

SHORT COMMUNICATION

Neoplastic Cells of Primary Cutaneous CD4⁺ Small/Medium-sized Pleomorphic T-cell Lymphoma Lack the Expression of Follicular T-helper Cell Defining Chemokine Receptor CXCR5Dóra Krenács^{1,2}, Annamária Bakos^{1,2}, László Török³, Lajos Kocsis⁴, Enikő Bagdi¹ and László Krenács^{1*}¹Laboratory of Tumor Pathology and Molecular Diagnostics, Jobb fasor 23/B, HU-6726 Szeged, ²2nd Department of Internal Medicine, Faculty of Medicine, University of Szeged, Szeged, Departments of ³Dermatology and ⁴Pathology, County Hospital, Kecskemét, Hungary. *E-mail: krenacs1@vipmail.hu Accepted Feb 29, 2016; Epub ahead of print Mar 1, 2016

Primary cutaneous CD4⁺ small/medium T-cell lymphoma (PCSM-TCL), a provisional peripheral T-cell lymphoma entity of the WHO-EORTC classification, is defined by the predominance of small-to-medium-sized CD4⁺ pleomorphic T cells without the features of mycosis fungoides (1–4), and is characterized by a non-epidermotropic polymorphic infiltrate presenting clusters of follicular T-helper (T_{FH}) cell marker-positive atypical T cells (5–7).

We report here that cluster-forming PD-1⁺ medium-to-large-sized T cells are the neoplastic cells of PCSM-TCL; furthermore, these cells demonstrate cerebriform nuclei and lack the expression of T_{FH}-cell defining C-X-C chemokine receptor CXCR5.

METHODS (See Appendix S1¹)

RESULTS

Patients' data and the results are summarized in Table S1¹. None of the 13 patients showed evidence of systemic dissemination within the follow-up periods of 1–36 months (median 7.5 months). All but one patient presented with lesions on the head and neck (Fig. 1A) or trunk with stable solitary lesions in 10 patients.

Morphologically, all cases displayed a dense polymorphous non-epidermotropic infiltrate (Fig. 1B) predominated by small-to-medium-sized CD3⁺/CD4⁺ T cells, which extended into the subcutaneous fat in 3 cases. Up to 40% of the lesional cells and up to 60% of the CD3⁺ cells demonstrated intense PD-1 positivity (Fig. 1C). Scattered mitotic cells were encountered exclusively in the PD-1⁺ populations. In general, the PD-1⁺ cells formed clusters and constituted medium-to-large-sized T cells with hyperconvoluted cerebriform nuclei (Fig. 1B–D).

In the context of T-cell clonality analysis, unequivocal dominant TCRγ product was detected in 77% (10/13) of cases when whole-section DNA extracts were tested, whereas all (10/10) tested cases revealed monoclonal pattern using microdissected samples (Table S1¹).

Neoplastic cells, recognized as PD-1⁺ cluster forming cells, were consistently CXCR5-negative (Fig. 1D, F, G), while displaying BCL6, CXCL13, and ICOS positivity in the great majority of cases. The reactive T_{FH} cells of the hyperplastic lymphoid follicles (in 3 cases

of PCSM-TCL and in the control tonsils) as well as the neoplastic cells of the control AITL and primary cutaneous T_{FH} cell lymphoma cases demonstrated unequivocal T_{FH} cell antigen positivity, including a robust CXCR5 staining (Fig. 1D, E).

Up to 40% CD20⁺ B cells were found in all lesions studied, comprising 2 populations, CXCR5⁺/CD21⁺ small B lymphocytes mostly arranged in sheets and scattered large B cells with variable CD30 positivity, both in close vicinity to PD-1⁺ T cells (Fig. 1F, G). The consistent presence of CXCR5⁺ small B cells also served as an endogenous positive control for this marker. Double staining revealed that no lesional B cells expressed BCL6 and MEF2B (10), indicating the lack of germinal centre (GC) B cells within the lesions. In addition, CD21 and CXCL13 failed to demonstrate follicular dendritic cell (FDC) meshwork within the lesional infiltrates.

DISCUSSION

This study assessed the characteristics of the proposed neoplastic cells of PCSM-TCL, performing detailed immunomorphological and clonality analysis in 13 cases.

The clinicopathological characteristics of the cases studied here were in accordance with previous reports (3–7) and support the indolent course of PCSM-TCL.

PCSM-TCL has been reported consistently to present rosette or cluster forming PD-1⁺ atypical cells (5, 7). The current study established that these cluster forming PD-1⁺ cells are medium- to-large cells with hyperconvoluted cerebriform nuclei (Fig. 1B, C), which latter feature has not been observed previously in PCSM-TCL.

Although PD-1⁺ atypical cells have been proposed as the neoplastic cells of PCSM-TCL (5, 7), T-cell clonality has not previously been assigned to them. Using microdissection, we demonstrated that T-cell clonality is linked to the PD-1⁺ cells, supporting that these are the neoplastic cells of this lymphoma entity.

T_{FH} cells are crucial in humoral immunity; they express PD-1, BCL6, ICOS and CXCL13, together with the highest levels of CXCR5, a receptor which defines follicular homing (11, 12). Due to the expression of PD-1, BCL6, ICOS and CXCL13, PCSM-TCL has been suggested to be a T_{FH}-cell-derived neoplasm (5–7), nevertheless, CXCR5 has not yet been tested on it. Our findings are in accordance with previous studies regar-

¹<http://www.medicaljournals.se/acta/content/?doi=10.2340/00015555-2386>

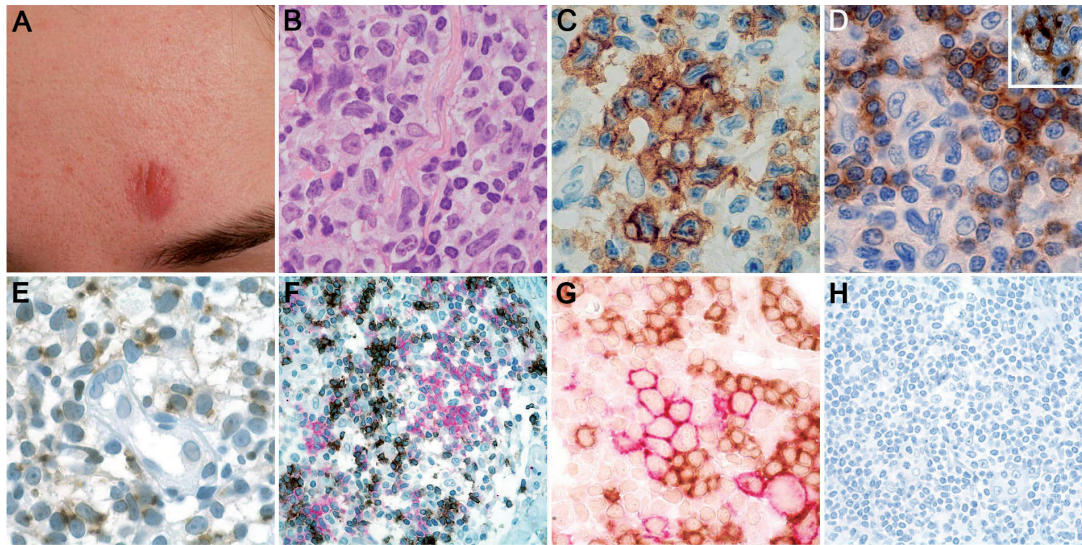


Fig. 1. Phenotypic characteristics of primary cutaneous CD4⁺ small/medium T-cell lymphoma. (A) Typical solitary erythematous macule presenting on the forehead (patient 6). (B) Characteristic polymorphous infiltrate containing medium- to-large-sized cells with hyperconvoluted nuclei (haematoxylin and eosin (H&E), original magnification $\times 100$). (C) PD-1⁺ neoplastic cells (brown) arranged in a cluster and demonstrating cerebriform nuclei ($\times 100$). (D) CXCR5-negative cluster of neoplastic cells with cerebriform nuclei surrounded by CXCR5⁺ lymphocytes ($\times 100$). *Inset:* Neoplastic cells of a control angioimmunoblastic T-cell lymphoma case show strong CXCR5 positivity ($\times 100$). (E) Neoplastic cells of the primary cutaneous T_{FH}-cell lymphoma case show CXCR5 positivity ($\times 100$). (F) Double immunostaining shows that the PD-1⁺ neoplastic cells (red) and the CXCR5⁺ lymphocytes (brown) are distinct populations ($\times 20$). (G) Double immunostaining (DI) demonstrates that the PD-1⁺ neoplastic cells (red) adjacent to the CXCR5⁺ lymphocytes (brown) lack the CXCR5 (original magnification $\times 100$, no counterstaining). (H) Negative control of CXCR5+PD-1 DI ($\times 20$).

ding the commonly used T_{FH} markers (5–7); however, the consistent lack of CXCR5 in the neoplastic cells, in conjunction with the absence of FDCs and GC B cells within the lesional infiltrate, argues against T_{FH}-cell derivation. PCSM-TCL may represent the neoplastic counterpart of a unique T helper-cell population that provides help to B cells outside the follicles (11, 12). Unlike T_{FH} cells, these extrafollicular BCL6⁺/PD-1⁺/ICOS⁺ T helper cells do not express CXCR5 (12).

This study established that the neoplastic cells of PCSM-TCL are medium- to-large-sized T cells with cerebriform nuclei, which lack the expression of T_{FH}-cell defining chemokine receptor CXCR5. These features are highly distinctive and can be invaluable in separating this rather indolent cutaneous T-cell lymphoid neoplasm from more aggressive lymphomas of T_{FH}-cell phenotype, including primary cutaneous T_{FH}-cell lymphoma (13) and skin involvement of AITL, which should be CXCR5⁺ (14, 15).

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