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Chemokine receptor repertoire reflects mature T-cell lymphoproliferative disorder clinical presentation

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ABSTRACT

The World Health Organization classification of mature T-cell lymphoproliferative disorders, combines clinical, morphological and immunophenotypic data. The latter is a major contributor to the classification, as well as to the understanding of the malignant T-cell behavior.

The fact that T-cell migration is regulated by chemokines should, in theory, enable us to identify tissue tropism and organ involvement by neoplastic T-cells by monitoring chemokine receptor surface expression. To address this issue we compared the expression of several early and late inflammatory, homeostatic, and organ specific chemokine receptors on blood T-cells from normal individuals and patients with T-cell large granular lymphocytic leukemia and peripheral T-cell lymphoma.

T-cell large granular lymphocytic leukemia cells mainly express late inflammatory chemokine receptors (CXCR1 and CXCR2), whereas peripheral T-cell lymphoma cells usually express one or more organ homing receptors (CCR4, CCR6 and CCR7). Nevertheless, no clear correlation was found between CCR4 and CCR7 expression and skin and lymph node involvement, respectively. Compared to their normal counterparts, lymphoma T-cells displayed an exaggerated CCR4 expression, whereas leukemic T-cells had abnormally high CXCR1 and CXCR2 expression.

Further analysis revealed that, in leukemia patients, the percentage of neoplastic cells expressing CCR5 correlates directly with lymphocytosis. In addition, in the case of CD8 T-cell leukemia patients, an inverse correlation with neutropenia was found. In lymphoma patients, higher CCR4 and CCR7 expression is accompanied by lower to absent CCR5 expression.

Introduction

T-cell lymphoproliferative disorders (LPD) are uncommon diseases, arising from an abnormal proliferation or accumulation of neoplastic mature T-cells. They are broadly divided into leukemia or lymphoma depending on their presentation at the time of diagnosis. The World Health Organization (WHO) classifies mature T-cell neoplasms according to clinical, morphological and phenotypic characteristics. Some of the most representative groups of T-cell LPD are the large granular lymphocytic leukemia (LGLL) and the peripheral T-cell lymphoma (PTCL).

PTCLs are usually sub-classified into nodal and extra-nodal lymphomas [1]. While the former affect the lymph nodes at diagnosis, the latter is evident in other organs and tissues, such as the skin, the gastrointestinal and the respiratory tract. Most nodal and extra-nodal cutaneous T-cell lymphoma cases arise on T-cell receptor α/β^+ $CD4^+$ T-cells, and cutaneous T-cell lymphomas account for the majority of extra-nodal PTCLs [2].

T-cell LGLL, one of the most common T-cell leukemias, is characterized by the proliferation of monoclonal large granular lymphocytes (LGL) in the blood, usually of the CD8 subset, and is diagnosed mainly based on the morphologic and phenotypic characteristics of the monoclonal T-cell population, including the absence of the CD28 co-stimulatory receptor, the expression of NK-cell associated molecules and the presence of cytotoxic granules containing perforin and granzymes [3,4]. $CD8^+$ T-cell LGLL usually is accompanied by neutropenia and other cytopenias, whereas $CD4^+$

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T-cell LGLL frequently is associated with other hematological or non-hematological neoplasms [3]. Except for splenomegaly, which is relatively frequent in patients with $CD8^+$ T-cell LGLL, other organ enlargement is rarely observed and, the marrow and spleen are the principal sites of accumulation of LGLL T-cells [4]. Unlike T-cell prolymphocytic leukemia [5], LGLL doesn't display any recurrent cytogenetic abnormality, nor does it usually result in high blood lymphocyte counts [6].

These diverse characteristics of T-cell diseases make the differential diagnosis difficult and demand for a different and better approach to classification.

Over the years, the classification systems have evolved from purely morphologic systems combined with prognostic categories (Working Formulation) to classification systems which integrate clinical, biological and laboratorial data (REAL, WHO and EORTC classifications) [7,8].

T lymphoblastic lymphoma/leukemia originates from the stages of T-cell differentiation in the thymus and peripheral T-cell neoplasms originate from post-thymic mature T-cells. T-cell prolymphocytic leukemia is thought to originate from naive T-cells whereas PTCL and LGLL originate from various stages of antigen-dependent T-cell activation, with LGLL resembling the phenotype of terminally activated cytotoxic T-cells [7]. The fact that the normal blood T-cell population is a mixture of cells in different activation stages, from naïve to effector, in conjunction with their resemblance to pathological T-cells, makes the identification of neoplastic T-cells difficult [9]. The same line of thought applies to migration in that lymphoid neoplasms should follow the homing patterns of their normal counterparts, despite the fact that no clear correlation has yet been made between the CR repertoire and cell activation on normal T-cells.

To test this hypothesis we looked at chemokine receptor (CR) expression, since T-cell traffic can be indirectly assessed by the expression of these CRs [10,11].

For this, we compared the expression of organ specific (CCR4, CCR6 and CCR7) [12–14], early inflammatory, Th1 related (CCR5 and CXCR3) [15,16] late inflammatory (CXCR1 and CXCR2) [17], and homeostatic (CXCR4) [18] CRs between the abnormal peripheral blood (PB) T-cells from patients with $CD4^+$ PTCL and both $CD4^+$ and $CD8^+$ LGLL patients. We also compared the CR repertoire from the abnormal T-cells of these LPD with normal PB T-cells with an equivalent phenotype.

Materials and methods

Patients and controls

The CR repertoire was studied on the abnormal PB T-cells from 18 $CD4^+$ PTCL, 10 $CD4^+$ LGLL and 18 $CD8^+$ LGLL patients and twelve normal age-matched adult individuals (blood donors) were used as controls.

All patients had normal hemoglobin values (11–13 g/dL), as well as normal platelet counts ($150–300\times10^3/\mu L$). White cell counts were usually high ($5–25\times10^3/\mu L$) due to lymphocytosis caused by T-cell proliferation. The majority of PTCL patients had either lymph node or skin infiltration by the neoplastic T-cells. The percentage of abnormal T-cells per total lymphocytes was always above 15%.

T-cell LPD was diagnosed and classified based on clinical and laboratorial data according to the WHO schema [7]. Abnormal T-cell identification was accessed by aberrant expression of cell-surface markers by flow cytometry [3]. T-cell clonality was accessed by at least one of the two following methods: a) expression of a single family of the TCR beta chain accessed by flow cytometry [3] and, or b) monoclonal TCR gamma and/or beta chain genes rearrangement accessed by molecular PCR-based studies [19].

Samples

PB samples were collected on EDTA-K3 containing tubes.

Four-color flow cytometry

Immunophenotypic analysis of surface antigen expression on PB T-cells was performed in all cases using a stain-and-then-lyse four-color direct immunofluorescence technique, as previously described in detail [3]. Briefly, 100 μl of whole blood containing $1–2\times10^6$ cells was incubated for 15 min at room temperature with antigen-specific fluorochrome-conjugated monoclonal antibodies (mAb), followed by erythrocyte lysis and cell fixation using FACS lysing solution (Becton-Dickinson BioSciences, BD, San José, CA USA), according to manufacturer instructions. The mAb indicated in Table 1 were used in different combinations in order to ensure the best selection of the abnormal T-cell population. In accordance, allophycocyanin conjugated anti-CD4 or anti CD8 mAb (for $CD4^+$ and $CD8^+$ T-cell LPD, respectively) were combined with Phycoerythrin (PE) conjugated anti-CR mAb in all cases. The specificity of the other mAb used was variable, depending on the best combination to identify the phenotypically abnormal T-cells in blood. In accordance, Cy5-Phycoerythrin (PC5) conjugated anti-CD28 and fluorescein (FITC)-conjugated anti-CD27 mAb were used in all LGLL cases, whereas Peridin–chlorophyll–protein complex. (PerCP) conjugated anti-CD3 and FITC conjugated anti-CD7 mAb was the preferred combination to identify PTCL cells. $CD8^+$ LGLL cells were further subdivided into three groups according to their CD11c and CD57 expression pattern ($CD11c^+CD57^-$, $CD11c^+CD57^+$ and $CD11c^-CD57^+$).

$CD4^+$ and $CD8^+$ LGLL cells were selected based on the absence of CD27 and CD28 expression whereas $CD4^+$ PTCL cells were identified based on their abnormal light scatter characteristics and abnormal levels of CD3 and/or CD7 expression.

All experiments were done on a FACSCalibur cytometer (BD Biosciences), using CellQuest software version 3.1 (BD Biosciences) for sample acquisition and Paint-a-gate Pro software (BD Biosciences) for data analysis.

Comparison between neoplastic T-cells and their normal counterparts

Normal terminally activated $CD4^+CD27^-CD28^-$ and $CD8^+CD27^-CD28^-$ T-cells were considered as the normal counterparts for $CD4^+$ and $CD8^+$ LGLL T-cells.

$CD4^+$ PTCL cells have no clear homology to any specific subset of normal PB $CD4^+$ T-cells except for the fact that they are always $CD28^+$ and usually express activation-related molecules [20]. Considering these two aspects normal PB $CD4^+CD28^+CD45RO^+$ T-cells were used as normal counterparts for $CD4^+$ PTCL cells in this study.

Table 1
Specificities, clones and sources of the monoclonal antibodies used

Specificity	Clone	Conjugate[a]	Source[b]
CD3	SK7	PerCP	BD
CD4	SK3	APC	BD
CD7	CLB-3A1/1,7F3	FITC	CLB
CD8	SK1	APC	BD
CD11c	SHCL-3	PE	BD
CD27	9F4	FITC	CLB
CD28	CD28.2	PC5	IOT
CD57	HNK-1	FITC	BD
CCR4	1G1	PE	PH
CCR5	3A9	PE	PH
CCR6	11A9	PE	PH
CCR7	3D12	PE	PH
CXCR1	5A12	PE	PH
CXCR2	6C6	PE	PH
CXCR3	1C6	PE	PH
CXCR4	12G5	PE	PH

[a] APC — Allophycocyanin; FITC — fluorescein isothiocyanate; PC5 — Cy5-Phycoerythrin; PerCP — Peridin–chlorophyll–protein complex.
[b] IOT — Immunotech, Marseille, France; BD — Becton-Dickinson, San José, CA, USA; CLB — Sanquin, Amsterdam, The Netherlands; PH — Pharmingen, San Diego, CA, USA.