger CXCL13 reactivity, and 8 cases showing stronger PD-1 reactivity Image 3I. The basis for the variation in staining intensity of these 2 markers in cases of AITL is not clear.

Discussion

Neoplastic cells in the majority of cases of AITL have been reported to express CD10, a marker of germinal-center B cells and B-cell precursors. Attygalle and coworkers identified CD10 as an immunophenotypic marker of neoplastic T cells in AITL lymphoma in 87% of cases and found that CD10 was not expressed in other peripheral T-cell lymphomas. CD10 coexpression was also demonstrated in neoplastic T cells in 6 of 8 AITL cases by flow cytometric analysis. Similarly, Ree and coworkers reported that bcl-6 protein, a transcription factor involved in B-cell proliferation and differentiation characteristically expressed by germinal-center B cells and the neoplastic cells in follicular lymphoma, was expressed by a subset of neoplastic CD3+ T cells in AITL. Both CD10 and bcl-6 are well-known as markers of follicle center B cells. Therefore, their expression on neoplastic cells in AITL confers an immunophenotype that is unique among T cells and suggests that the neoplastic cells in AITL may be derived from a unique, possibly germinal center–associated T-cell population. Jones et al showed that FDCs characteristically proliferate and are associated with the neoplastic cells in cases of AITL, which also suggests that the cell of origin of AITL may be closely associated with FDCs and possibly of germinal-center origin.

Recently, Chtanova and coworkers used gene expression array analysis to identify transcription factors, cytokines, and cell surface molecules that underlie the differentiation pathways and functional properties of a T-follicular helper cell subset. PD-1 and CXCL13 were both identified as unique markers for this subset of T cells and were not expressed at significant levels in other T-cell subsets. Kim and coworkers also reported a gene expression profile of germinal-center T-helper cells using complementary DNA microarray analysis and identified genes differentially expressed by the germinal-center T-helper cells compared with other T-cell subsets. They found that GCTh cells displayed substantial differences in messenger RNA for adhesion molecules, chemoattractant receptors, and cytokines compared with other populations. CXCL13, a chemokine specifically expressed in lymphoid follicles and required for B-cell entry into germinal centers, was found to be one of the most highly up-regulated genes in this subset of T cells.

Recently, we reported that PD-1 was expressed by neoplastic cells in AITL and by T cells associated with neoplastic B cells in nodular lymphocyte predominant Hodgkin lymphoma. PD-1 is a member of the CD28 family of receptors that includes CD28, cytotoxic T lymphocyte–associated antigen 4, inducible costimulator, and B- and T-lymphocyte attenuator, which have a
Image 2 PD-1 and CXCL13 immunostaining in angioimmunoblastic T-cell lymphoma. A, Lymph node involved by angioimmunoblastic T-cell lymphoma showing vascular proliferation and numerous intermediate-sized lymphoid cells with clear cytoplasm. B and C, CD20 highlights rare B cells (B), whereas CD3 highlights numerous T cells (C). D, CD4 also is reactive in the majority of T cells. E, Expanded CD21+ follicular dendritic cell meshwork is associated with the neoplastic T-cell proliferation. F and G, A significant subset of T cells expresses PD-1 (F) and CXCL13 (G).
Image 2 (cont) G (inset), A perinuclear dot pattern of CXCL13 is evident. H, Double immunostaining for PD-1 (brown) and CD21 (red) shows similar distributions of these 2 markers but separately stained cells. I, Double immunostaining for CXCL13 (brown) and CD21 (red) shows similar distributions of these 2 markers but separately stained cells. J, Double immunostaining for PD-1 (brown) and CD4 (red) shows dually stained cells. K, Double immunostaining for CXCL13 (brown) and CD4 (red) shows dually stained cells. L, Double immunostaining for CXCL13 (brown) and PD-1 (red) shows dually stained cells. (A-L, ×400; inset in G, ×1,000).
CXCL13 and PD-1 show variable staining intensity among different cases of angioimmunoblastic T-cell lymphoma, with some cases showing stronger staining for CXCL13 (A) than for PD-1 (B), some cases showing stronger PD-1 staining (D) than for CXCL13 (C), and some cases showing the same intensity for CXCL13 (E) and PD-1 (F) (A-F, ×400).
role in regulating the cellular immune response.\textsuperscript{15,16} PD-1 is also expressed on activated T cells, B cells, and myeloid cells.\textsuperscript{17}

In the present study, we compared the expression of PD-1 and CXCL13 in angioimmunoblastic lymphoma and other peripheral T-cell lymphomas. We found that PD-1 and CXCL13 had similar expression patterns in reactive lymphoid tissue and in AITL. Neither was significantly expressed in other types of peripheral T-cell lymphoma. The expression pattern of CXCL13 was slightly different from that of PD-1. PD-1 expression was on the cell surface and cytoplasmic, whereas CXCL13 reactivity was mainly cytoplasmic with a perinuclear dot pattern. In reactive lymphoid tissue, PD-1 and CXCL13 expression was seen in a small subset of T cells mainly located in the germinal centers, with only occasional cells in the interfollicular areas, as would be expected for germinal center T-helper cells. In cases of AITL, the PD-1+ and CXCL13+ cells were mainly confined within the expanded follicular dendritic meshwork highlighted by CD21, as might be expected of germinal center T-helper cell–derived neoplastic cells. Double staining experiments showed that PD-1 and CXCL13 were not expressed by CD21+ FDCs but were expressed in a subset of CD4+ T cells. PD-1 and CXCL13 dual staining was seen in this T-cell population, indicating that they highlight the same population of neoplastic T cells. Previously, we showed that PD-1 staining in AITL was present in a distribution similar to that of CD10+ neoplastic T cells and bcl-6+ cells.\textsuperscript{8} Therefore, expression of both markers, CXCL13 and PD-1, further supports the hypothesis that AITL is derived from follicular or germinal center–associated T cells.

Recently, Grogg and coworkers\textsuperscript{18} reported that 31 of 35 cases of AITL and only 2 of 20 peripheral T-cell lymphoma cases showed CXCL13 expression in a pattern similar to that seen for CD10. Fourteen cases of paracortical lymphoid hyperplasia cases were negative for CXCL13 expression,\textsuperscript{18} suggesting that CXCL13 is a useful marker of AITL and that the GCTh cell is the possible origin of this neoplasm.\textsuperscript{18}

We have demonstrated that PD-1 and CXCL13 identify GCTh cells and show a similar pattern of expression in AITL, without significant staining in other T-cell lymphoproliferative disorders. Both PD-1 and CXCL13 should serve as useful new markers for AITL, in addition to CD10 and bcl-6. The similar pattern of expression of CXCL13 and PD-1 in AITL provides further evidence that AITL is a neoplasm derived from germinal center–associated T-helper cells. The intensities of staining for PD-1 and CXCL13 vary in a subset of AITL cases, suggesting that the use of both markers may improve the sensitivity of detection of a germinal center T-helper cell–derived cell population in cases of AITL.

References


