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CASE REPORT

T-cell prolymphocytic leukemia and tuberculosis: a puzzling association

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Key Clinical Message

T-cell prolymphocytic leukemia can result in severe immune T-cell deficiency. Clinicians should be aware of this complication in this rare lymphoid malignancy, and opportunistic infections should be ruled out before the use of usual immunosuppressive procedures such as alemtuzumab and hematopoietic stem cell transplantation.

Keywords

Immune deficiency, JAK3, T-cell prolymphocytic leukemia, tuberculosis.

Introduction

T-cell prolymphocytic leukemia (T-PLL) is a rare lymphoid malignancy characterized by a proliferation of mature CD4+ T-cell lymphocytes. Diagnosis is based on cytologic analysis that identifies lymphoid cells with intermediate size, irregular nuclear contours, condensed chromatin, prominent round to oval single nucleoli, and basophilic cytoplasm with cytoplasmic blebs. By immunophenotyping, lymphocytes express pan-T antigens CD2, CD3, CD5, and CD7, as well as CD4 antigen and the targetable CD52 antigen. Conventional cytogenetic study analysis typically reveals inversion of chromosome 14 [inv(14)] or t(14;14) and/or rearrangement of the *TCL1* gene by FISH studies. Abnormalities of chromosome 8, 12p, and deletions of the long arm of chromosomes 5, 6, 11, and 13 are also common [1, 2]. Prognosis is dismal, and the recommended management includes front-line treatment with the anti-CD52 monoclonal antibody alemtuzumab (Campath®, Genzyme, Cambridge, MA, US) followed by hematopoietic stem cell transplantation [3]. More recently, activating mutations on the JAK (Janus kinase)/STAT (signal transducer and activator of transcription) signaling pathway were reported, opening perspectives of targeted therapies [4, 5].

Immune cellular deficiency is not a usual complication of lymphoid malignancies of T-cell origin, with the exception of patients with adult HTLV-1 T-cell leukemia/lymphoma (ATL) who are prone to develop opportunistic parasitic infections as a result of increased CD4+/Foxp3+ cells [6]. So far, no opportunistic infections were directly attributed to T-PLL. Here, we report an informative patient who presented concomitantly a T-PLL associated with asymptomatic tuberculosis. Given the risk of exacerbation of tuberculosis infection under alemtuzumab, clinicians should be aware about this possible association.

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Methods

All experiments performed with the patient's lymphocytes were carried out before treatment. Informed consent was obtained from the patient and the study complied with the human experimentation guidelines of Saint-Antoine Hospital.

Samples

Mononuclear cells were obtained after ficoll separation and cryopreserved at the tumor bank of Saint-Antoine Hospital, Paris. Fresh sample was used for molecular exploration. The blood cells were sorted to isolate the CD3+ fraction. Negative selection of B lymphocytes was performed with avidin-coated magnetic beads.

Next-generation sequencing analysis

A panel of 35 genes was designed to identify important mutations for lymphomagenesis (Table S1). Amplicon libraries were obtained from 104 ng of the CD3+ fraction blood DNA, using HaloPlex Target Enrichment System (Agilent technologies), according to the manufacturer's protocol. Sequencing was performed using a MiSeq platform (Illumina) using the manufacturer recommendations.

For all samples, average depth in target regions was 619 (range 270–1650) and 98.26% (range 93.5–99.5) of target regions were covered by at least 100 reads, with a general sensitivity and specificity of more than 96% and 99%, respectively [http://www.e-cancer.fr/Professionnels-de-sante/Les-therapies-ciblees/Les-plateformes-de-genetique-moleculaire-des-cancers/Le-programme-d-assurance-qualite-des-plateformes], with a normal deamination score (Table 2).

Results were analyzed after alignment of the reads using the SureCall Software version 3.0.1.4 (Santa Clara, CA, US) from Agilent Technologies. Minimum allele frequency for variant calling was set at 5% with a minimum local depth at 40. All variants were manually checked using IGV 2.3 software and confirmed with Sophia Genetics software (Sophia Genetics, Saint-Sulpice, Switzerland).

Detection of described polymorphism was performed by an in-house software using Ensembl and Alamut database.

Flow cytometry analysis

For lymphocyte phenotyping, CD4+ T cells, CD8+ T cells, B cells, and natural killer (NK) cells were first numerated on fresh blood using a XL Flow Cytometer (Beckman Coulter, Brea, CA, US). Differentiation and activation phenotyping was performed on fresh blood cells using following antibodies: anti-CD8-PE, CD8-PerCP, CD8-PE (BD Biosciences), and anti-CD4-PerCP-Cy7 (Becton Dickinson Biosciences, Franklin Lakes, New Jersey, US) and acquired after fixing cells with PBS BSA on a FACS-Canto I flow cytometer (Becton Dickinson Biosciences).

Intracellular cytokine staining was performed as previously described [7]. Briefly, purified peripheral blood mononuclear cells (PBMC) (10^6 cells/mL) were studied at baseline and after stimulation with PMA (phorbol myristate acetate)/ionomycin. RPMI 1640 was used as a negative control. Cells were harvested, washed, and stained with CD8-FITC A700, then permeabilized with the Intra-Prep Kit (Beckman Coulter) and intracellularly stained with the following antibodies: CD3-APC, CD4 PE-Cy7, IL-2-PE, and IFNγ-PE (Becton Dickinson Biosciences). Cells were acquired on a FACSCanto I flow cytometer (Becton Dickinson Biosciences). At least 20,000 events were acquired within the CD3+ or CD3-/CD4+ or CD3+/CD8+ lymphocyte population.

Clonality tests

Clonality of expansion was routinely tested using TCR-γ and IGH Polymerase Chain Reaction (PCR) followed by fragment length analysis on 3500 xL Dx Genetic Analyser (Applied Biosystems®, Foster City, CA, US) according to Biomed-2 standardization protocol [7].

Case Report

A 63-yo male with arterial hypertension as the only medical past history was referred for chronic mild hyperlymphocytosis (6 G/L) discovered fortuitously. Performans status was normal, and clinical examination was unremarkable. Platelet count was mildly decreased, and C-reactive protein was normal. Blood smear disclosed small monomorphic lymphocytes with a mature chromatin and irregularly outlined nucleus. Cytoplasm was basophilic with cytoplasmic expansions (Fig. 1A). Immunophenotyping revealed that expanded lymphocytes were positive for CD4 and CD7, partially expressed CD25 and TCR (T-cell receptor)$\alpha\beta$ and were negative for CD2 and CD5 (Table 1). Metaphasic cytogenetic analysis was normal. Rearrangement for γT-cell receptor locus was consistent with a clonal T-cell expansion (Fig. 1B). Total body computerized tomography revealed hyperdense pictures on superior lobe of right lung, consistent with an infectious process (Fig. 2A). Broncho-alveolar lavage disclosed a hypercellular liquid whereas bacterial examination identified acido-alcoholo-resistant bacilli, related to a Mycobacterium tuberculosis infection. A positron emission tomography found no other infectious localization (Fig. 2B). Tuberculosis resolved with adapted standard

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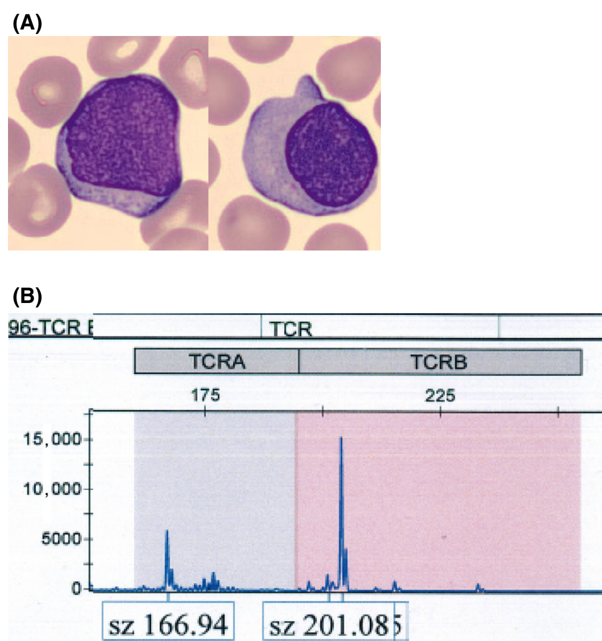

Figure 1. (A) Blood smear showing malignant cells. (B) T-cell receptor-γ locus rearrangement analysis of peripheral lymphocytes showing that the CD4+ T-cell expansion was clonal.

Table 1. Peripheral blood cell count on diagnosis.

	Value
White blood cells (G/L)	8.84
Hemoglobin (g/dL)	14.7
Platelets (G/L)	131
Neutrophils (G/L)	1.77
Total lymphocytes (G/L)	6.38
CD3-/CD4+ (%)	69
CD3+/CD4+ (%)	11
CD3+/CD8+ (%)	6
CD19+ (%)	11
CD16+/CD56+ (%)	3
Reticulocytes (G/L)	54
LDH level (xN)	1.3

LDH, lactate dehydrogenase; xN, number of times the upper normal value. Results are given as percentage of normal values.

antibiotherapy and T-cell proliferation was formerly not treated.

Three years later, however, peripheral T-cell lymphocytosis increased to 80 G/L in association with mild cytopenias and spleen enlargement. A new diagnostic work out was performed and found similar findings. Moreover, generation sequencing (NGS) identified a M511I mutation of JAK3, in association with a mutation of enhancer of zeste homolog 2 (EZH2) (Table 2). We found that both mutations were somatic, as they were not present in nonhematopoietic cells (mucosal cells from oral cavity). From these results, the final diagnosis of T-PLL was retained. A treatment with alemtuzumab was undergone, which allowed achieving a complete remission with no detectable malignant cell by immunophenotyping in blood and bone marrow. A therapeutic intensification with allogeneic hematopoietic stem cell transplantation was performed.

To determine a possible relationship between tuberculosis and T-PLL, we hypothesized that T-PLL could have facilitated tuberculosis by inducing an acquired immune cellular deficiency. For this, we explored peripheral CD4+ T-cell function. Interestingly, we found that peripheral monoclonal CD4+ T cells were unable to secrete interferon-γ and interleukin-2; moreover, the residual polyclonal CD4+ T-cell population poorly secreted interferon-γ and interleukin-2 (6% and 21% of residual CD4+ T cells, respectively) (Table 3).

Discussion

Our observation provides two original messages. First, we provide evidence that T-PLL can result in a severe acquired immune cell deficiency, related to a severely decreased capacity of both malignant and residual polyclonal T cell to secrete the T-helper 1 cytokines interferon-γ and interleukin-2. As patients with T-PLL are usually treated with immunosuppressive strategies including alemtuzumab followed by autologous or allogeneic hematopoietic stem cell transplantation [3], opportunistic infections may occur or exacerbate and thus need to be identified early in the management, such as in the present case. Clinicians should therefore be aware of this previously unreported association when they manage patients with T-PLL. The mechanisms leading to the absence of interferon-γ and interleukin-2 secretion by malignant cells remains unexplained, although they could result from oncogenic processes. Similarly, we hypothesized that the severely decreased cytokine secretion by residual polyclonal CD4+ T cells could result from an excessive secretion of T-helper 2 cytokines such as interleukin-4 and 10 by malignant cells. However, our experiments did not support this hypothesis (data not shown). Taken together, these results are consistent with the view that T-PLL resulted in an acquired cellular immune deficiency by the absence of T-helper 1 cytokines interferon-γ and interleukin-2 secretion by malignant cells, but also by residual polyclonal cells, through unknown mechanisms. Alternatively, one could suggest that a chronic stimulation of T cells by tuberculosis could have favored the occurrence of T-PLL. Although we cannot totally exclude this scenario, the complete eradication of tuberculosis had no effect on lymphocytosis, rending this hypothesis less likely.

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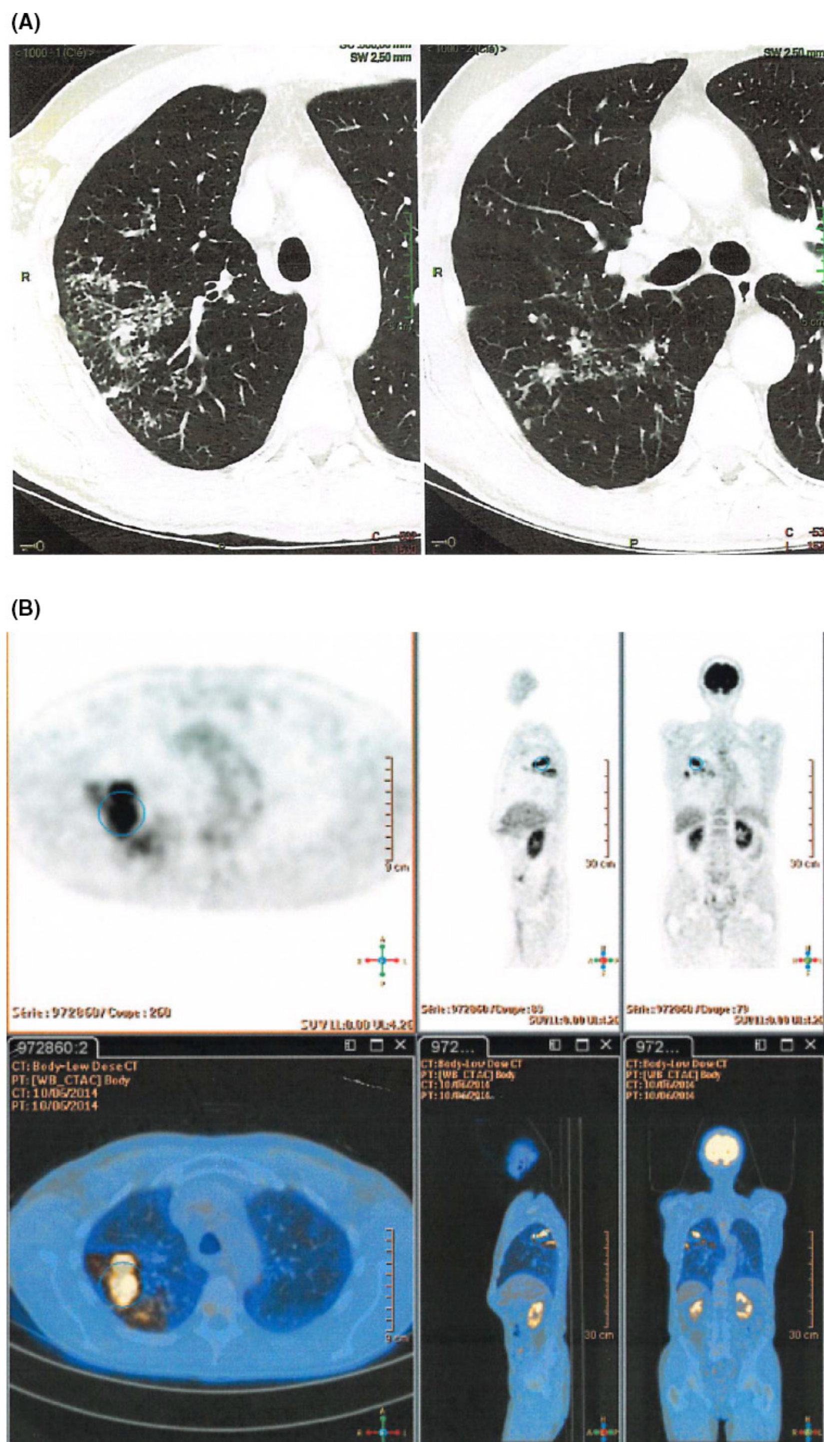

Figure 2. (A) Computerized chest tomography and (B) positron emission tomography on admission.

The second finding emphasizes the importance of next-generation sequencing as a diagnostic tool in malignancies. Indeed, our patient initially had a former diagnosis of chronic T-cell expansion with no other specification, and the accurate diagnosis of T-PLL was only made after the identification of typical molecular abnormalities by next-generation sequencing analysis in this malignancy, that is, JAK3, ATM, and EZH2 mutations [5], which allowed subsequently providing an adapted treatment. It is therefore likely that malignancies poorly characterized

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Table 2. Next-generation sequencing: variants.

Gene	Mutation type	cDna	Protein	VAF	Coverage	Pathway	SIFT deleterious score	Mutation taster	Domain	Sensitivity	Deamination: proportionC>T et G>A
JAK3	Nonsynonymous SNV	NM_000215.3, chr19(GRCh37): c.1533G>A	Met511Ile	27.41	2481	JAK/STAT pathway	0.34	0.927	Tyrosine-protein kinase non receptor Jak/TyK2	10^{-4}	Normal
EZH2	Nonsynonymous SNV	NM_001203247, chr19(GRCh37): c.803A>T	Asn268Ile	41.98	2144	Epigenetic regulation	0.02	1	Interaction zone with DNMT3	10^{-4}	Normal
DNMT3A	None									10^{-3}	Normal
STAT5B	None									10^{-3}	Normal
NRAS	None									10^{-3}	Normal

cDNA, complementary DNA; JAK, Janus kinase; DNMT3A, DNA methyltransferase 3A; STAT5B, Signal Transducer and Activator of Transcription 5B; VAF, variant allele frequency; SIFT, Sorting Intolerant from Tolerant.

Table 3. Interferon-γ and interleukin-2 secretion by CD4+ T-cell lymphocytes.

	Baseline		After stimulation	
	IFN-γ	IL-2	IFN-γ	IL-2
Malignant population CD3-/CD4+ lymphocytes (%)	0	0	0.5	8
Polyclonal population CD3+/CD4+ lymphocytes (%)	0	0	6	21
CD3+/CD8+ lymphocytes (%)	0	0	20	51

IFN-γ, interferon-γ; IL-2, interleukin-2. T-cell lymphocytes were stimulated with PMA (phorbol myristate acetate)/ionomycine.

by traditional tools should be significantly revisited by the use of next-generation sequencing [8].

Authorship

PCe, AG, CM, FF, and RT: performed experiments. NJ and PCo: managed the patient. PCe and PCo: wrote the manuscript. All authors reviewed and approved the final version of the manuscript.

Conflict of Interest

The authors declare no competing interests.

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Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Table S1. Next generation sequencing lymphoma panel.

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